INTRODUCTION

SORGHUM AS A CEREAL CROP

*Sorghum bicolor* (L.) Moench is one of the important cereal crops of the world. It ranks fifth in position following wheat, rice, maize and barley (Stenhouse *et al*., 1997) and is cultivated annually in about 50 million hectares of land (Teetes *et al*., 1999). It is predominantly cultivated in tropical and sub-tropical parts of the world such as Africa, India and China but also in temperate zones. Due to this climatic versatility, sorghum has been called a ‘physiological marvel’. Today, it is a dietary staple for more than 500 million people in more than 30 countries, especially for the poorer sections of society in tropical and subtropical countries. In spite of its importance and potential, sorghum is often described under such misleading categories as coarse grains, minor crops, famine foods, feed grains, broom corncobs and food of peasant classes (National Research Council, 1996).

Sorghum researchers have estimated that the demand for this crop as a staple food has increased in recent years and in future it can become the food of all classes of people. Because of its adaptability sorghum holds promise. Other factors influencing the future of sorghum include major environmental changes such as diminishing area of fertile lands, temperature rise and unpredictable availability of water due to greenhouse effect. Researchers have also predicted that the 21st century could be the century of sorghum since this crop can be an appropriate substitute for the other major cereals used as food for the increasing human population and as feed for livestock. Sorghum is also used increasingly in agricultural industries (National Research Council, 1996). Currently, a considerable quantity of this grain is used only for the manufacture of
animal feed in many parts of the world. Only in some countries of Africa, and in China, India and a few other countries it is consumed as a food grain (House, 1985).

The popularity of sorghum cultivation in the United States is attributed to the appreciation that sorghum is drought tolerant, grows in wide range of soil pH and moderate salinity and production cost is less when compared with corn (Texas Grain Sorghum Association, 2002). Sorghum cultivation also leaves large amounts of residue thus improving soil condition and helps to prevent water run off. Post-harvest handling of sorghum is economical as it has 25% longer shelf life than corn and shows less shrinkage of grains. Processing cost of sorghum is said to be 30% more economical than wheat.

Sorghum is a millet. Therefore, it is often associated with other millets such as the pearl millet (Pennisetum typhoides), kodo millet (Paspalum scrobiculatum), foxtail millet (Setaria italica), proso millet (Panicum miliare), finger millet (Eleusine coracana) and barnyard millet (Echinochloa colona). These other millets are commonly referred to as minor millets and poor man’s cereals. Consumption of millets in many parts of the world is often restricted to tribal people. Millets form an integral part of tribal cultures where they play an important role in religion and rituals besides serving as food. It is in the tribal tracts that many genetically rich land races of millets have evolved (Seetharam, 1995). Teshome et al. (1996) studied the sorghum landrace diversity, ethnobotanical knowledge and agricultural system in Ethiopia. They suggested that ethnobotanical knowledge for crops like sorghum could be used to develop elite crop varieties and that farmers’ knowledge and farmers’ role in developing new varieties and crop genetic diversity should be properly recognized.
It is difficult to estimate the current production of sorghum accurately due to the lumping of sorghum together with other millets. Production of millets has increased steadily in the world since 1960 with sorghum and pearl millet making the major contribution to this increase (Hanna, 1998). Sorghum is cultivated in about forty-two million hectares of land in the world. In India sorghum cultivation occupies about 10 million hectares, of which 6.7% is irrigated (Anonymous, 1998). Average world productivity of sorghum is 7-9 tones per hectare (Teetes et al., 1999). In India average productivity of sorghum is 0.7-0.85 tones per hectare (Rai, 2002). In comparison the yield of corn and pearl millet in India is 1.7-1.8 and 0.6-0.8 tones per hectare, respectively. The current production of coarse cereals (corn, sorghum and pearl millet) in India is about 30 million tones (Anonymous, 2001). Table 1 summarizes 5-year data released by the FAO on area of cultivation, annual production and yield of sorghum in the world and in India.

**Table 1. Five-year data on area of cultivation, production and yield (FAO)**

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Rao (1982, 1985) presented an account of how the traditional varieties of sorghum were transformed into the currently more productive types. He described the contributions of India and West Africa in developing new hybrids, and the influence of these hybrids on sorghum-based cropping systems in these countries. The short-duration new hybrids now make a significant contribution to the overall sorghum production in India. Hybrids of sorghum such as the Coimbatore cultivars (CSH-1, CSH-5, CSH-9, CSH-10 and CSH-11) suited for the *kharif* season (rainy season, June-August) have replaced the traditional cultivars because of their high-yielding potential, especially when combined with fertilizer use.

Recent efforts in sorghum improvement have resulted in extensive collections of germplasm from areas of origin as well as from around the world where sorghum is under cultivation. The International Crops Research Institute for Semi-Arid Tropics (ICRISAT) is one of the centers of the Consultative Group on International Agricultural Research (CGIAR). Located in Hyderabad, India, ICRISAT is a storehouse for germplasm of sorghum, chickpea and groundnut. This center holds more than 35,000 accessions of sorghum. These germplasm collections are available for research and breeding activities. The center releases many improved hybrids. Most of these hybrids are generated by the exploitation of cytoplasmic male sterility system (CMS). The traditional option of using landraces that have natural resistance to pests, good environmental adaptation and yield stability is also an important means of improvement of sorghum (Hanna, 1998).

Apomictic propagation of sorghum has been tried in recent years and it was found to have many added advantages like simplifying planting, harvesting and germplasm maintenance (Hanna, 1995). In India, Murty and Rao (1979) and Murty *et*
al. (1984a; 1984b), have extensively studied apomictic embryo production and its genetic control of development. Schertz (1992) described some facultative apomictic types in sorghum.

In recent years, there has been significant advance in crop modeling of various cereals including sorghum that has enabled the prediction and understanding of crop response to various inputs. Hammer et al. (1996) outlined crop modeling of sorghum in Australia. Crop modeling in sorghum is still in its infancy since the knowledge of sorghum crop physiology is inadequate to provide the inputs needed for modeling.

Many institutes are currently experimenting with tissue culture mediated propagation of sorghum. In India, seven cultivars, namely, CO-21, CO-22, CO-23, CO-24, TNS-24, TNS-25 and TNS-30, have been attempted for micropropagation. Nahdi et al. (1995) developed direct in vitro regeneration of plantlets from shoot apical meristems. In vitro culture of anthers and direct somatic embryogenesis were also achieved in sorghum (Kumaravadivel and Rangasamy, 1996). Battraw and Hall (1991) investigated the stable transformation of Sorghum bicolor protoplasts. Sairam et al. (1996) described appropriate in vitro culture systems for successful genetic transformation of sorghum. Leaf cells were found to be very suitable for callus production in many cultivars. More recently, production of virus free plants was also attempted through tissue culture (National Research Council, 1996). Fluorescence in situ hybridization (FISH) was also used to analyze the genetic architecture of the S. bicolor (Zwick et al., 2000).
ECONOMIC VALUE OF SORGHUM

Sorghum is eaten in many ways. In India, it is cooked and eaten as porridge or gruel by boiling the grains (House, 1985). It is often milled into flour for preparation of unleavened flat bread known as *chapattis* and biscuits (Maiti, 1993; National Research Council, 1996). Popped sorghum, like popcorn, is a favorite food in some parts of India, Asia and Africa. 36 cultivars of ICRISAT possessing corneous endosperm have popping qualities. Sorghum with sugary endosperm, rich in glycogen, is consumed like sweet corn. Sweet sorghums with sugar filled stems are rich in sucrose and fructose, but they are not as well known as sugarcane and sugar beet. Sweet sorghum also has high biomass and it is used as a raw material in the fermentation industry in USA, Japan and other countries (Tsuchihashi *et al.*, 1997). Sweet sorghums are utilized to produce sugar, ethanol, syrups and molasses. Gluten-free sorghum is ideal for making breads, cereals, pastries, ethnic food, beer and wine. White food sorghum has no phenolic pigments and the food cooked from its flour has no discoloration. The flour of white food sorghum can be mixed with flour of wheat, rice and potato. Some cultivars such as the rice-like sorghum, cold-tolerant sorghum, heat-shock sorghum, aromatic sorghum, and quality protein sorghum are still in the exploratory stages of research as potentially valuable genotypes for large-scale cultivation (National Research Council, 1996).

The nutrient composition of sorghum is similar to most other cereal grains. On dry weight basis sorghum has about 10.4% protein, 72.6% carbohydrates and 1.9% fat (Rai and Mauria, 1999). Sorghum cultivars do not significantly differ among themselves in nutritional value (Belavady and Deosthale, 1980). The yellow colored varieties of sorghum, however, are rich in vitamin A.
In addition to human consumption, sorghum is also equally valuable as a fodder plant. It is extensively used as a good fodder crop in many countries. Generally, feed sorghum is softer than food sorghum and it is often colored. Foliage and stem are used as green chop, hay and silage. Sudan grass (*Sorghum sudanense* (Piper) Stapf), a relative of sorghum, is also cultivated as a forage plant for pasture feed and hay. Another relative of grain sorghum, the Johnson grass (*Sorghum halepense* (L.) Pers.) is also used as a fodder plant and it often becomes weedy and tends to be cyanogenic. Cyanide concentration in grain sorghum shoot is not, however, a serious problem for use as cattle feed. Usually cyanide concentration is high in the seedling stage and this can be overcome by selecting low cyanide cultivars (House, 1985).

Other economic uses of sorghum include the use of stem as building material, fermentation of flour for the production of alcohol and beer, and for extraction of vegetable oil from the grains. Sorghum also finds use in the production of adhesives, waxes, dyes, and lubricants for oil-well drills and as sizing material for paper and cloth. Some cultivars of sorghum are the principle source of domestic brooms and brushes (National Research Council, 1996; Mabberley, 1997).

**BOTANY OF SORGHUM**

*Sorghum bicolor* is a typical panicoid grass and an extremely complex crop comprising wild and weedy perennials and cultivated annual forms. It is known by different names such as great millet, Guniea corn, sorgo, milo, kafir corn, jowar and cholam (National Research Council, 1996; Stenhouse et al., 1997). The genus *Sorghum* is placed in the subfamily Panicoidae (Grass Phylogeny Working Group, 2001) and the tribe Andropogoneae and subtribe Sorghiniae (Clayton and Renvoize, 1986). Among the
cultivated cereal grasses *Sorghum* superficially resembles maize in height and external vegetative features of leaf and stem.

According to Clayton and Renvoize (1986) the subtribe Sorghinae consists of 14 genera including *Sorghum*, *Chrysopogon*, *Dicanthium* and *Vetiveria*. The genus *Sorghum* consists of about 20 species distributed in Old World tropics and subtropics. One endemic species occurs in Mexico. The genus is also divided into four subgenera, namely, *Chaetosorghum*, *Heterosorghum*, *Parasorghum* and *Stiposorghum*. Members of the two later subgenera possess \( x = 5 \) as the basic chromosome number while the former two subgenera have \( x = 10 \).

As a \( C_4 \) photosynthetic plant sorghum uses NADP-dependent malic enzyme for decarboxylation of the four-carbon malate within the bundle sheath cells. Leaf blade anatomy shows typical NADP-ME features such as the absence of a xylem mestome layer and the presence of centrifugal chloroplasts in the bundle sheath cells where carbon reduction takes place. The outline of the bundle sheath is typically uneven (Hattersley, 1992; Samson, 1997).

After germination the sorghum plant continues vigorous vegetative growth for about 30 to 40 days. Rapid cell division and maximum cell elongation result in the elongation of internodes and leaves. Flowering in warm climates occurs between 55 and 70 days after germination (House, 1985). As in other cereal plants, sorghum has a typical fibrous root system (Maiti, 1993). The seminal roots arise directly from below the scutellar node of the germinating seed and the adventitious roots arise from the node of the coleoptile. In most soils sorghum roots are known to establish vesicular arbuscular mycorrhizal associations in the cortical tissues (Menge *et al.*, 1980).
The culm or stem is made up of a series of alternating nodes and internodes enclosed by leaf sheath. A bud is present at each node except at the flag leaf. Sometimes the buds develop into axillary tillers with the very first node providing the basal tiller. These lateral shoots are frequently referred to as suckers (Gould and Shaw, 1983). The vascular bundles are scattered throughout the stem and the vascular bundles at the center are larger than those at the periphery (House, 1985). In sweet sorghum a significant proportion of the photosynthate is stored as sugars in the parenchyma cells of the stem (Nakamura et al., 1997).

Sorghum leaf is made up of a thin flat lamina with prominent midrib and a rigid leaf sheath clasping the internode. A membranous ligule is present at the base of the leaf blade. The length of the leaf may be as long as 1 m and the width 10-15 cm. The leaf blade is provided with longitudinal and parallel vascular traces of two different types along side the midrib, and transverse cross veins (Dayanandan, 2000). Dale (1982) has described the development of leaf in sorghum.

The emergence of the 'boot' resulting in the pushing up of the flag leaf is generally considered to be the beginning of the reproductive phase in sorghum, although the inflorescence had already been initiated before booting. Sorghum is a short-day plant; and flowering is delayed in most genotypes when the photoperiod exceeds 12-14 h (Doggett, 1988). Eastin and Lee (1984) critically reviewed panicle initiation and flowering in sorghum. The inflorescence of sorghum is a panicle consisting of groups of spikelets clustered on short racemose branchlets (Gould and Shaw, 1983; House, 1985). The panicle consists of a furrowed central axis with whorls of lateral branches. A panicle has about ten whorls, each having 7 to 8 branches. The panicle may range in length from 4 to 25 cm, and in width from 2 to 20 cm. There is considerable variation in the shape
and branching pattern and clustering of the branchlets resulting in inflorescences that may be short, loose, open, compact or bent in appearance.

There are two kinds of spikelets in each cluster. The terminal spikelet is sessile and fertile, and the lateral spikelet is pedicellate but may be sterile or staminate. The terminal regions of a branchlet may bear two pedicellate and one sessile spikelet. (Gould and Shaw, 1983) The shape of the sessile spikelet varies from lanceolate to ovoid. It has an upper and lower glume both of which are green in color during the flowering stage and change to purple or straw color at seed maturity. There are two delicate lemmas, the lower one elliptic or oblong in shape, and the upper one shorter. The flower is surrounded by a short palea. There are two membranous lodicules, three stamens and an ovary with two feathery stigmas. The pedicellate spikelet is narrower, lanceolate in shape, and may possess only stamens and sometimes a rudimentary ovary.

The order of anthesis is basipetal, proceeding from top to bottom (Ayyangar and Rao, 1931) requiring about 6 days for anthesis to be completed in a panicle. Anthesis involves the opening of the lemma and palea, followed by the elongation of the filaments with their terminal anthers. Flowering is influenced by prevailing weather conditions but is generally maximum between 6 to 8 a.m. Sorghum is a self-pollinated crop with only up to a maximum of 6% of cross fertilization (Maiti, 1993). During seed development three stages can be recognized: ‘milky stage’, ‘early dough stage’ and ‘late dough stage’. These terms are commonly used and are not specifically defined. The dry fruit of sorghum is a caryopsis; however, terms such as grain and seed are commonly used interchangeably to refer to the caryopsis. Sorghum caryopsis attains physiological maturity in about 30 days after flowering (DAF). The maximum dry weight of the grain is reached between 25-55 DAF, depending on prevailing environmental conditions.
Grains are normally harvested at 15% moisture level after additional 10 days of dry matter accumulation. (Stenhouse et al., 1997). Grains produced in different parts of the same inflorescence may differ in weight. Seeds that mature last on the basal part of the panicle weigh less than seeds from the top of the panicle. Spikelet removal in sorghum increases the weight of the remaining grains (Hamilton et al., 1982).

ORIGIN, DOMESTICATION AND DISTRIBUTION OF CULTIVATED SORGHUM

Sorghum was first domesticated in Africa. However, the time and place of domestication and the ancestral species from which grain sorghum was derived are poorly understood. Some authorities suggest that sorghum might have been cultivated as early as 7000 to 5000 years ago (Doggett, 1965; Randhawa, 1980; Mann et al., 1983; House, 1985; Maiti, 1993). However, De Wet (1978) found no conclusive evidence for such early dates and suggested that sorghum was domesticated only about 3000 years B.P. It was clearly domesticated by 1000 B.C. in the area of Sudan. Harlan and De Wet (1972) used paleobotanical, archeological, botanical and anthropological evidence and traced the origin of sorghum as spreading from near Lake Chad in Africa. De Wet and Huckbay (1967) suggested that cultivars of sorghum might have had separate origins from wild species in western, eastern and eastern-central Africa. Snowden (1936) also suggested that the different races of sorghum might have had different centers of origin. Harlan (1975) was of the view that the current distribution of genetic variability in sorghum revealed that there was no single center of origin and diversity, and that sorghum was a ‘non-centric crop’ with initial domestication occurring as a long belt in Central Africa. The progenitors of domesticated *S. bicolor* might have been *S. arundinaceum* (Desv.) Stapf. *S. verticilliflorum* (Steud.) Stapf. occurs in areas of Africa
where many ancient varieties of grain sorghum are grown. Therefore, this species also has been suggested as a probable ancestor of cultivated grain sorghum. From Africa, sorghum spread to other regions of the world through land and sea routes. It was introduced to Egypt from Ethiopia. Sorghum might have initially reached India during the latter part of the second millennium B.C. From India it was carried to China through the silk route about 2000 years ago (National Research Council, 1996).

Snowden (1955) made significant taxonomic contribution by classifying sorghum into 31 cultivated and 17 wild species. The 31 taxa described by him as separate species are now considered to be races of a single species, namely, *S. bicolor*. According to Clayton and Renvoize (1986) there are about twenty species of sorghum in the tropics and sub-tropics of the Old World. One endemic species occurs in Mexico. Gould and Shaw (1983) list about 35 species of sorghum found mostly in the warmer parts of Africa. Mabberley (1997) indicates that there may be about 8 major groups of cultivated sorghum. In general, cultivated sorghum can be divided into grain sorghum (nonsaccharine), sweet-juice or forage sorghum (saccharine) and brush manufacturing sorghum (broom corn). Three additional races, namely, saccharatum, subglabrescens and technicum are described by others (Mabberley, 1997).

Harlan and De Wet (1972) provided an informal scheme of classification that is found most useful by plant breeders (House, 1985). This is based on previous schemes of classification of Snowden (1955) and De Wet et al. (1970). This scheme recognizes the following three species of sorghum in the section (=subgenus) *Sorghum*:


*S. bicolor* is further subdivided into three subspecies:


Harlan and De Wet (1972) further classified the cultivated taxa of *S. bicolor* subsp. *bicolor* into 15 races on the basis of mature spikelet characteristics and head type. Five of these are basic races and the remaining 10 are intermediate hybrid races.

The five basic races of cultivated sorghum (*S. bicolor* subsp. *bicolor*) are:

1. **Bicolor**
2. **Guinea**
3. **Caudatum**
4. **Kafir**
5. **Durra**

Three independent centers of domestication of sorghum have been proposed, namely, Ethiopian region of Eastern Africa, Tropical Western Africa and South Eastern Africa. Durra sorghum was probably domesticated in Ethiopia. Eastern Nigeria through
Chad and Western Sudan might have been the center of diversity for caudatum race. The guinea race might have evolved in the region of Western Niger to Senegal. Tanzania to South Africa is considered to be the center for the kafir race (National Research Council, 1996).

Although an ancient crop of Africa, sorghum is now widely cultivated in countries such as Australia and North and South America where it was introduced only about a hundred years ago (Peacock, 1984). In India, sorghum is the third most important cereal crop after rice and wheat, with approximately 10 million hectares under cultivation, occupying about 35% of world’s sorghum growing area (Anonymous, 1983; Maiti, 1993; National Research Council, 1996; Table 1). Sorghum is mostly a rain-fed crop in India. The traditional landraces and modern cultivars are cultivated in about equal area of land signifying the strong association between ancient cropping patterns and the variety of growing seasons found in the country (Stenhouse et al., 1997). 80% of India’s cultivated sorghum belongs to durra race of Ethiopian origin (National Research Council, 1996). Mostly the white colored variety is preferred for milled flour used for making traditional unleavened bread. Sorghum is routinely cultivated in the following states: Maharashtra, Andhra Pradesh, Madhya Pradesh, Karnataka, Tamil Nadu, Gujarat, Rajasthan, Uttra Pradesh and Haryana (Srivastasa, 1985). Of these states, Maharashtra has the largest area devoted to the cultivation of sorghum. Crop yield is generally higher in South India than in the states in North India (Ryan and Von Oppen, 1982). In Tamil Nadu, cultivars developed in Coimbatore and Kovilpatti are most popular; some popular cultivars are: Co1 (Peria manjal cholam) Co2 and Co3 (Talai virichan cholam). Fodder varieties include Irungu cholam, Talai virichan, Chinna manjal and Vellai cholam (Chadha, 1972).
Worldwide about 50-70 million tons of sorghum are produced annually, forming a dietary staple food for some 500 million people in 30 countries (National Research Council, 1996). The average yield of sorghum ranges from 3-4 t/ha under normal conditions, sometimes with the values as high as 7-9 t/ha where moisture is not a limiting factor. This value tends to be as low as 0.3-1 t/ha where moisture becomes a limiting factor (House, 1985).

ECOLOGICAL ADAPTATIONS OF SORGHUM

Sorghum use the C₄ photosynthetic pathway, an advantage found only in certain other economic crops such as maize and sugarcane. It is one of the most drought-resistant plants; in relation to moisture content it has been described as ‘the camel’ of the world's crops (Ustimenko-Bakumovsky, 1983). Sorghum is well adapted to extreme conditions of growth such as arid climate, saline and flooded soils and soils with excessive amounts of toxic aluminum. It tends to “hang on” during the dry period and resumes growth with the return of rain (House, 1985). Sorghum can be grown either as a rain-fed or irrigated crop. Occasionally it is transplanted like rice, ratooned like sugarcane and allowed to resprout from cut bases. It is one of the quickest maturing (about 75 days) of food plants, so that it can be harvested thrice a year. Another important adaptive feature of this crop is that it has a well-penetrated root system providing greatest efficiency of water transport under limited soil water conditions. The leaves conserve moisture by closing stomata and rolling the blades at higher temperatures.

Based on surface characteristics of the seedling leaves, sorghum is classified into glossy and non-glossy genotypes (Maiti, 1993). Part of the reason for the glossy appearance is the presence of a layer of epicuticular wax on the leaf surfaces.
Epicuticular wax in sorghum provides multiple resistance to insect pests and abiotic stress such as high temperature, salinity and drought (Jordon et al., 1984; Maiti, 1993). The major constituents of the epicuticular wax are free fatty acid, trace amounts of esters, alcohols, n-alkanes and sterols (Bianchi et al., 1977). Many cultivars of sorghum also produce poisonous hydrocyanic acid. Halkier and Moller (1989) studied the biosynthesis of the cynogenic glucosides dhurrin, in sorghum seedlings. Gorz et al. (1977) measured the HCN content using spectrophotometer, which was used as an efficient tool in screening for low-cyanide cultivars.

Productivity and distribution of dry matter in sorghum is strongly influenced by environmental factors such as water stress and biotic factors such as attack by pests, viral, bacterial and fungal diseases, and by the root parasite, Striga and birds. About 75 species of insects are known to attack sorghum with varying degrees of affect on productivity (Srivastava, 1985; Teetes et al., 1999). Among the more damaging pests are sorghum midge (Stenodiplosis sorghicola), greenbug (Schizaphis graminum) and shoot fly (Atherigona soccata). The mode of infection, symptoms, and control measures for various diseases infesting sorghum such as smuts, rusts, mildews, rots, and leaf blights are now well established (Ramakrishnan, 1971). Singh and Rana (1996) have summarized the recently emerging strategies for integrated pest management in sorghum.

In many parts of the world Striga has become a major determinant of yield in sorghum. Striga asiatica, commonly referred to as witch weed, is a red or white flowered root parasite that infects sorghum and corn. Reddy and Rao (1996) summarized current research interest on Striga at the global level. Striga reduces yield as much as 70% in sorghum and has been the leading cause for famine in some parts of Africa.
Much effort over a long period of time has been devoted to the isolation and identification of stimulating chemicals in root exudates of sorghum that promote germination of Striga seeds. The stimulant strigol was first isolated from Gossypium hirsutum (cotton) a non-host species (Cool et al., 1966). A host-derived germination stimulant for Striga isolated from the root exudates of sorghum was identified as sorgoleone. Another germination stimulant of Striga known as sorgolactone has also been isolated from sorghum root exudates.

The presence of tannins in the grains of sorghum is of particular importance in relation to bird damage and human nutrition. Tannins are deposited in the testa during the milky stage of grain development. The tannin containing sorghum cultivars produce grains that are red in color. The tannins of sorghum affect many animals including ruminants, poultry, rats and mice (Butler et al., 1986). In humans, dietary consumption of sorghum tannins has resulted in the incidence of oesophageal cancer (Morton, 1978). Mammals including humans also show adaptation to different levels of sorghum tannins. They synthesize a proline-rich salivary protein in their parotid glands and this salivary protein, because of its great affinity to sorghum tannins, bind to them and make them inactive. Without this adaptation, sorghum consumption in semi-arid tropics would have been impossible (Butler et al., 1986). Hoshino and Duncan (1981) stated that the tannin content and bird damage in sorghum varies depending upon different environmental conditions. In general a number of varieties are protected from bird damage because of the presence of tannins located in the testa (Blakely et al., 1979). Tannin may also help prevent microbial attacks during seed germination (Swain, 1979).
STRUCTURE OF CEREAL CARYOPSIS

The dry indehiscent fruit developed from a superior monomeric gynoecium of Poaceae is termed as caryopsis. Richard (1811) coined this term using two Greek root words, *karyon* and *opsis*, which essentially means “appearance of nut”. Based on the path of translocation of solutes and associated anatomical peculiarities the caryopsis of cereals were classified into two types, namely, the elongated and cylindrical type as in wheat and rice and the distally flattened type as in sorghum and maize (Srinivas et al., 1985). Cereal caryopsis has been intensely studied from various perspectives by several workers (Bechtel, 1983). O’Brien (1983) traced the nearly 400-year history of study of cereal grain structure. As might be expected structural studies have focused mostly on wheat, barley, rice and maize more than on the millets (Kiesselbach and Walker, 1952; Frazier and Applanidau, 1965; Ingle et al., 1965; Utsunomiya et al., 1973; Bechtel and Pomeranz, 1978; Hoshikawa, 1983, 1984a and b; Harris and De Mason, 1989). Narayanaswami made significant contributions to our understanding of the structure and early stages of development of caryopsis of Indian millets such as *Pennisetum typhoides* Rich (1953), *Paspalum scrofulatum* L. (1954), *Panicum miliazeum* L. (1955a), *Echinochloa frumentacea* Link (1955b), *Eleusine coracana* Gaertn. (1955c) and *Setaria italica* (1956).

A mature cereal caryopsis consists of remnants of maternal tissues surrounding the filial tissues. The former is derived from the maternal ovarian tissues, namely the pericarp, integuments and nucellus. The filial tissues consist of the diploid embryo and the surrounding triploid endosperm. During development the original ovary wall consisting of 5-6 layers of cells get stretched and some or all of them remain collapsed and attached to the seed coat. During active grain-filling stages the pericarp may be
Differentiated into an epicarp with a waxy cuticle on the outer epidermal surface, and a middle layer of mesocarp. In sorghum Rooney and Miller (1982) recognized four types of cells in the pericarp. The inner epidermis of the pericarp differentiates into elongated tube cells that surround the seed. The tube cells might be involved in providing mechanical support to the developing caryopsis (Krishnan, 1996). The inner layers of the mesocarp are known as cross cells and they are elongated perpendicular to the long axis of the caryopsis. The cross cells have chloroplasts and are provided with abundant intercellular air spaces. They are interconnected by numerous plasmodesmata (Morrison, 1975). This layer is of particular interest to the cereal physiologist as it has the capacity to photosynthesize and provide assimilates to the developing grain.

The ovule has two **integumentary layers** namely, the outer and the inner. The outer integument is usually absorbed during the early stages of ovary development. The inner integument typically has two layers of cells. During development of the caryopsis the inner layer of the integument may persist for a long period. In some cereals the inner integument is completely absorbed. In others it may persist and get filled with pigmented material and is known as the testa.

The **nucellus** is a mass of tissue enclosed by the integuments. The embryo sac develops within the nucellar tissue. The nucellus is composed of rectangular or elongated parenchymatous cells. At the time of fertilization the outermost layer of the nucellus is well differentiated as the nucellar epidermis. This is an important layer of cells involved in apoplastic transfer of solutes to the developing endosperm. In the course of endosperm development the nucellar tissue becomes reabsorbed and the nucellar epidermal layer becomes gradually disintegrated in the late grain-filling stages.
In wheat, barley and rice the region which connects the nucellus with ovular vascular bundle is clearly distinguished and is known as the nucellar projection.

The triploid endosperm develops soon after double fertilization. The initial free-nuclear division is followed by cellularization from the periphery towards the center. The outer most layer of the endosperm is constituted as the aleurone layer. Esau (1977) described the endosperm of Poaceae as consisting of the outer aleurone and the starchy inner endosperm. Rice, barley and oats may possess multiple aleurone layers at least in some regions. Rye and triticale contain a single aleurone layer. Sorghum also has a single aleurone layer but it is discontinuous at the scutellar region. Aleurone cells are filled with protein and phytin-rich aleurone grains and lipid bodies. Often the aleurone cells also contain a few amyloplasts. In Zea mays and millets such as Setaria and Echinochloa, the aleurone in the vicinity of the placental vascular supply possesses numerous invaginations in the inner wall. These cells are called transfer aleurone cells (Rost and Lersten, 1970).

The cereal embryo is the germ or future plant of the grass caryopsis. Reeder (1957) classified grass embryos into six groups namely: Festucoid, Panicoid, Chloridoid-Eragrostoid, Bambusoid, Oryzoid-Olyroid and Arundinoid-Danthonoid. Negbi and Koller (1962) investigated the homologies of embryonic organs of grass embryos. Grass embryos are characterized by a distinct single cotyledon termed as scutellum (Barnard, 1964). The embryonic organ enclosing the shoot is called coleoptile and that of the root is called coleorhiza. The coleorhiza develops an outgrowth called epiblast. The grass embryo is a complex structure and the homology of the different organs of the embryo, such as the epiblast, scutellum and coleoptile have been variously interpreted by different authors (Brown, 1960; Negbi and Koller, 1962).
PREVIOUS STUDIES ON CARYOPSIS OF SORGHUM

In comparison with wheat, rice and maize relatively little has been published on the structure and development of tissues of the caryopsis of sorghum. Winston (1903) provided the earliest description of the sorghum caryopsis. The development of pericarp, seed coat and endosperm was investigated by Sanders (1955) and Paulson (1968). The structure of caryopsis of sorghum was also described by Hubbard et al. (1950); Rooney and Clark (1968); Wall and Blessin (1969); Rooney and Sullins (1977); Rooney and Miller (1982) and Rooney et al. (1983). Zeleznak and Varriano-Marston (1982) investigated the ultrastructure of pearl millet and grain sorghum caryopsis. Hulse et al. (1980) pointed out the essential similarities between pearl millet and sorghum. Kersting et al. (1959) described the changes in chemical composition that occurred during the development of sorghum caryopsis.

Maness and McBee (1986) reported that the tissues associated with carbohydrate import into the endosperm such as vascular trace, chalazal tissue, nucellar tissue, placental sac and aleurone transfer cells, were well differentiated by 10 DAF. Persistent integument has been reported in sorghum (Naryanaswamy, 1953). The scanning electron microscopic study of cereal grains by Rooney et al. (1983) included a description of the endosperm of sorghum. The sorghum endosperm was described as consisting of an outer corneous and inner floury region. The corneous zone had cells that contained protein in two forms, as an amorphous mass surrounding the starch grains and as discrete protein bodies. The starch grains were of the simple type as in maize and pearl millet. The starch grains were polygonal in the corneous zone while they tended to be spherical in the soft floury zone. The aleurone cells were reported to contain protein bodies, lipid droplets and phytins as in other cereals.
GRAIN-FILLING IN CARYOPSIS OF CEREALS

Translocation and distribution of the photoassimilates are considered to be the major determinants of plant and crop productivity (Patrick, 1988). Grain-filling in cereals is a combination of events such as photosynthesis, phloem loading, assimilate transport, phloem unloading and utilization of sugars and other nutrients in the synthesis of starch and other storage components within the caryopsis (Felker et al., 1983). According to Jenner et al. (1991), grain-filling is the deposition of polymeric product in cells and organelles formed during the grain enlargement phase. Developing fruits and seeds are strong sinks exhibiting a very high amount of assimilate import. The mechanisms of phloem loading and unloading are still not well established in various plant groups and research in this field is restricted to economically important plants (Haupt et al., 2001).

Two kinds of loading mechanisms operate in plants. One is apoplastic way of transport of photoassimilates resulting in active uptake into phloem cells (Van Bel, 1993). The second is symplastic, which involves transport through plasmodesmatal-cytoplasmic connections from mesophyll to the sieve elements (Turgeon, 1996, 2000). Plasmodesmata are dynamic structural channels (Oparka, 1993) capable of regulating molecules of different sizes to pass through (Robards and Lucas, 1990). To understand the functional role of plasmodesmata, fluorescent probes were microinjected into plant cells and the size exclusion limit (SEL) of plasmodesmata was determined (Oparka and Roberts, 2001). Haupt et al. (2001) made use of a number of techniques including electron microscopy and the symplastic tracer carboxyfluorescein, and the systemic movement of barley stripe mosaic virus expressing the green fluorescent protein, to understand phloem unloading in barley leaves. They concluded that unloading was a
symplastic process. Turgeon and Beebe (1990) favoured phloem-loading process to be symplastic by various analyses such as plasmodesmatal ultrastructure and distribution, loading mechanisms of exogenous sugars, pattern of sugar synthesis and dye coupling methods.

There are two major types of sinks in plants namely, permanent or reproductive sinks and temporary or growth or vegetative sinks. Transport into the permanent sinks tends to be apoplastic while transport in temporary sinks is mostly symplastic (Porter et al., 1987). Many researchers have employed quantitative and qualitative procedures to understand grain-filling in cereals and legumes. Techniques such as indicator-dilution (Fisher and Wang, 1990), microperfusion (Wang and Fisher, 1994a), empty seed-coat technique (Thorne and Rainbird, 1983) and microautoradiographic analysis (Fisher and Wang, 1993) were employed to study and measure the transport rate of phloem in different cereals. The empty seed-coat technique clearly showed that there was no symplastic connection existing between endosperm, embryo and surrounding maternal tissues.

Elucidation of the cellular structures involved in assimilate transport pathway in the caryopsis is of paramount importance in understanding grain-filling and assimilate partitioning (Offler and Patrick, 1986). The structures associated with assimilate unloading from sieve tubes are not well understood (Wolswinkel and Ammerlaan, 1983). The movement of fluorochromes into the cereal caryopsis was investigated by several researchers for elucidating the pathway of translocation (Schumacher, 1933; Peterson et al., 1981; Cook and Oparka, 1983; Grignon et al., 1989; Oparka, 1991; Wang et al., 1994; Wang and Fisher, 1994b; Wright and Oparka, 1996). Fluorescein, a symplastic phloem tracer was routinely employed in the earlier investigation; but this fluorochrome
was found to show significant leakage through the plasma membrane (Schumacher, 1933). Later, carboxyfluorescein (CF) having an additional carbonyl group in either 5th or 6th position was considered to be less permeant to biological membranes than fluorescein and was extensively used as a useful phloem tracer. Cook and Opara (1983) studied the movement of fluorescein starting from phloem, through pigment strand, nucellar projection and out into the endosperm cavity in barley and wheat. Opara (1991) assessed the uptake of different fluorescent probes involved in phloem translocation in rice. Wang et al. (1994) and Wang and Fisher (1994b) used an ester form of CF called carboxyfluorescein di acetate (CFDA) to understand the post-phloem unloading pathways in the sink region. Raghavan (1997) studied the pattern of grain-filling in rice using the 5(6)-carboxyfluorescein and established it to be an efficient phloem tracer. Wright and Opara (1996) have made an attempt to understand the loading and movement of 8-hydroxypyrene-1, 3, 6-trisulphonic acid (HPTS) in barley caryopsis. After 1 hr of supply it appeared inside the grain. They suggested that HPTS possesses good membrane impermanent properties and might be a valuable alternate to CF. In addition to the use of fluorescence microscopy for the determination of the pathway and transport of solutes, confocal laser scanning microscopy (CLSM) has been recently used to evaluate the transport of HPTS in cereal grains (Wright and Opara, 1996).

Thorne (1985) suggested that the continued supply of assimilates in developing caryopsis is facilitated by: 1. Chemical alteration of translocates 2. Compartmentation into symplastic and apoplastic pathways, and 3. Utilization of assimilates for storage and embryo development. He reviewed the anatomical features of maternal tissues such as placenta, chalaza, and nucellar projection which facilitated translocation of solutes into the embryo. Smart and O’Brien (1983) reported that modified nucellar epidermal cells
are involved in the short distance transport of nutrients into the embryo. The driving force of sugar allocation is mainly influenced by the sink strength, based on the activity of embryo sac and the developing endosperm and embryo (Zamski, 1995). The major sources of assimilates during grain development is derived from the current photosynthesis of flag leaf, stem and ear, each of which contribute different amounts of carbohydrates (Carr and Wardlaw, 1965; Evans and Rawson, 1970; Thorne, 1985). Hydrolysis of sucrose to monosaccharides by invertase activity in the unloading site could act as a ‘reflux valve’ which might prevent reloading of assimilates into the sieve elements (Eschrich, 1980).

In rice, transfer of solutes from the phloem into the endosperm occurs via longitudinally oriented continuous ovular vascular trace (Oparka and Gates, 1981a and b; Ugalde and Jenner, 1990; Krishnan, 1996). There are two pathways of transport from the ovular vascular trace. One is the ‘dorsal pathway’, which involves direct transfer from nucellar projection and the second is ‘nucellar epidermis pathway’, where solute enters the nucellar epidermis and then apoplastically moves into the endosperm. In wheat, an endosperm cavity accumulates solutes and then transfers them to the endosperm. In corn the vascular bundle terminates at the base of the ovule and solute transfer is facilitated by the presence of transfer cells. Krishnan (1996) has provided an account of grain-filling in rice and has compared the pattern of grain-filling in rice with other cereals.

Several investigators have examined various aspects of **grain-filling in sorghum** since the pioneering work of Artschwager and McGuire (1949) on anthesis, pollination, fertilization and development of the ovary in sorghum. The estimation of growth rate of the caryopsis, net assimilation rate and distribution of dry matter were determined in a
sorghum hybrid and its parents by Gibson and Schertz (1977). Similarly the
development of caryopsis in the CSH1 hybrid was studied at ICRISAT under an All-
India Coordinated Project (Maiti, 1993). Subramanian et al. (1982) compared the
amounts of starch, protein, fat, ash, dry weight and soluble sugars in developing grains
of eight sorghum cultivars. Quinby (1972) studied the period of grain-filling in sorghum
and determined that maximum grain weight was reached between 30 and 40 DAF.

Maness and McBee (1986) reported that the various tissues and cells such as
vascular tissue, chalaza, remnants of nucellar tissue, placental sac and aleurone transfer
cells associated with solute import into the endosperm were well differentiated by 10
DAF. The aleurone cells were in fact already differentiated by 5 DAF in the region of
the endosperm adjacent the placental sac. Felker and Shannon (1980) and Maness and
McBee (1986) suggested that the chalaza and placenta in sorghum function as temporary
storage organs and that the inversion of sucrose into hexose takes place here prior to
import into the endosperm region. The placento-chalazal tissue cell walls are rich in acid
invertase (Shannon and Dougherty, 1972). The placental sac region was identified to be
the site of hexose import as evidenced by high glucose and fructose concentrations in the
placental sac fluids (Maness and McBee, 1986). Fischer and Wilson (1975a; 1975b)
determined that starch accumulation was at a maximum about 15 DAF. Protein synthesis
during grain-filling was investigated by the incorporation of radioactive leucine into the
caryopsis (Johari et al., 1977).

Our understanding of the process of grain-filling in sorghum will be discussed in
detail in the Discussion section of this dissertation. However, it may be stated by way of
summary that the pattern of grain-filling in sorghum shares some common features with
wheat and corn. As in other cereals the final event of transfer of assimilates into the
endosperm is apoplastic. Sorghum has a placental sac as does wheat. A placental sac is absent in corn. However, the vascular bundle of sorghum terminates at the base of the ovary, as in corn. Also, as in corn, well-developed transfer cells facilitate the transfer of nutrients from the placental sac into the endosperm. There is evidence that the aleurone layer too is involved in partial transport of the nutrients into the endosperm. By about 40 DAF the grain reaches physiological maturity. By this time the phloem parenchyma near the hilar region turns black and completely shuts off the translocation of assimilates (Quinby, 1972).

PRINCIPLE STORAGE COMPONENTS OF CEREALS

Starch, protein and lipid are the three major storage components that determine grain yield and grain quality of cereal grains. Minerals and vitamins constitute minor but nutritionally important constituents of cereal grains. A large number of researchers have contributed to the understanding of the structure, development and deposition of starch, lipids, protein and other storage components in cereal grains (Buttrose, 1960; Khoo and Wolf, 1970; Adams and Novellie, 1975; Brooner, 1975; Harris and Juliano, 1977; Miflin et al., 1983; Duffus, 1987; Bewley and Greenwood, 1990). Several sophisticated methods are also available for qualitative and quantitative determination of cereal components (Rooney and Clark, 1968; Johari et al., 1977; Paulis and Wall, 1979; Fulcher et al., 1981; Fulcher, 1982; Fulcher and Wood, 1983; Juliano, 1985 and 1992; Oparka, 1991; Harris, 1992; Oparka and Reed, 1994; Wang and Fisher, 1994a; Kavitha and Chandrasekar, 1997).

A comparison of nutritional composition of selected cereal grains obtained from several sources is provided below in Table 2 (Gopalan et al., 1991; Rai, 2002).
Table 2. Nutrients per 100 grams dry weight

<table>
<thead>
<tr>
<th>Plant</th>
<th>Protein (g)</th>
<th>Carbohydrate (g)</th>
<th>Lipids (g)</th>
<th>Minerals (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum</td>
<td>10.4</td>
<td>72.6</td>
<td>1.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Rice</td>
<td>7.5</td>
<td>78</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Wheat</td>
<td>11.8</td>
<td>71.2</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Maize</td>
<td>11.6</td>
<td>67.5</td>
<td>3.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Barley</td>
<td>11.5</td>
<td>69.6</td>
<td>1.3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Starch is the major carbohydrate present in cereal grains. Most of the cereal carbohydrate is derived from CO₂ fixed during the grain-filling period. Starch is a semi-crystalline, three-dimensional structure consisting of about 30% amylose and 70% amylopectin glucose polymers. Starch is stored as granules temporarily within chloroplasts and amyloplasts. The chloroplast starch is of assimilatory nature and is in a transient state, while the starch of amyloplasts is stored as reserve substances (Buchanan et al., 2000). Starch is synthesized in the leaves during daytime and transported to the storage organs such as seeds, fruits, tubers and storage roots (Martin and Smith, 1995). Buttrose (1960) assessed the nature of amyloplasts and the development of starch granules in cereal endosperm tissue. Bhatia et al. (1972, 1974 and 1975) investigated the formation of simple sugars by the photosynthetic organs, their temporary storage in the stem and remobilization into the developing grains for starch synthesis in different millets.

Starch can be classified as simple and compound types. Simple starch occurs in wheat, corn, sorghum and rye while cereals such as rice and oats possess compound starch grains (Varriano-Marston, 1983). Starch grains vary in size from less than 1 μm in diameter to greater than 100 μm. In cereal endosperm the size of starch grains vary from
10-50 μm in diameter (Duffus, 1987). Starch grains grow in size by the addition of molecules around existing grains. This action gives characteristic concentric growth rings clearly visible in a microscope. The point around which starch is deposited is known as the hilum, and the hilum may be either in the center or the side of a starch grain. When viewed between cross-polarized light, starch grains reveal typical ‘Maltese cross’ (Bennet, 1950; Varriano-Marston, 1983). The addition of a First Order Red Plate clearly reveals the orientation of the molecules within starch grains.

Three different enzymes are involved in starch synthesis. These are ADP-glucose pyrophosphorylase, starch synthase and starch branching enzyme. Starch synthase adds glucose from ADP-glucose to amylose or amylopectin chain. The starch branching enzyme is responsible for the (1-6)α-bond that results in branching of starch at approximately 20 glucose residues apart, leading to the formation of amylopectin.

The submicroscopic structure of starch grains was investigated by Buttrose (1960) and Hood and Liboff (1983). Payen (1838) made early diagrams of the starch of maize and sorghum. Sorghum and corn starch are simple granules and are similar in shape and dimensions. Sorghum starch is polygonal or spherical in shape and range between 4-25 μm in diameter and possesses a centric hilum (Varriano-Marston, 1983). Sorghum starch is usually white in color. However some cultivars have dull colored starch; this appears to be due to the presence of certain alcohol-soluble substances (Subramanian et al., 1994). Sorghum starch forms an opaque paste of medium viscosity with water.

Proteins in cereals constitute about 8-15% of the dry grain weight and they are an important dietary source for human beings and livestock (Shewry et al., 1995). The history of cereal protein research can be traced back to Beccari’s (1745) description of
wheat flour consisting of starch and gluten. Protein bodies probably occur in all seeds, with characteristic structure, chemistry, ontogeny and patterns of digestion (Rost, 1971). Proteins in the endosperm occur as membrane bound deposits called protein bodies. The protein body or protein granule found in aleurone cells is known as aleurone grains. The structure of protein bodies, their synthesis and time of accumulation during seed development have been well researched. Several investigators have used the transmission electron microscope to determine the ultrastructure of protein bodies, and electrophoresis to separate the various components of proteins. Bechtel and Juliano (1980), Oparka and Harris (1982) and Krishnan and White (1995) investigated the structure and properties of rice storage proteins. Khoo and Wolf (1970) studied the proteins of maize while Rost (1971) examined the cereal proteins of *Setaria lutescens*. Wolf and Khoo (1970) found that α-amylase treatment of the endosperm resulted in the digestion of starch and better visualization of the endosperm protein bodies. Lott (1981) made electron microscopic studies of seed protein bodies and suggested their usefulness in plant systematics.

Following T.B. Osborne’s (1924) classical studies on solubility, storage proteins have been traditionally classified into four kinds, namely: albumins, globulins, prolamins and glutelins. These terms are still used although the classification scheme on which they were based has undergone changes. Globulins are the major storage proteins in dicots, especially in the legumes. The glutelins of wheat offer unique visco-elastic properties suitable for baking process (Shewry *et al.*, 1994). Cereal grains possess two classes of major storage proteins: alcohol-water soluble prolamins and acid (or) alkali soluble glutelins. A third class of globulins is present in relatively small amounts in the embryo. Prolamins are the major storage protein of sorghum, maize and wheat. Prolamins are reported in all the major cereals and grasses; however, rice and oats
contain glutelin-type prolamins and globulins (Shewry and Tatham, 1990; Shewry et al., 1994).

Cereal chemists use specific terms to refer to the proteins present in various cereal grains. Thus, the prolamin types present in sorghum are known as kafirins. Secalins occur in rye, hordeins in barley, zein in maize, and gliadins and glutenins in wheat. The prolamins found in *Coix lacryma-jobi* are called coixins (Leite et al., 1992). Prolamin constitutes the major component of endosperm protein in most cereals (Larkins, 1981). Most cereal prolamins have evolved from a single ancestral gene by duplication, insertions, and deletions (Buchanan et al., 2000). Based on amino acid sequences proteins are classified into three groups: sulfur-rich, sulfur-poor and high-molecular-mass proteins. The sulfur-rich component accounts for about 90% of the prolamins.

The synthesis of proteins and development of protein bodies have been investigated in several cereal grains, especially in rice, wheat and maize. The general picture emerging indicates that protein bodies develop differently in aleurone and starchy endosperm cells. The protein bodies of aleurone cells may contain globulins and albumins but do not store prolamins and glutelins. Protein body formation takes place in two different ways, either inside vacuoles or rough ER. In rice and oats the 11S globulins are synthesized inside cisternal ER and from there are transported to vacuoles via Golgi bodies. The vacuoles fragment to produce many protein bodies. In the second pathway of protein body formation, protein sequestered in the ER lumen directly distends to produce protein bodies. The ER that stores prolamins in this manner is known as protein body ER (PB-ER). Within the PB-ER of maize are stored four different kinds of zein proteins (Buchanan et al., 2000).
Lipids are the third major biochemical component of cereal caryopsis including sorghum grain. The living aleurone layer and embryo are the major storehouse for most of the lipids present in a mature caryopsis. In addition to storage lipids, structural lipids are associated with cuticles that occur in the cereal grain. Lipids are made up of triacylglycerols. Lipids are stored in membrane-bound structures variously described in the literature as spherosomes, lipid bodies and oleosomes. However, the membranes appear to be ‘half-unit’ and only about 3 nm thick rather the usual 8 nm thick bilayer membrane found in plant cells (Hulse et al., 1980). The polar hydrophilic surface of the membrane appears to be exposed to the aqueous cytosol while the inside of the ‘half-membrane’ that is non-polar and hydrophobic faces the lipid.

The structure of lipid bodies and the composition and synthesis of lipids in sorghum grains have been investigated by Baldwin and Sniegowski (1951); Stemler et al. (1976); Paul et al. (1972); Rooney (1968 and 1978). The white food sorghum has 2.867 g of total lipids per 100 g of grain. It is considered to be nutritionally desirable since the value of saturated and unsaturated fats are low, 0.5 g and 2.36 g respectively, as against 0.22 g and 3.38 g in corn (Texas Grain Sorghum Association, 2002).

In addition to starch, proteins and lipids, cereal grains also accumulate minerals whose content is usually in the range of about 1% dry weight. The cations are associated with the negatively charged myo-inositolhexaphosphoric acid, also known as phytic acid (Johnson and Tate, 1969). About 90% of the phosphorus found in cereals comes from phytic acid. The salt formed by the association of cations with phytic acid is known as phytate of phytin (Greenwood, 1989). The most common cations associated with phytins are Mg and K. Lesser amounts of Ca, Mn, Ba and Fe also occur. Phytate occurs either dispersed around the proteinaceous matrix of protein bodies or in discrete
electron-dense aggregates known as globoids or globoid crystals (Lott, 1981; Lott and Ockenden, 1986).

In seeds, phytin appears to accumulate mostly in the aleurone layer of the endosperm and in the embryonic tissues. In maize grain, 90% of phytin deposit is found in the protein bodies of the scutellar tissues of the embryo (Lott, 1984). Lott et al. (1995) and Greenwood (1995) have described the synthesis, accumulation and deposition of phytin and the possible physiological roles of phytin in plants. Greenwood and Bewley (1984) suggested that phytic acid may be synthesized in the endoplasmic reticulum and transported to the vacuoles of developing protein bodies. Wada and Maeda (1980) examined the structure and distribution of globoids in aleurone and scutellar tissues of major and minor cereals. Fulcher et al. (1981) microscopically detected phytin reserves in cereals.

**SEED GERMINATION STUDIES IN CEREAL GRAINS**

The germination of a seed is the beginning of a new generation of a plant. Germination is also the end of several structures associated with the previous generation as well as tissues that developed along with the embryo. It is therefore of interest to understand the fate of structures and storage material that were recently deposited during the development of the seed. In the case of cereals, since the seed is inseparable from the dry fruit wall, seed germination is intimately associated with changes that occur in the caryopsis as a whole.

Seed germination is one of the most important and widely studied physiological processes (Fincher, 1989; Bewley and Black, 1994; Baskin and Baskin, 1998). Germination is a complex process that involves three phases namely: 1. Imbibition
phase, 2. Lag or plateau phase and 3. Post germination phase. Since no visible growth occurs during the first two phases the last phase is often taken as the phase of germination proper. In many seeds a pregermination phase of dormancy is often encountered even when water and other favorable conditions are provided (Baskin and Baskin, 1998). Minutes after absorption of water, solutes, especially the low molecular metabolites leak out from the cell (Buchanan et al., 2000). At this stage the membranes are said to be in a gel phase or in a liquid-crystal phase. Soon after rehydration the membranes assume normal integrity and the leakage is stopped. The enzymes and mRNA and ribosomes stored earlier begin to function. This is also the time when the preexisting mitochondria function to provide energy, and new mitochondria are produced to keep up with the new demand. During phase 3 protein synthesis takes place as new mRNAs are transcribed. DNA synthesis and cell division are followed by cell elongation. This is when growth is visible to the unaided eye as the radicle emerges and elongates. It is during this phase that mobilization of stored reserves occurs; starch, proteins and lipids and phytin are digested to provide metabolites for the growing seedling.

Cereal grains have become a favorite material for the study of the role of hormones, especially the gibberellins, in seed germination and reserve mobilization (Jones, 1969). The gibberellins apparently act on the living aleurone cells and promote de novo synthesis of mRNA leading to the production of a variety of hydrolytic enzymes, chief among them being alpha amylase. These enzymes in turn help breakdown the storage material and supply low molecular metabolites for the developing seedling. The scutellum especially the scutellar epithelium of the embryo performs the important functions of secreting enzymes into the endosperm, and absorbing low molecular metabolites and transferring them to the developing seedling. Negbi (1984)
identified four different types of scutellum in the grass family. Negbi (1986) also studied the scutellum of *Avena* with respect to reserves mobilization during seed germination. Smart and O'Brien (1979a) investigated the structure and development of scutellum in wheat, barley, oats and rye. Several researchers have investigated the changes that occur in the storage components and the role of the scutellum during germination in various cereals (Niewdorp and Buys, 1964; Swift and Buttrose, 1972; Swift and O'Brien, 1972; Smart and O'Brien, 1979a, b; Miyata et al., 1981; Xu Shixiong, 1983; Fincher, 1989; Ebenezer, 1997). The embryo, of which the scutellum is an integral part, rather than the endosperm cells, appears to control the hydrolysis of starch in the cereal endosperm (Negbi, 1984). Although starch is the most important carbohydrate reserve utilized during germination mannans, $\beta$-glucans and arabinoxylans stored in the endosperm cell walls are also known to be utilized during germination in barley (Morrall and Briggs, 1978). Yet another peculiarity among cereals is the ability of rice grains to germinate and elongate the coleoptile under strict anoxia (Perata et al., 1997).

**Seed germination in sorghum** is fairly well understood. Murty and Aswathaiah (1986) investigated the sowing quality and germination percentage of sorghum grains. Grains harvested 36–42 DAF were found to be physiologically mature and yielded best results in germination tests. Higher contents of moisture in the grains tended to lower the percentage of germination. The availability of mineral nutrients to the mother plant during grain-filling appears to have a positive influence on the vigor of germination of the grain (Benench, 1993). Bijagare (1994) studied the effect of seed size and vigor of germination in sorghum. Gritton and Atkins (1963) and Clark et al. (1967, 1968) examined the factors influencing dormancy in sorghum grains. Dormancy can be retained by maintaining the moisture content at or less than 25% or until the grain
reaches maximum dry weight. Evans and Sticker (1961), Evans et al. (1961) and Kasalu et al. (1993) studied the influence of factors such as moisture and temperature on seed germination sorghum. Higher soil temperature affected emergence of seedlings. Peacock et al. (1990) also found that high soil temperatures inhibited seedling establishment even when adequate soil moisture was available.

The presence of plant growth regulators such as ABA, cytokinins, isopentenyladenine, zeatin and zeatin riboside, in mature seed and the changes in their concentration during germination of sorghum seeds were investigated by Dewar et al. (1998). While there appeared to be no change in the concentration of gibberellic acids there was significant drop in the concentration of other plant growth regulators. The pregerminated grains contained high amounts of ABA. Thus, native plant growth regulators appear to be involved in the control of seed germination and seedling establishment in sorghum.

According to Negbi (1984) sorghum has the typical ‘shield-like’ scutellum and this structural feature is maintained during germination and early stages of seedling growth. As in other cereal grains the activity of $\alpha$-amylase increased during the early stages of seed germination in sorghum (Kneen, 1945). Analysis of the sorghum embryo during early stages of germination revealed an increase in the content of various metabolites about 24 hr after germination (Philomena and Shah, 1980). Afria and Mukherjee (1980) determined that during root and shoot development protein content declined in the endosperm.

Panchaksharappa and Annigeri (1980) and Philomena and Shah (1986) have carried out limited histochemical studies on the germinating grains of sorghum; these will be discussed later in connection with the findings of the present study.
HISTOCHEMISTRY OF CEREAL GRAINS

Histochemistry is a microscopic technique, which makes use of many microscopic procedures and bright-field dyes, reagents and fluorochromes to characterize the various structural and biochemical constituents of cells and tissues. The presence of chemicals such as carbohydrates, proteins, lipids, nucleic acids, lignin, cutin, suberin, tannin, phytin, minerals, vitamins, amino acids and various enzymes could be determined in tissues and organs during various stages of development. It is also possible to enhance the usefulness of this technique by appropriate use of electron and confocal microscopes. The presence of trace amounts of chemicals can be demonstrated with such procedures. The importance of histochemical techniques in the study of plant structures and chemicals components is attested by the publication of several major books on Histochemistry: Gomori (1952); Jensen (1962); Pearse (1972); Conn (1977); Pearse (1980); Clark (1981); Horobin (1982, 1988); Gahan (1984); Olga Bayliss High (1984); Krishnamurthy (1988); Harris and Oparka (1994).

Fluorescence microscopy has now gained a prominent place in histochemical research. Fluorescence microscopy can be used to locate minute quantities of substances by exciting under shorter wavelengths such as UV, violet, blue and observing the reemitted longer wavelengths. Some cellular components such as cutin, lignin and chlorophyll are autofluorescent by nature. Many other non-fluorescent biological substances can be visualized by the judicious use of specific fluorochromes. Fluorescence microscopy was employed in the study of cereal grains to localize various storage materials (Fulcher, et al., 1977; Wood and Fulcher, 1978; Rost, 1980; Stewart, 1981; Fulcher et al., 1981; Fulcher, 1982; Cook and Oparka, 1983; Xushi-xiong et al., 1983; Felker, 1986; Philomena and Shah, 1986; Jones and Rost, 1989). Fluorescence
microscopy has also helped in understanding transport pathways in cereal grains during grain-filling (Oparka, 1991; Harris, 1992; Wang et al., 1994). Jones and Rost (1989) combined histochemistry and electron microscopy to analyze the changes occurring during zygotic embryogenesis in rice. Morrison et al. (1975) made histochemical investigation on development of wheat aleurone cells. Harris and De Mason (1989) made a comparative account of the structure and histochemistry of endosperm in different varieties of corn. Immunohistochemical techniques were used by Wang et al. (1995) to identify and characterize a phloem-specific α-amylase. More recently fluorescence microscopy was employed to identify the presence and content of lignin in corn plants that were transformed by Bacillus thurigiensis (Saxena and Stotzky, 2001).

In our laboratory, Krishnan (1996, 2001) made use of a variety of histochemical techniques to understand the development of the rice grain from anthesis to maturity. Ebenezer (1997) extended this investigation to understand the structural and chemical changes associated with seed germination in rice. Jonathan (1999) examined the chemical constituents of mature bamboo caryopsis using a variety of histochemical procedures. Raghavan (1997) made use of the phloem tracer, carboxyfluorescein, to investigate the path of transport of assimilates through the panicle and ovary in rice.

AIMS OF THE PRESENT INVESTIGATION

This investigation was carried out to understand aspects of grain-filling in sorghum using histochemical techniques. This study on sorghum follows previous investigations in this laboratory on histochemical analysis of caryopsis of rice during development and germination. I wished to study sorghum both because of its importance in Indian economy and also because, unlike rice or wheat, sorghum is a C₄ cereal grass and no detailed histochemical investigations of C₄ cereals caryopsis is available.
In this study I decided to examine the structural and chemical changes associated with the development of the ovary and the mature caryopsis of a few cultivars of sorghum. The changes that occur during germination were also investigated in order to appreciate the role of embryonal organs and the time of mobilization of storage substances. The major biochemical constituents of the caryopsis and the time and pattern of deposition were determined using bright field and fluorochrome dyes, and a variety of light microscopic techniques. The ultrastructure of selected tissues was investigated using an electron microscope. The results obtained in the present investigation were compared with the available published information on related C₄ cereals and the findings of Krishnan (1996) and Ebenezer (1997) who investigated the histochemical aspects of the rice caryopsis, a C₃ grass.