International Journal of Bio-resource and Stress Management 2011, 2(3):250-256

Comparative Anatomy of Maize and its Applications

R. K. Maiti*, S. K. Ghosh, S. Koushik, A. Ramasamy, D. Rajkumar and P. Vidyasagar

Vibha Seeds, Vibha Agrotech Ltd, Inspire, Plot no. 21, Sector 1, Huda Techno Enclave, High Tech City Road, Madhapur, Hyderabad, Andhra Pradesh (500 081), India

Article History

Manuscript No. 176
Received in 1st July, 2011
Received in revised form 3rd August, 2011
Accepted in final form 4th September, 2011

Correspondence to

*E-mail: ratikanta.maiti@gmail.com

Keywords

Maize anatomy, stereome, bulliform cells, endosperm, sclerenchyma

Abstract

The present study reveals that there are large variations in anatomy of maize genotypes, which help in selecting drought tolerance lines, stem borer resistant as in tropical region. In some genotypes, presence of thick exodermis and higher lignification provide better structural integrity, and higher root diameter is associated with higher xylem diameter which helps in better solute transport. Considerable variations exist among genotypes in distribution and amount of mechanical tissue (stereome) and vascular bundles, and in the size and shape of the protoxylem cavity. In some genotypes, there is strong steroeme system in stem and long bulliform cells, thick sclerenchyma patches in leaf to survive in severe drought condition. Leaf anatomy possesses typical Kranz anatomy characteristics of C_4 plant showing the variation in trichome density, silica crystal deposition on the epidermis which offers insect and fungal resistance. Maize genotypes show variability in seed anatomy with distribution and amount of corneous and floury endosperm which may be related to imbibitions capacity.

1. Introduction

Maize (*Zea mays*, 2n=20) is the most important food and animal feed crop of the Western Hemisphere. It is grown throughout the world under wide range of climates. Since pre-Hispanic time, it has been the basic food for the majority of people in Mexico and Latin American countries. From about 900 A.D. to 1300 A.D., there was a shift from intensive foraging with some agriculture to intensive farming with some foraging. This was accompanied by more densely settled population and greater cultural complexity among people in Mid-west. In scientific and formal usage, 'maize' is normally used in a global context. Equally, in bulk-trading contexts, 'corn' is used most frequently. Mexico is one of the centers of origin of maize where several native species grow in abundance in diverse environments. Several factors were involved in the diversification of maize in Mexico—the existence of primitive landraces; the influence of exotic varieties from the northern part of the country, teosinites that underwent crossing with other races leading to the origin of new races; geographical conditions favoring a rapid differential and diversification due to varying factors. More than 65 races of maize have been identified in Mexico, while in USA, only 14 races are observed. The number of races of maize in South American countries like Bolivia (77) and Peru (68) are higher than that of North American countries.

Maize seeds are used in different forms throughout the world. Maize and corn meal (ground dried maize) constitute a staple food in many regions of the world. Maize meal is also used as a replacement for wheat flour, to make corn bread and other baked products. Pop corn and corn flakes are a common breakfast cereal found in many other countries all over the world. Sweet corn, a genetic variety that is high in sugars and low in starch, is usually consumed in the unripe state. Certain varieties of maize have been bred to produce many additional developed ears. These are the sources of the 'baby corn' used as a vegetable in Asian cuisine. Starch from maize can also be made into plastics, fabrics, adhesives, and many other chemical products. Grain alcohol from maize is traditionally the source of bourbon whiskey. The corn steep liquor, a plentiful watery by-product of maize wet milling process, is widely used in the biochemical industry and research as a culture medium to grow many kinds of microorganisms.

2. Materials and Methods

Plant materials which include roots, stem portion, leaves were collected from the field and put in a polythene zip-lock bags containing water. Thin sections were cut using sharp razor blade. The sections were immersed in water to avoid the formation of air bubbles. Sections were stained with safranine. Excess of stain was washed with water. Then sections were stained with fast green solution. Excess of stain was washed with water. Sections were mounted in glycerol, covered with cover slip and observed under microscope. The anatomy of vegetative parts (stem, leaves and root) with respect to organization of tissues was taken on the basis of transverse sections. At least 20 observations were taken into consideration for deciding the nature and structure of a particular character (part/tissue).

The seed anatomy with respect to distribution of corneous and floury endosperm was undertaken on the basis of longitudinal section of seeds. Seeds of maize genotypes were soaked separately for each entry in water (glass beaker) for 15-20 h. Seeds were completely submerged in water. Uniform and ideal size of 20 seeds was taken from each entry after 20 h. Seeds were cut longitudinally through the embryo (longitudinally made two equal halves from the center of the seed) and observed for floury and corneous endosperm.

3. Results and Discussion

3.1. Root

3.1.1. Root anatomical features

There are two distinct types of roots in the maize root system- the embryonic roots that develop from young embryo, and the post-embryonic (adult) roots that develop during different stages of crop growth. The general anatomical features of both the roots are similar and are typical of monocots. The maize root is composed of epidermis; ground tissue (cortex); endodermis surrounding the vascular bundles; vascular bundles consisting of xylem and phloem present in alternate bands (Plate 1). The epidermis consists of elliptical cells subtended by two layers of hypodermis. In early stage of root development, the cortex is made up of ovoid parenchymatous cells with considerable intercellular space. As the root ages, the cortical cells elongate to assume a plate like appearance. A single layer of ovoid to cubical cells of cortical cells of cortical parenchyma encircles the endodermis. The endodermal cells are barrel to boat shaped and are thickened with suberin on the inner tangential wall. Crystals are present in the endodermal cells; their shape and size vary with cultivar and with age of the root. The pericycle below the endodermis is composed of one to several layers of thick-walled sclerenchymatous cells, of which the outer most layer is highly lignified. Xylem parenchyma surrounding the metaxylem may be thick-walled or lignified. The xylem and phloem show a typical closed radial arrangement. Protoxylem bundles are present on the exterior side of the metaxylem. The size of the metaxylem bundle varies according to cultivar. The pith is solid with round intermediate to compactly arranged parenchyma cells.

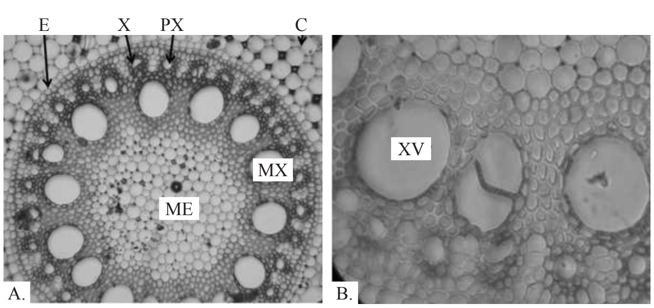

Plate 1: A. Transverse section of root (100X) showing different parts- cortex (C), endodermis (E), protoxylem (PX), metaxylem (MX) and medulla (ME); B. Sector enlarged (400X) xylem vessels (XV)

3.1.2. Significance of variations in root anatomy

Cultivar differences are expressed in a number of root characteristics in maize. These are:

- Presence or absence of sclerenchymatous exodermis in the cortex
- Thickness of endodermal cell walls
- Size and shape of crystals in the endodermal cell cavity
- Intensity of lignification in the pericycle cell layers and in cells surrounding the vascular bundles

Presence of exodermis, higher thickness and higher lignification provide better structural integrity to the root system. Thus genotypes exhibiting these characteristics are expected to have better crop stand and resistance to lodging. Heavy lignification of exodermis of maize root gives higher bending strength, thus providing better anchorage (Ennons et al., 1993). Thicker roots are advantageous for higher root growth and help in better water transport. Higher root diameter is associated with higher xylem diameter in maize, which helps in better solute transport. A number of drought tolerant maize lines developed by the International Maize and Wheat Improvement Center (CIMMYT) have thicker root systems; thus they are able to penetrate deeper in the soil for extraction of available moisture compared to drought susceptible lines (Hund et al., 2009).

The Plates 2, 3 show the highly lignified pericycle region comparing with having lignified pericycle and thickened vessels help in better conduction in drought condition.

3.2. Stem

3.2.1. Stem anatomical features

In a transverse section, the epidermis consists of cubical to boat-shaped epidermal cells containing elongated crystals. Just below the epidermis, and opposite the ridges of the stem, there are alternate bands of large and small thick-walled sclerenchymatous patches. Each sclerenchymatous band alternates with a broad band of chlorenchymatous tissue corresponding to the furrows of the stem outline. These hypodermal bands

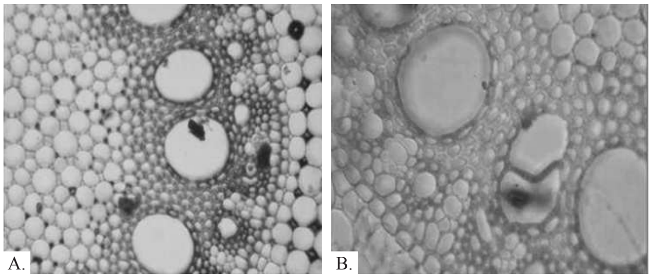

Plate 2: Transverse section of root (400X). A. Casparian thickening in endodermal cells; B. Casparian thickening in endodermal cells and crystals in periycle

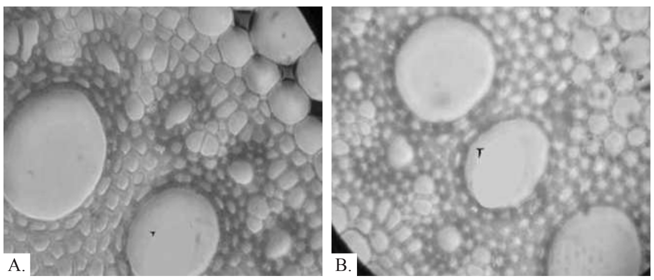

Plate 3: Transverse section of root (400X) showing intensity of mechanical tissue in pericycle region. A. High amount of sclerenchyma; B. Low amount of sclernechyma

of sclerenchyma are intern connected to the subtending broad cylinder of sclerenchyma, which contains the ring of vascular bundles. There are four to five rows of vascular bundles, the outer most in general being the smallest in size (Plate 4). In some sections, just below the outer most layers, large and small vascular bundles alternate with each other. The central vascular bundles are scattered in the ground tissue. Mechanical tissues in these are not as extensive as in the outer bundles, particularly in the peripheral region. They contain only a semi-lunar band of sclerenchyma adjacent to the protoxylem cavity towards the center of the pith. The peripheral region of the central vascular bundles does not contain sclerenchyma. The ground tissue is composed of round parenchymatous cells. Pith is solid.

3.2.2. Significant variation in stem anatom

Internodes of maize vary in the strength and supporting ability. Examination of transverse sections from seventh and eighth internodes collected at physiological maturity reveals considerable variation in distribution and amount of mechanical tissue (stereome) and vascular bundles, and in the size and shape of the protoxylem cavity. The vascular bundles are associated with protective fiber cells, which originate primarily from procambium and provide strength to the stem system (Esau, 1943).

Mechanical tissue consisting of sclerenchyma forms the stereome system of the plant. Maize genotypes show large variations in anatomical structure of stem with respect to the

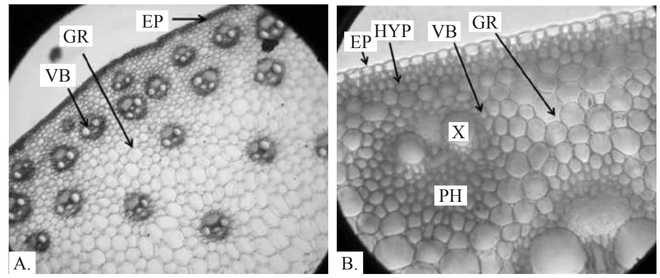

Plate 4: A. Transverse section of stem (100X) showing atactostele; B. Sector enlarged (400X) showing epidermis (EP), hypodermis (HYP), scattered vascular bundles (VB) in ground tissue (GR), xylem (X) and phloem (PH)

distribution and intensity of sclerenchyma, on the basis of which we can classify the genotypes having weak stereome system, intermediate system and strong stereome system. Variability were observed in thickness of sclerenchyma tissue below the epidermis. Strong stereome system consists of 4 layers of cells of sclerenchyma and genotypes with thin sclerenchyma possess only 2 layers. While breeding for new genotypes, anatomical studies thus may help in better selection of genotypes according to the target environment and breeding objectives. Genotypes having strong stereome are expected to be resistant to lodging and exhibit better resistance to stem borer insects. High amount of sclerenchyma gives mechanical support (Murdy, 1960) and reduces the loss of water from internal parenchyma tissue by evapo-transpiration. Medium to large size and high number of xylem vessels are required for efficient translocation of water under drought condition. Thickening of hypodermis and higher sclerociation of vascular bundles provide better tolerance to water stress (Murdy, 1960).

Anatomical variations in stem can also be used for prediction of growth behavior of the plants. Genotypes having higher thickening of hypodermis usually show slower rate of internode growth, thus increasing the crop growth duration. Modern short duration corn varieties of USA have high rate of internode growth and less thickening of hypodermis compared to slow growing races of Mexico and Peru.

A number of stem borers infest maize by boring and feeding on the internode tissues. Stems having higher thickness of parenchymatous tissues and long internode have been correlated with resistance to stem borers (Santiago et al., 2003), indicating these can be used as reliable predictors in developing insect resistance genotypes.

Stem anatomy study of mutants have also allowed identification of important genes involved in cell wall biosynthesis in maize. A good example is the brittle stalk mutant of maize, which has reduced mechanical strength in the stem. Anatomical investigations revealed that the brittleness is due to reduced deposition of cellulose and uneven deposition of secondary cell wall material in the subepidermal and perivascular sclerenchyma (Ching et al., 2006). By using transposon tagging approach, a gene *Bk2* controlling cellulose deposition has been identified which shows high expression in the vascular bundle in the wild type (*Bk2/bk2*) and reduced expression in the mutant (*bk2/bk2*).

Plate 5 and 6 depict the variations in epidermis and sclerenchyma in stem anatomy. A. Showing highly lignified epidermis, thickened sclerenchyma surrounding the vascular bundles. This line remains green in water stress field condition. Having strong steroeme system in stem, it is somewhat resistant to stem borer, comparing with weak steroeme.

3.3. Leaf anatomy

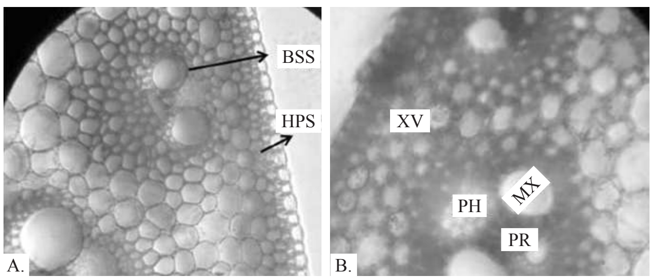

Plate 5: Transverse section of stem 400X. A. Lignified patches of hypodermal sclerenchyma (HSP) and bundle sheath cells (BSS); B. Vascular bundles with protoxylem cavity (PR), metaxylem (MX) and phloem (PH)

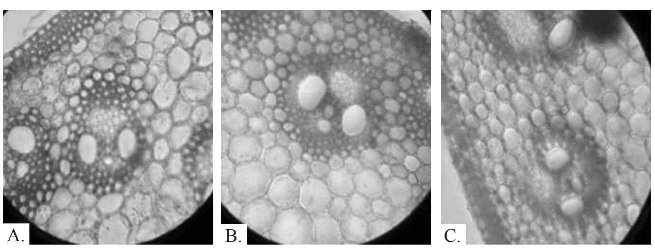

Plate 6: Transverse section of stem (400X). A. Strong stereome with large vascular bundles and high amount of sclerenchyma; B. Intermediate stereome with medium size vascular bundles; C. Weak stereome with small bundles and less amount of sclerenchyma patches below the epiderm

3.3.1. Leaf anatomical features

The anatomy of the maize leaf is characteristic of that of a mesophytic grass. There are some variations among the genotypes studied, but in general leaf anatomy does not very significantly from genotype to genotype. The epidermis is cuticularized, and composed of rectangular or oval epidermal cells. The lower epidermis is entire in outline. Silica crystals, prominent in some genotypes and not so in others, protrude from the adjoining cell walls of two adjacent epidermal cells towards the cuticle. The upper epidermal cells are wavy in outline and interspersed with zones of bulliform cells, the size of which differ in different genotypes. The chlorophyllous tissue surrounding the vascular bundles may be loose or compact. Surface structure shows variation in stomatal frequency size, and trichome. The shape of the mid-rib in transverse section is almost semi-lunar. Bulliform cells are absent in the upper epidermis of the mid-rib and vascular bundles of different sizes are present below the upper epidermis. In some genotypes, the mid-rib has three large vascular bundles, of which one is in the center and one is to each side at the junction of leaf lamina mid-rib. Chlorophyllous tissue is present between the vascular bundles, but confined to the lower portion of the mid-rib. The sclerenchyma forms a band below the upper epidermis. The vascular bundles are of three types in the lamina and mid-rib: 1) the large central vascular bundle corresponding to the main vein, 2) medium size vascular bundles, and 3) very small vascular bundles. The first two types are generally fibro-vascular, containing patches of sclerenchyma on both sides (connecting the lower or upper epidermis), or at one side only (connected to the lower epidermis). The third type-small vascular bundles- are generally present in the lamina, and are without any fibrous bands. These laminar bundles are again of two sizes, alternating with each other. Each vascular bundle remains surrounded by a bundle sheath consisting of thin-walled parenchyma cells (Plate 5, 6). The cells of bundle sheath generally contain big size chloroplasts and form starch; grana are absent. This special structure was first identified by Haberlandt in 1882. The structure appears like garland (Kranz=wreath, garland), so it is known as Kranz anatomy. Kranz anatomy is invariably associated with C_4 photosynthesis. A typical feature of mesophyll cells are toward the exterior of the leaf so that they can be in contact with the intercellular air space. The bundle sheath cells are arranged in the interior of the mesophyll cells.

3.3.2. Significance of variations in leaf anatomy

Maize leaf structure is not only important for understanding Kranz anatomy and C_4 carbon cycle, but also for various modifications such as leaf cuticularization, deposition of silica crystals in the upper epidermal cells (offering rigidness to the leaf as well as resistance to insects), size and shape of bulliform cells (help in rolling of the leaf thereby avoiding loss of transpiration under drought situations).

The Kranz anatomy, which is essential for C_4 photosynthesis, provides distinct photosynthetic advantages to the maize plants under higher CO_2 concentration by overcoming photorespiration (Sage and Monson, 1999). C_4 photosynthesis system is particularly advantageous under high temperature and high light intensity conditions.

Maize leaves have developed a number of mechanisms for reducing evapo-transpirational loss. Presence of thick cuticle on epidermis of leaf reduces traspirational loss in many xerophytic species. Thickness of cuticle is inversely proportional to leaf water loss in maize, particularly when the stomata are closed (Ristic and Jenks, 2002). Maize genotypes exhibit high variation in leaf glossiness. Non-glossy leaves show higher cuticular wax deposition and higher amount of trichome hairs. Glossy leaves show much higher cuticular transpiration than the non-glossy leaves (Traore et al., 1989). High density of trichomes covering the surface reduces the direct sunlight effect and minimizes leaf temperature, which in effect reduces transpirational loss. Reduction in stomatal density on lower and upper epidermis minimizes the loss of water from leaf. Besides, presence of long size bulliform cells prevents evapo-transpiration. Thus in breeding for drought tolerant maize varieties and hybrids, these leaf anatomical traits are of considerable importance. Trichomes also offer insect resistant specially sucking pest tolerance (Plate 7). Studies have shown that maize genotypes having higher leaf trichome density provides resistance to stem borers by creating barrier to insect oviposition (Durbey and Sarup, 1982).

High density of silica crystals reduces the fungal growth by acting as physical barrier. Incorporation of high amount of silica leads to abrasion of cuticle feeding inhibition (Plate 8). Above Plate 9 shows the variations in size of the thick cuticle, silica deposition, bulliform cells and epidermal cells. A. Having more number of silica crystals and long bulliform cells leaves remain green under water stress condition due to low transpiration, comparing with other. B. Having fibrous patches around the vascular bundles reduces water loss by avoiding absorption of water by surrounding tissue under water deficit condition and active in translocation of water and minerals in drought condition.

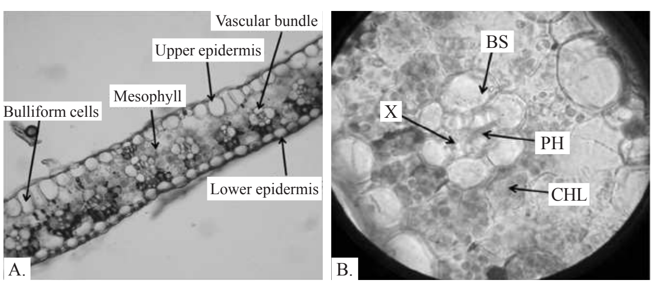

Plate 7: A. Transverse section of leaf (400X) showing different parts; B. Sector enlarged (400X) fibro-vascular bundle showing chloroplasts (CHL), bundle sheath (BS), xylem (X) and phloem (PH)

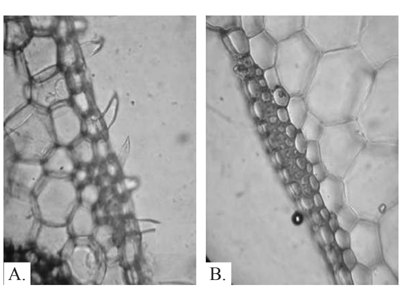

Plate 8: Transverse section of leaf showing variation in silica crystals (400X). A. High density; B. Very low density

Thickening of cell walls increases toughness of tissues causing interference with feeding and oviposition mechanism. High density of trichomes covers the leaf surface and minimizes the direct sunlight effect, creates microenvironment and reduces transpirational loss of water. Transverse section of leaves shows variation in thickness of sclerenchyma patches. Sclerenchyma reduces transpirational loss of water from leaf lamina (Plate 10, 11).

3.4. Seed anatomy

3.4.1. Seed anatomical features

The embryo of the maize grain is located beneath the endosperm. The details of the seeds anatomy has been discussed through the pictorial presentation (Plate 12, 13, 14). It is demarcated from the latter by a single layer of epithelial cells. The embryo consists of a radicle and a plumule. The radicle is partially covered and protected by colerorhiza. Width of the pericarp at the hilar region is much reduced. The radicle is partially covered and protected by colerorhiza. The plumule is partially covered and protected by the aleurone cells in this region are small in size, compact, and rectangular in shape. The endosperm cells between the aluerone layer and the scutellum are compact. The scutellar cells are elongated and form a single layer surrounding the embryo. The embryo cells are highly compact, especially at the hilar region. The black layer shows a semi-lunar ring of vacuolated cells in a network pattern, which appears to cut off the vascular connection from the pedicel to the grain. Formation of this

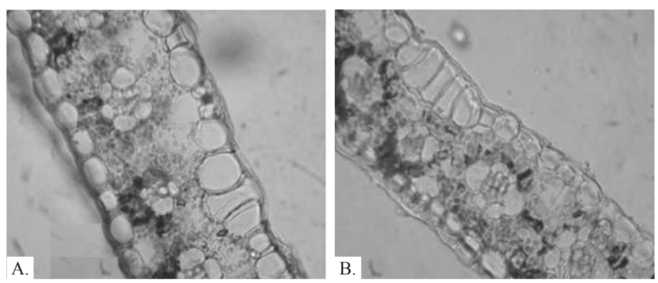

Plate 9: Transverse section of leaf through leaf lamina (400X) showing variation in epidermal cell thickness and size of the bulliform cells. A. Thick, large size epidermals, thick cuticle and bulliform cells; B. Small size epidermal cells and elongated bulliform cells

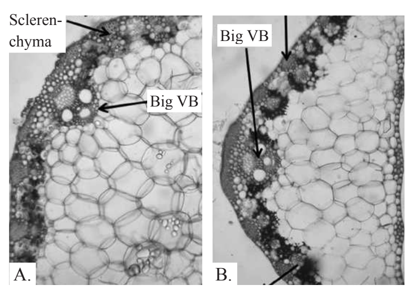

Plate 10: Transverse section of leaf (100X) showing variation in thickness of sclerenchyma and distribution of vascular bundles (VB). A. High amount of sclerenchyma; B. Less amount of sclerenchyma

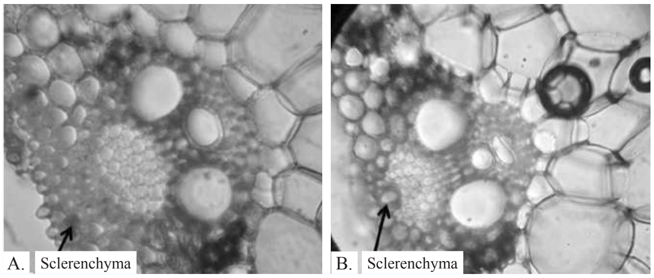

Plate 11: Transverse section of leaves showing variation in thickness of sclerenchyma patches. A. High amount of sclerenchyma; B. Low amount of sclerenchyma

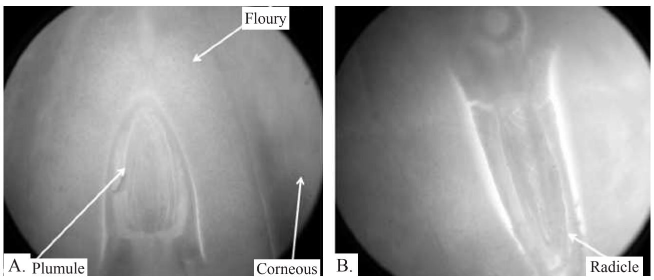

Plate 13: Longitudinal section of grain (caryopsis) showing different parts A. Plumule, floury, corneous, radicle B. Radicle

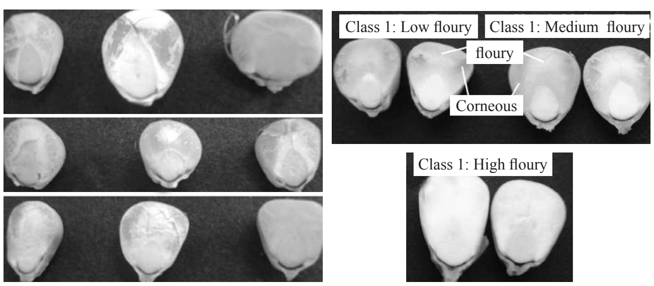

Plate 14: Classification of maize seeds based on amount of floury and corneous endosperm

black layer coincides with the termination of grain development.

A longitudinal section of the maize grain soaked for 2 or 3 days should be taken along the endosperm-embryo axis and stained with dilute iodine solution in order to study its structure. The outer most coat enclosing the entire grains formed of inseparably fused fruit coat (pericarp) and seed coat, and is called hull. The hull is not stained by iodine. In longitudinal section, the seed shows two distinct regions, viz. the endosperm and embryo.

3.4.2. Significance of variations in seed anatomy

Lot of variation was observed in maize lines with respect to type and amount of endosperms and pericarp thickness. Amount of foury and corneous endosperm shows variation with respect to each genotype. Amount of floury and corneous in the is vice versa. Lines are classified in to 3 groups based on the amount of floury and corneous endosperm.

Class-1: Consists of entries with high amont (proportion) of floury endosperm and less amonut of corneous endosperm.

Class-2: Consists of entries with almost equal amont (proportion) of floury and corneous endosperm.

Class-3: Consists of entries with less amont (proportion) of floury and high amount of corneous endosperm.

In general, stem anatomy of maize consists of epidermis and ground tissue. But there is variation in epidermis thickness and presence or absence of sclerenchyma patches in ground tissue among different genotypes of maize. Stem anatomy has been studied in a number of genotypes to determine variability, if any, in internal structure. Weak stereome system figures:

- Intermediate stereome system
- Strong stereome system
- Weak stereome system

It is expected that the genotypes having strong stereome will be resistant to drought. There is some variation among the genotypes studied, but in general leaf anatomy does not very significantly from genotype to genotype. Silica crystals, prominent in some genotypes and not so in others, protrude from the adjoining cell walls of two adjacent epidermal cells towards the cuticle. The upper epidermal cells are wavy in outline and interspersed with zones of bulliform cells, size of which differ in different genotypes. Chlorophyllous tissue surrounding the vascular bundles may be loose or compact.

We observed based on anatomical characters that the lines which show better adaptation to drought having strong steroeme system in stem, thick cuticle, long bulliform cells in leaf, strong vascular bundles in stem and leaf. In root system also there is a lignified pericycle region; thickened vessels are present when comparing with sensitive lines.

4. Conclusion

Based on anatomy there is large variations in drought tolerant line and sensitive line. Tolerant lines have thick cuticle, strong steroeme system, long bulliform cells, and better conduction system. Considerable variation was observed in the distribution and amount of mechanical tissue (stereome) and vascular bundles, and in the size and shape of the protoxylem cavity. In some genotypes, there is strong steroeme system in stem and long bulliform cells, thick sclerenchyma patches in leaf to survive in severe drought condition. Leaf anatomy possesses typical Kranz anatomy characteristics of C_4 plant showing the variation in trichome density, silica crystal deposition on the epidermis which offers insect and fungal resistance. Maize genotypes show variability in seed anatomy with distribution and amount of corneous and floury endosperm which may be related to imbibition capacity. Anatomical studies must be applied in selecting abiotic stress resistance.

5. References

Ching, A., Dhuggam, K.S., Appenzeller, L., Meeley, R., Bourettm, T.M., Howard, R.J., Rafalski, A., 2006. Brittle stalk 2 encodes a putative glycosylphosphatidylinositol-anchored protein that affects mechanical strength of maize tissues by altering the composition and structure of secondary cell walls. Planta 224, 1174-1184.

Durbey, S.L., Sarup, P., 1982. Morphological characters-development and density of trichomes on varied maize germplasms in relation to preferential oviposition by the stalk borer, *Chilopartellus* (Swinhoe). Journal of Entomology Research 6, 187-196.

Ennons, A.R., Crook, M.J., Grimshaw, C., 1993. The anchorage mechanics of maize, *Zea mays*. Journal of Experimental Botany 44, 147-153.

Esau, K., 1943. Ontogeny of the vascular bundle in *Zea Mays*. Hilgardia 15, 327-368.

Hund, A., Ruta, N., Liedgens, M., 2009. Rooting depth and water use efficiency of tropical maize inbred lines, differing in drought tolerance. Plant and Soil 318, 311-325.

Murdy, W.H., 1960. The strengthening system in the stem of maize. Annals of the Missouri Botanical Garden 47, 205-226.

Ristic, Z., Jenks, M.A., 2002. Leaf cuticle and water loss in maize lines differing in dehydration avoidance. Journal of Plant Physiology 159, 645-651.

Sage, R.F., Monson, R.K., 1999. C_4 Plant Biology. Academic Press, USA, 596.

Santiago, R., Souto, X.C., Sotelo, J., Butrón, A., Malvar, R.A., 2003. Relationship between maize stem structural characteristics and resistance to pink stem borer (*Lepidoptera noctuidae*) attack. Journal of Economic Entomology 96, 1563-1570.

Traore, M., Sullivan, C.Y., Rosowski, R., Lee, K.W., 1989. Comparative leaf surface morphology and the glossy characteristic of sorghum, maize, and pearl millet. Annals of Botany 64, 447-453.