Abstract: Legumes are mostly grown rainfed and are exposed to various types of drought ranging from terminal drought to intermittent drought. The objective of this study was to compare the root anatomy of six major legume crops in relation to their drought adaptation strategies. Plants of chickpea (*Cicer arietinum* L.), groundnut (*Arachis hypogaea* L.), pigeonpea (*Cajanus cajan* [L.] Millsp.), cowpea (*Vigna unguiculata* L. walp.), soybean (*Glycine max* [L.] Merr.) and common bean (*Phaseolus vulgaris* L.) were grown along with pearl millet (*Pennisetum glaucum* [L.] R. Br.) in a Vertisol field during the rainy season of 2010. Four root segments from 35-day-old plants of each crop species were collected, 10 cm from the root tip and used for making transverse sections. These root segments were thinner in both groundnut and pigeonpea than in other legumes but similar to those of pearl millet. Soybean and pigeonpea had a relatively thinner cortex than the other legumes. Xylem vessel size and the numbers were apparently the most discriminating traits of legumes. Pigeonpea is equipped to conduct small quantities of water per unit time with a few narrow xylem vessels and that explains the conservative early growth of pigeonpea. Chickpea and cowpea showed moderate xylem passage per root indicating that they are capable of absorbing water moderately and are well equipped for regular drought episodes. The development of cortical and stele tissue and their proportion is markedly influenced by moisture availability to the root system.

Key words: Cortex, Hydraulic conductance, Parenchyma, Vascular tissue, Xylem vessels.

Plants have evolved to adapt to their ecological environment. Therefore, their morphology, anatomy and physiology are likely to provide clues towards their adaptation to various growing conditions (Chandler and Bartels, 2008). Crop plants respond to variations in water and oxygen status of the soil through morphological, anatomical and physiological adjustments that help them cope with such variations and the associated stress (Krishnamurthy et al., 1998, 1999; Chandler and Bartels, 2008). This capability may have high adaptive value in plants in an environment with frequent episodes of drought.

Legumes are mostly grown rainfed and experience water deficit at different developmental stages. The intensity of drought depends on the environment and the crop species. Chickpea, pigeonpea and groundnut are often subjected to terminal drought stress while pigeonpea and groundnut are subjected to intermittent drought resulting in heavy yield losses (Subbarao et al., 1995). Cowpea is a versatile crop mostly grown in regions across various climatic zones in semi-arid regions like the savannas of Africa (Singh and Allen, 1980) where terminal drought events are frequent. Many such characteristics make it more tolerant to drought than other legume crops (Turk et al., 1980; Gwathmey and Hall, 1992; Itani et al., 1992; Mia et al., 1996; Singh et al., 1999). Soybean is planted at the start of spring or later in USA, South America and China and experience relatively less drought compared with other legumes with a high water requirement (450–700 mm) (Dogan et al., 2007). Soybean is found to be well-adapted to the rainy season in South East Asia (Srivastava et al., 1996). Common bean has a short growth period, under the rainfed condition requiring about 300 to 500 mm water for a 60- to 120-day growth period (FAO, 2012). This crop is highly responsive to irrigation and spells of drought during reproduction adversely affect...
grain yield and seed quality (Upreti et al., 1997). Therefore, these crop species may have different adaptive mechanisms to drought at various organizational levels including morphology, anatomy and physiology due to differences in growing season and the agro-ecological conditions of the growing environments. The development of deep roots is one common example of both the adaptation and avoidance mechanisms of drought (Chandler and Bartels, 2008). Roots are usually the site showing the highest resistance in the pathway for liquid-phase movement of water through the soil-plant-atmosphere continuum (Kramer and Boyer, 1995). The efficiency of soil water uptake by the root system is, therefore, a key factor in determining the rate of transpiration and the varying strategies of adaptation to drought. Water uptake by the root is a complex process which depends on root structure, root anatomy, and the pattern by which different parts of the root contribute to overall water transport (Steudle et al., 1987; Cruz et al., 1992).

A major difference between the monocots and dicots is the continuing presence of endodermis and pericycle, which are two highly suberized layers that may play a major role in selective absorption and regulation of mineral and water intake. In monocot roots, the endodermis tissue develops within the cortex and is characterized by a more compact, generally unicellular layer that has no air space. It also has casparian bands in the radial and transverse anticlinal walls (Vasquez, 2003). These cells have lignin and suberin deposits making them impermeable to water and ions. Any substances that they allow through to the stele must pass through the protoplasm of the casparian band cells which are accomplished through the plasmalemma or the plasmodesmata. In many plants, just beyond the endodermis is a parenchymatous layer called the pericycle. This layer is meristematic and is the source of lateral roots, although it can sometimes lignify. Beyond the pericycle is the vascular cylinder or stele. The roots of some monocotyledons, like corn, have a medulla as well as a vascular cylinder (Vasquez, 2003).

Xylem vessels play a key role in water movement. An increase in root hydraulic resistance can best be achieved by decreasing the diameter of the main xylem vessel in the seminal roots (that absorbs water from the deeper soil horizon). Also this trait was found to be useful in enhancing grain yield selectively under drought in wheat while not found to impose yield penalty in good seasons (Richards and Passioura, 1981a, 1981b). Subsequently, this trait had been successfully utilized in breeding for better yield under receding soil moisture conditions (Richards and Passioura, 1989).

The objective of this work was to explore the possibilities of identifying differences in anatomical traits that differentiate drought adaptation strategies of legumes.

### Material and Methods

#### 1. Plant material and crop management

Six legumes with different drought tolerance abilities and pearl millet, a cereal crop adapted to semi-arid environments, were tested for variation of their root anatomy in relation to their level of drought tolerance. Varieties Annigeri (chickpea), ICPL 87119 (pigeonpea), TAG 24 (groundnut), Suvita (cowpea), JS 9305 (soybean), Topcrop (common bean) and ICMV 155 (pearl millet), were sown on 1 Jul 2010 in a Vertisol field at ICRISAT, Patancheru. The soil (fine montmorillonitic isohyperthermic typic pallustert) was heavy and retained about 230 mm of plant available water in the 120-cm soil profile. The field was prepared into a flat seed bed and fertilized before sowing by surface application and incorporation of 18 kg N ha$^{-1}$ and 20 kg P ha$^{-1}$ as diammonium phosphate. Each crop species was planted in a 3 m long row and in 2 such rows with 30×20 cm spacing. Four crops (adjacent to one another) on one side and three more on the other with no borders were planted. Seeds were treated with 0.5% Benlate$^\text{®}$ (E.I. DuPont India Ltd., Gurgaon, India) + Thiram$^\text{®}$ (Sudhama Chemicals Pvt. Ltd. Gujarat, India) mixture before planting and the plots were kept insect pest and weed free until the roots were harvested.

#### 2. Root sampling and root sectioning

Roots were harvested from 3 Aug 2010 to 8 Aug 2010 at 34 to 39 days after sowing (DAS) after digging a trench in a way to expose all the rows of four crops on one side and three on the other and washing the exposed walls with a fine spray of water so as to expose roots to a certain length from the tip. A 2-cm long piece of the tap root, 10±2 cm above the root tip and where the secondary thickening is expected to be complete, was collected from each crop and kept in distilled water after washing them. Freehand sections of about 50 μm thick were cut and the selected sections were stained with 50% toluidine blue, a polychromatic stain that gives different colors with different tissues, and mounted in distilled water. For each genotype, ten uniform sections were selected at random for observation. The following measurements were performed using an optical microscope (Olympus, USA) connected to a digital camera: (i) thickness of the whole root, (ii) thickness of cortex and stele, (iii) diameter of the xylem vessels (All the transverse section of the roots were printed separately after magnifying the size for a single frame for a conveniently enlarged form and diameter of all the vessels were measured using a scale. The readings were readjusted (reduced) considering the magnification of the picture (both microscopic and the print frame). The cell wall was not included (Carlquist, 1988). It was difficult to identify the metaxylem vessels for the protoxylem. Therefore, all the xylem vessels were grouped into two...
groups, 1. large metaxylem vessels and 2. small vessels (protoxylem vessels and small metaxylem vessels). The collected data were used to compute the percentage of large metaxylem vessels in roots (ratio between the area occupied by the large metaxylem and total cross sectional area).

3. **Chickpea roots from hydroponics and pot culture**

   External environment is known to affect the plant morphology and is also expected to affect the anatomy. The anatomical variation across growth medium environments was studied in chickpea. Five-day-old seedlings of variety Annigeri were transferred to a well-managed and a well-aerated hydroponics for 35 days in a greenhouse and the roots were harvested similar in position to the field-grown plants. Also two plants were grown for 40 days in 20 cm diameter pots containing 5 kg of Vertisol fertilized with 300 mg kg⁻³ of diammonium phosphate. The roots (intact with plants) of these pots were washed in running water and the root portions 10 cm away from the tap root tip were selected for sectioning using the same protocol as above.

   **Results**

   1. **Root growth**

   Visual observations on the exposed trench wall showed that the branching of the roots in pearl millet was profuse whereas branching was less and limited to the second order level in legumes (data not shown). Though the roots could be traced to depths of more than 60 cm at 35 DAS the rooting depth did not differ with the crop. The prolificacy of roots in the top 30 cm soil horizon was the highest in pearl millet followed by chickpea. Groundnut and pigeonpea had the least prolificacy of the root system (data not shown). The differences in root distribution of chickpea and cowpea can be seen in Fig 1.

   2. **Root diameter**

   A wide range of root diameter at the proximal portion of the growing root tips, i.e. 10 cm above the root tip, was observed among the six crops studied (Fig. 2). Pearl millet had the thinnest roots (705 μm) followed by groundnut (728 μm) and pigeonpea (833 μm) (Fig. 3). The remaining crops produced relatively thicker roots with root diameter ranging from 975 to 1200 μm. Soybean roots, maintained in dry pots, were shown to be thinner than these roots (Rieger and Litwin, 1999) likely due to the wet growing conditions of the Vertisol soil.

   3. **Cortex and endodermis**

   The cortex is made of parenchyma tissue and plays a critical role in the regulation of the transport of water and other substances via the apoplast and symplast pathways. In dicotyledons, the cortex is shed when secondary growth begins while in monocotyledons, the cortex is maintained throughout the plant’s life and the cells can develop secondary walls and lignify. The crops that are used in this study had the root cortex proportion in the range of 31% to 49% of the cross section area (Fig. 2 and Fig. 4). Pearl millet had the largest cortex area of about 50% of the whole root section. Soybean followed by pigeonpea presented smaller cortex than the other legumes. Pearl millet had a clear endodermis layer in the center that surrounds the vascular cylinder. However, in all the legumes both the endodermis and the pericycle layers were missing. The cortex was found intact in all legumes at this stage though loss of major cortex was reported as a consequence of secondary thickening (Vasquez, 2003).

   4. **Vascular tissue**

   The primary tetrarch arrangements of the vascular bundles, characteristic of the examined six legumes at the start of secondary thickening (chickpea: Fatima and Chaudhry, 2004; pigeonpea: Bisen and Sheldrake, 1981; groundnut: Tajima et al., 2008; cowpea: Lawton, 1972; soybean: Kumudini, 2010; common bean: Jaramillo et al., 1992), are lost due to secondary thickening in all the legumes. The whole inner core is fully occupied by the xylem vessels with medullary rays barely visible (Fig. 2). The centripetal pattern of maturation, reported in dicotyledons in the early stages of secondary thickening, is lost. The narrow xylem elements were seen interspersed with metaxylem vessels throughout the central xylem core. However, the crushing and loss of protoxylem as a consequence of secondary thickening in the stems of *Medicago sativa* is reported by Esau (1977). No symptoms of such crushing and loss of protoxylem were seen in the roots of any of the legumes that were studied. The phloem is pushed more into the cortex towards the periphery of the central xylem-dominated core. The vascular cylinder of the root is very different from that in the stem. In stems, the xylem and the phloem are found in continuing rings, xylem occupying a more central position and the phloem on scattered patches well into the cortex. In pearl millet, either one single xylem element or a few in a cluster surrounded by phloem cells are placed closely inside the pericycle and a large central medulla (Fig. 5). In many dicotyledons, secondary growth develops later where the cambium and the peridermis play an important role.

   5. **Xylem vessels**

   Among the crops studied, chickpea had the maximum number of large metaxylem vessels (32) as well as small xylem vessels (44) but with the narrowest average diameter of these vessels (9.5 μm) (Table 1). Cowpea and common bean had the least number of total xylem vessels but their average diameter was moderate. The total xylem passage (number of xylem vessels × average vessel diameter) of a
Fig. 1. The differences in rooting patterns of chickpea (two rows on the right) and cowpea (two rows on the left). Note the profuse surface rooting in chickpea on the surface soil horizon.

Fig. 2. Transverse sections of roots of six legume species in comparison with pearl millet. A= pearl millet (×80), B= chickpea (×120), C= pigeonpea (×100), D= groundnut (×100), E= cowpea (×200), F= soybean (×200) and G= common bean (×300).

Fig. 5. Stelar portion of roots of A= pearl millet (×200), B= chickpea (×200), C= pigeonpea (×300), D= groundnut (×400), E= cowpea (×400), F= soybean (×400) and G= common bean (×400). LMX, large metaxylem; SXV, small xylem vessels; EN, Endodermis.

Fig. 6. Transverse sections of chickpea roots that were grown for 40 days in (A) hydroponics (×100), (B) well-watered Vertisol-filled pot (×100) and (C) under receding soil moisture (×120) in a Vertisol during rainy season 2010.
shown all the characteristics of a plant grown by hydroponics, but the secondary thickening seemed to have progressed by producing relatively fewer and narrower vessels (Fig. 6). Also the tetrarch formation of the xylem bundles was seen intact while newer large metaxylem vessels were added between the gaps of the tetrarch arms and below the phloem bundles. Also the round parenchyma cells seen in the hydroponics had turned hexagonal seemingly with the internal pressure of secondary thickening. A clear endodermis layer and cambium are intact.

In a field-grown plant, with the advance in secondary thickening, all these early stage characteristics are lost with the enormous addition of xylem vessels in number and size (Fig. 6). However the cortical layer remained 6-7 layers thick irrespective of the stele growth or the growing environment. The cortical cells were centripetally compressed, relatively small and dense with no intercellular spaces. With increasing levels of water deficit the cells tend to be more compact and tightly packed.

6. Influence of growing environment on root anatomy

The roots of chickpea grown in well-managed hydroponics showed a large number of branches arising from the base of the tap root. These branches measured not more than 25 cm in length and did not branch much further (data not shown). This morphological modification is probably due to less resistance to root elongation compared with soil-grown plants. Roots grown in this environment clearly showed the characteristic tetrarch pattern of xylem bundles that alternated with strips of phloem bundles (Fig. 6). The stele size as well as number of xylem vessels was limited. All these stele characters indicated that either the secondary thickening was delayed or the roots will not thicken at all. However the cortex was proportionately thick with round, large and loosely packed parenchymatous cells indicating a very poor centripetal growth.

The chickpea roots grown in regularly well watered pots,
limited environments but exposed to varying intensities and periods of drought. Chickpeas are usually grown under progressively receding soil moisture conditions whereas the other pulses also experience intermittent drought that is relieved with subsequent rain or irrigation. Efforts have been made to understand the differences in the strategy for drought adaptation with the legume, by studying the root anatomy of the crop. One of the most functional aspects related to root anatomy is water and nutrient transport capacity, because it is highly influenced by the number and size of the water conducting elements (Esau, 1965; Steudle and Peterson, 1998). Roots, the primary organs for the absorption of water and minerals, ironically offer the greatest resistance to liquid water flow in the soil-plant inter-phase simply to regulate the absorption process with minimum energy (Rieger and Litvin, 1999).

Pearl millet was included in this study as a representative of dry land cereals and to compare legumes with cereals. Roots of pearl millet branch into higher orders and are thin and have a definite but fewer narrow xylem vessels arranged in a single layer below the endodermis (Figs. 2, 5), with a low range of xylem vessel diameter. This fine root development and limitation in xylem vessel number is likely to be a compensation for a large root length density of finer roots that are known to be produced in cereal crops as in wheat (Gregory and Eastham, 1996). Cereals are known to have a greater root length density than the legumes (Hamblin and Tennant, 1987; Brown et al., 1989; Petrie and Hall, 1992). The presence of highly suberized exodermis, a definite cortex, a pericycle and the endodermis are clearly meant for better regulation and resistance that ensured very effective but a conservative absorption of soil moisture making the plants more suited to lighter soils with minimum water holding capacity as well as longer periods of water deficit. Thinner roots, wider xylem vessels and a thin cortex were positively related to the hydraulic conductivity (Rieger and Litvin, 1999) while maintaining the minimum water potential gradient in the soil-plant-atmosphere continuum.

Chickpea had relatively thicker roots than pearl millet, groundnut and pigeonpea. It also had a larger number of thinner vessels in a wider range of sizes than common bean, cowpea and soybean that had broader vessels. In heavier soils such as Vertisols with finer soil particles, the lateral movement of water is relatively restricted and therefore finer vessels coupled with dense root lengths can lead to better absorption of the available soil water. Therefore, chickpea seems more suitable to dense heavier soils while common bean, cowpea and soybean are better adapted to coarse soils and rapid absorption of available soil water than chickpea.

Groundnut had the thinnest roots along with very slender vessels though the number of vessels was similar to that of cowpea and common bean. Groundnuts also seemed to be well adapted to conservative use of soil moisture and are known to produce less prolific root system and thus poorly equipped with a rapid absorption of soil water. Groundnuts have leaves that are better equipped for a prolonged drought. After temporary wilting and drooping of leaves, the plants are capable of complete recovery when watered.

Pigeonpea is a special legume that had few and narrow xylem vessels. The stele contained a large number of xylem fibres mimicking the stems where these cells are certainly needed for providing mechanical strength to the tall plants. A large number of xylem fibres with thickened walls, similar to those seen in pigeonpea (Bisen and Sheldrake, 1981), were also seen in soybean. On the contrary, there were few such fibres in groundnut (Fig. 5). Pigeonpeas have a relatively long growth period with a very low crop growth rate in the early vegetative growth (Sheldrake and Narayanan, 1979). Therefore, this conservative approach of soil water absorption can be an appropriate match for the slow growth of this crop.

Common bean, soybean and cowpea had a moderate number of broad vessels, and thick roots indicating that their roots are capable of absorbing a larger amount of water when available; which explains their good adaptation to rainy seasons. Even within these three legumes, common bean had the thinnest cortex with more uniformly broader xylem vessels indicating that this crop is well adapted to soils with better water regimes and can be highly productive under regular irrigation.

Root water uptake of the whole plant is a function of both hydraulic conductivity and water potential gradient across the root or the whole plant (Rieger and Motisi, 1990). Considering the low root prolificacy and narrowest xylem vessels in groundnut, this crop is expected to develop a high gradient of water potential across the soil-plant continuum for the necessary water uptake whereas chickpea, with thicker roots and large number of xylem vessels, may not need such a wide gradient of water potential for the necessary water uptake. However, both crops are adapted to water-limited environments with a different strategy.

Crop plants are better equipped with an appropriate type of anatomy, largely constitutive in nature, to cope with the surrounding (soil moisture) environment (Rieger and Litvin, 1999). However environment also seems to play a major role in modifying the anatomical features. In response to the changing water regime of the growing environment major changes do occur in selective growth of component tissues. During the secondary thickening, very little change seems to occur in the volume of cortical layer and the phloem bundles whereas the number and size of the xylem vessels and other xylem components seem to increase with water scarcity. In situations of severe
drought stress further increase in vessel number and size seems likely. Also these root growth changes are structural and once secondary thickening is completed, no further changes are possible even when alternate moisture environments are provided. This could be more harmful to crops where the rooting front descends with the receding soil moisture. Development of permanent conducting tissues that can support less volume passage can act as a bottleneck when better soil moisture conditions are provided. For example, in chickpea grown in lighter soils with a drier soil environment until flowering, yield is not increased even if a very comfortable moisture regime is provided at a later crop growth stages. This may be why vegetative stage irrigation is invariably inevitable, while most economical limited lifesaving irrigation techniques have been tested. This may be why new axillary roots are initiated when late crop growth stage irrigation is practiced or rainfall is experienced.

**Conclusion**

Among the six legumes examined, the portion of the root 10 cm behind the tip was the thinnest in groundnut and pigeonpea and was similar to that of pearl millet. The presence of thinner roots and thinner cortex that offers less root resistance to hydraulic conductance in groundnut makes this crop more adapted either to a regularly irrigated environment or to a very dry environment. The early growth of pigeonpea is conservative and the presence of very few thin xylem vessels in pigeonpea explains the low passage of water. Chickpea and cowpea had a thicker cortex along with a moderately high xylem passage per root indicating that they are capable of absorbing water moderately and are well equipped for regular drought episodes. Soybeans with a thin cortex and the common beans with their broad and fewer vessels are well suited for locations with optimum water supply. Legumes, as demonstrated in chickpea, are capable of regulating the necessary tissue development for appropriate hydraulic conductance during secondary thickening of the root system depending on the soil moisture status. Further studies using genotypes with contrasting responses to drought would provide more information on the extent of the adaptive nature anatomical reaction in response to drought in the legume species.

**Acknowledgement**

This work was supported by the Bill & Melinda Gates Foundation through the Tropical Legumes 1 (TL1) grant.

**References**


Fatima, T. and Chaudhary, NY. 2004. Morphogenetic effect of growth hormones i.e., indole-3-acetic acid, gibberellic acid and heavy metal i.e., lead nitrate on the external and internal morphology of seedlings of *Cicer arietinum* L. *Pak. J. Biol. Sci.* 7: 1443-1452.


Lawton, J.R. 1972. The use of common weeds and garden plants for...


