CHAPTER 1

INTRODUCTION
The plant seed is an organ of propagation and dispersal, and is also the major plant tissue harvested by human kind. The seed, containing the new plant in miniature, is equipped with structural, physiological, and biochemical devices to fit it for its role as a dispersal unit and well provided with food reserves which sustain the young plant until a self-sufficient, autotrophic plant can be established.

A typical angiospermic seed consists of a protective seed coat. Seed coat may consist of two layers called testa and tegumen. Embryo is present in the seed. Seeds which contain the endosperm are called endospermic or albuminous seeds, e.g. maize. Seeds without endosperm are called non-endospermic or exalbuminous seeds, e.g. beans. The embryos in seeds of different species of plants differ markedly by in size and appearance, but all mature embryos are composed of one or more cotyledons, a plumule and a hypocotyl. The cotyledons vary in number from one in monocotyledons to as many as fifteen in the embryos of some conifers (Gymnosperms). The embryos of dicotyledons have two cotyledons, as the name implies. The cotyledons are attached near the upper end of the short thick stem like axis of the embryo, the hypocotyl. The plumule or bud of the embryo is usually located just above the point at which the cotyledon or cotyledons are attached to the hypocotyl. The plumule consists of a meristem with rudimentary leaves. The primary root of the plant develops from the lower end of the hypocotyl. The tissue develops from the endosperm nucleus and usually contains considerable quantities of accumulated food reserves. In the seeds of legumes, which contain no endosperm, the cotyledons are usually enlarged and contain food reserves.
The seed is dispersed from the mother plant endowed with a store of food reserves of protein, carbohydrate and fat in a more concentrated package than occurs anywhere else in the plant. Animals exploit this property when using seeds as an extremely important part of their diet. Physiologically and biochemically 'dispersal units' should be considered as seeds. The great differences among seeds in their content of food reserves reflect the varied requirements of different seeds for supporting growth and development of seedlings.

Germination

Seeds do not normally germinate until they have undergone a considerable period of growth and development, accumulating reserves and finally becoming air-dry. These are quiescent. When a viable (i.e., living) seed is wetted, water is taken up, respiration, protein synthesis and other metabolic activities begin and after a certain period of time, the embryo emerges from the seed, generally radicle first, the seed has germinated (Bewley & Black, 1978; 1994).

Three phases of development can be recognized. (1) Imbibition; (2) Lag phase; and (3) Germination. Air-dry seeds have a low water content, around 15% or less, but given access to water they will imbibe rapidly, with tissues generally reaching a water content between 30% and 50% in a day or two. At this time the seed enters a lag phase; it has swollen and became heavier, and starts metabolizing actively. If the temperature is appropriate and the supplies of oxygen adequate, the seed will in due course enter the third phase, the germination. But once germination has occurred, growth of the young seedling continues supported by the mobilization of the food reserves.
Although the mobilization of food reserves from storage tissues is well known to occur during germination, the complexity of metabolic changes makes this topic interesting. The major metabolic events occurring during germination are not the same in seeds of different species and even in the different cultivars of the same species owing to their genetically determined diversity in seed morphology, physiological maturity, developmental pattern, chemical reserves and special requirements of signals for changing phase or of stimuli for depressing genes. Much of our knowledge of reserve mobilization and its control processes comes from studies with cereals. In contrast, available information on legumes is limited to a few species and no unanimous conclusions about food mobilization pattern and common control mechanisms for different species of this family have yet been reached (Ching, 1972; Bewley and Black, 1978; Mayer and Poljakoff-Mayber, 1982; Murray, 1984; Muntz, 1996).

Chemistry of Food Reserves

Seeds characteristically contain relatively large amounts of food reserves which support growth and development of the seedling until it can establish itself as a photosynthesizing, autotrophic plant. Based upon the major food reserves, seeds may be classified into carbohydrate storing seeds and lipid-containing seeds. Protein-containing seeds can belong to either group. Almost no seeds are known in which the predominant storage material is protein, although soybeans are an exception and *Machaerium acutifolium* has been reported to contain 66 % protein. The biochemistry of food reserves has not been studied in detail in many leguminous seeds of semi-arid regions and in seeds of wild plants (Mayer & Poljakoff Mayber, 1982).
Carbohydrates

Starch, cell wall polysaccharides, sucrose and their derivatives are known to function as storage carbohydrates in seeds. Hemicelluloses and galactomannans also are frequently present as main storage material. Hemicelluloses, both pentosan and hexosan, occur in the endosperms of the palms as well as in the cotyledons of lupin, Primula and Impatiens. Galactomannans are present as the main reserve carbohydrate especially in the endosperm of Leguminoseae, for example Trigonella (Reid and Meier, 1973). These are deposited in varying amounts in different species during seed development and consumed during and mainly after germination.

Starch is laid down in discrete subcellular bodies, the starch grains. Granular size and shape is very variable between species, though how this is determined by the ordering of molecular chains within granules is incompletely understood (Bewley & Black, 1978; Halmer, 1985). In legumes, high amounts of starch along with protein are stored in the cotyledons. Pea contain 45-55% starch and in beans it is about 50-60% (Crocker and Barton, 1957), whereas Arachis hypogea contains only 12% (Halmer, 1985), horse gram contains about 59.3% carbohydrate (Manage and Schonie, 1972) and starch was isolated with an yield of 28% which has 34.3% amylose and 65.7% amylopectin (El Faki et al., 1983 a). Alaska peas contain 35% amylose and 65% amylopectin (Akazawa, 1965). Amylose content of many legumes varies between 25-35%. However, wrinkled peas contain 65% amylose (Badenhuizen, 1969; Banks and Greenwood, 1975). Amylopectin is other major constituent of starch in many plant tissues, usually comprising about 75-80% in most plants ranging from 60% up to 90% and reaches about 100% in waxy cultivars of rice, maize, sorghum, barley, millet, foxtail millet, Job's tears, Amaranthus hypochondriacus, pea and potato (Sakamota, 1982; Nakamura, 1996).
Thick cell walls act as a carbohydrate reserve. They may also have roles in protecting delicate seed structures or in storing water under dry conditions or conducting water (Reid and Bewley, 1980). Several types of hemicellulose which differ in their major sugar composition and bond linkage pattern are known. Galacto \((1 \rightarrow 4) \beta\)-mannans are stored in the endosperms of many legumes (Dea and Morrison, 1975). Galacto-xylo\((1 \rightarrow 4)\)-\(\beta\)-glucans ('amyloids') are known to be present in several legumes (Koimann, 1960; Courtois et al., 1976). Arabino\((1 \rightarrow 4)\)-\(\beta\)-galactans are stored in the cotyledonary cell walls of soybean and lupinus (Matheson and Saini, 1977; Clarke et al., 1979). \((1 \rightarrow 5)\)-\(\alpha\)-Arabinans serve as a major food reserve (9\%) of mustard cotyledons (Rees and Richardson, 1966) and galactomannan serve as a major food reserve in *Sesbania marginata* endosperm (Buckeridge and Dietrich, 1996) and other legumes (Mc Cleary and Matheson, 1974).

Most seeds retain small amounts of soluble sugars at maturity, in addition to other food reserves (Amuti and Pollard, 1977), but soluble sugars constitute the main carbohydrate reserve in some species such as *Acer saccharum* (Crocker and Barton, 1957), soybean (Adams et al.; 1981) and *Lupinus* spp (Saini and Lymborg, 1983). Most commonly found are the raffinose family of oligosaccharides (Dey, 1980) in *Trigonella foenumgraecum* (Reid, 1971), *Dimorphandra mollis* (Buckeridge et al., 1995) and *Sesbania marginata* (Buckeridge and Dietrich, 1996). Horse gram contains 3.6\% of the sugars while cow pea has 8.1\% and chick pea 7.1\%, all showing the presence of galactose, glucose, sucrose, raffinose, stachyose, and verbascose in varying proportions. Fructose was not detectable in horse gram but was present in small quantities in chick pea and cow pea. Stachyose was the predominant sugar in horse gram and cow pea (El Faki et al., 1983 b). Sucrose, raffinose, stachyose and verbascose were present in *Sesbania marginata* seeds (Buckeridge and Dietrich, 1996).
Lipids

Lipid reserves are laid down in the form of discrete subcellular organelles - 'oil bodies'. They are also known by other names such as spherosomes, oleosomes and lipid containing vesicles. The predominant storage lipids of seeds are the neutral fats, or oils if they are liquid at 'normal' temperatures. Details regarding the development of oil reserves in the oil bodies are lacking (Bewley and Black, 1978). Though saturated and unsaturated fatty acids are present in triacylglycerols, the predominant fatty acids are unsaturated. The saturated fatty acids contain an even number of carbon atoms, usually, between 4 and 24. Palmitic acid is the most common saturated fatty acid. Arachidic, behenic and lignoceric acids are also present in leguminous seeds. Most commonly oleic and linoleic acids are present in seeds. Variations in the amount of oil in any one species are due to seasonal and geographical factors. Not only the percentage of oil vary but also the fatty acid composition. The oil content of seeds may vary from 2% in Acacia spp to 56% in Arachis hypogea. Generally, seeds rich in oil tend to be high in protein but not in starch (Bewley and Black, 1978; Mayer and Poljakoff-Mayber, 1982). Interestingly, oil-rich seeds are smaller on average than those with abundant carbohydrate and protein. Leguminosae for example possess both kinds of seed.

Proteins

The storage proteins usually occur in well defined organelles, which can be distinguished in the microscope. Generally these are referred to as protein bodies or microbodies. (Mayer and Poljakoff-Mayber, 1982; Chrispeels, 1984). The amount of protein present in seeds varies from ~10% (in cereals) to ~40% (in certain legumes and oil seeds) of the dry weight, forming a major source of dietary protein (Shewry et al., 1995).
Although the vast majority of the individual proteins present in mature seeds have either metabolic or structural roles, all seeds also contain one or more groups of proteins that are present in high amounts and that serve to provide a store of amino acids for use during germination and seedling growth (Bewley and Black, 1994; Shewry et al., 1995). Dicotyledonous seeds such as legumes predominantly contain globulins, e.g. arachin and conarachin of peanuts. Legumin and vicillin are present in *Pisum sativum*, *P. vulgaris* and glycinin in soybean (*Glycine max*). The globulin storage proteins have been studied in most detail in legumes, notably peas, soybean, broad bean (*Vicia faba*) and French bean (*Phaseolus vulgaris*) (Staswick, 1994; Shewry et al., 1995). These proteins precipitate differentially when salt extractions were diluted and heat treated. Modern techniques like ultracentrifugation and electrophoresis have improved our knowledge on the structure of storage proteins. Separation of leguminous globulins by ultracentrifugation generally reveals two bands based on their sedimentation coefficients, the 7S vicillin-type globulins and the 11S legumin-type globulins distinguishable in size and sugar content (Gueguen and Azanza, 1984; Vitale and Bollini, 1995). They are mainly found in legume seeds. 7S globulins are major storage proteins in monocotyledons (Shewry et al., 1995). In oil palm embryos globulins of the 7S type predominate (Morcillo et al., 1997). In some species e.g. lupin 12 S globulin are predominant (Duranti et al., 1988). In *Glycine max* additional proteins 2.2 S are present during early stage of seed development, whereas in others e.g. *Acacia* spp 11 S proteins seems to be absent. Both 11 S and 7 S proteins split into subunits separable by electrophoresis. Aminoacid composition of legumes has some common characteristics. High levels of asparagine, glutamine and arginine are present. 7S proteins lack cysteine. In many cases these globulins are shown to be glycoproteins (Bewley and Black, 1994; Shewry et al., 1995). Vicillin in *Phaseolus aureus* is a glycoprotein containing neutral sugars and a
small amount of glucosamine. Legumin, previously also regarded as a glycoprotein appears merely to co-purify with a low molecular weight glycoprotein but can be separated from it. Legumins are not usually glycosylated, an exception being the 12 S globulin (Duranti et al., 1988; Shewry et al., 1995). Both proteins are made up of non-identical subunits, three for legumin and four for vicillin (Ericson and Chrispeels, 1973). Thus it appears that the individual proteins show a great deal of heterogeneity. This is made evident by their molecular weights and it seems to be a general phenomenon (Derbyshire et al., 1976; Wall, 1979). This heterogeneity finds application in seed quality control. Autran (1975) has shown that the storage proteins of each seed has a highly characteristic pattern which permits its identification and in detection of adulteration.

**Biochemical changes during germination**

The dry seed is characterized by a remarkably low rate of metabolism. This dry seed is a well equipped functional unit which can carry out a large number of biochemical reactions provided that the initial hydration of the proteins and more specifically of the enzyme proteins take place. The process of seed germination is accompanied by a variety of interesting and significant biochemical phenomena. The overall changes may be summarized as follows:

1. Seeds absorb water from the soil by imbibition.
2. In the presence of water the enzymes are activated.
3. By the action of enzymes, complex food reserves degraded to simple soluble substances.
4. The embryo absorbs these simple substances and grows in size by repeated cell division.
5. Seed coat rupture and embryo is exposed to air and light.

6. Aerobic respiration increases rapidly. Cells of primary axis (of the embryo) synthesize proteins and DNA.

7. Newly formed cells elongate rapidly.

8. Radicle emerges first and later develops root and shoot systems.

In seeds, each embryonic part has a predetermined metabolic pattern of its own as well as a blue-print for further development. However, the mature storage organ would usually have reached a committed phase of development before hand. Food reserves in storage organs of seeds are mobilized during seed germination and the post-germinative growth of seedlings (Ashton, 1976). Time-course studies between the decline in reserve materials and growth of the embryonic axis have indicated that the hydrolytic products are transported to the growing axis. The complete transfer to the axis, however, probably depends on the fate of the storage organ.

In germinating seeds, the metabolism is usually amphibolic, involving both catabolic and anabolic reactions. Catabolic reactions of food reserves provide energy and raw materials required for the early seedling growth. Through anabolic reactions, synthesis of proteins (needed for respiration and other cellular processes), biogenesis of organelles as well as the anabolic synthesis of new cells and tissues are achieved. Storage tissues such as the endosperm of seeds of both mono- and dicotyledons, mega-gametophytes of gymnospermous seeds and cotyledons of dicots form the major sites of catabolic reactions. True anabolism usually takes place in the embryo or embryonic axis.

**Carbohydrate metabolism**:

Although carbohydrate depletion in the endosperm and cotyledons of legumes has received considerable attention in a few species, the mechanism
by which it is achieved is less clearly understood than that in cereals. Mobilization of food reserves during germination in some endospermic and non-endospermic legumes have been studied. Most of our knowledge is based on work on peas (*Pisum sativum* and *P. arvense*), green beans (*Phaseolus vulgaris*), broad beans (*Vicia faba*), soybeans (*Glycine max*), mung bean (*Vigna mungo*), and *Arachis hypogea* have been studied to a more limited extent (Halmer, 1985).

Starch degradation in germinating seeds is mainly carried out by amylases or phosphorylases or a combination of these enzymes in conjuction with a debranching enzyme. $\alpha$-Amylase seems to be the key degradative enzyme in starch mobilization in cereal grains. It is present in low levels in mature seeds, and increases dramatically as starch degradation proceeds (Dale, 1969; Van Onckelen *et al.*, 1977; Allfrey and Northcote, 1977; Karunagaran, 1990; Nandi *et al.*, 1995; Taneyama *et al*. 1995).

Juliano and Varner (1969) studied the enzymic degradation of starch granules in the cotyledons of smooth pea cultivar, Early Alaska, during the first 15 days of germination. Slow degradation of starch was observed initially for 5 days which was followed by a rapid degradation. During the initial slow phase of degradation, phosphorylase activity increased gradually and reached a peak on the fifth day while $\beta$-amylase hardly changed and $\alpha$-amylase was low. In the next phase rapid degradation of starch occurred coinciding with $\alpha$-amylase production. Thus the $\alpha$-amylase is the major enzyme involved in the rapid degradation of starch into more soluble forms while phosphorylase and $\beta$-arpylase assist in its action. Neither free sugars nor dextrins accumulate in the cotyledons at any time which would indicate a rapid utilization of the products of starch hydrolysis. At least two distinct phosphorylases have been characterized in germinating seeds (Matheson and Richardson, 1976;
Richardson and Matheson, 1977). The initial activity of phosphorylase probably provides the substrate for glycolysis and respiration produce the ATP quickly needed at the very early stage of germination for the synthesis of enzymes and organelles necessary for further mobilization of reserves. In wrinkled varieties of peas, starch phosphorylase levels peak after those of α-amylase and β-amylase is undetectable throughout (Bain and Mercer, 1966; Shain and Mayer, 1968; Juliano and Verner, 1969; Abbott and Matheson, 1972; Yomo and Varner, 1973).

β-amylase appears not to be essential for starch mobilization in peas, and it seems to be the case in other legumes too. The enzyme is undetectable in germinated seeds (Fernandez-Tarrago and Nicolus, 1976), its levels increase in one cultivar of green beans but remain unchanged in another (Van Onckelen et al., 1977; Garcia-Luis and Guardiola, 1978; Ren et al., 1993). Neither β-amylase nor starch phosphorylase seem necessary for mobilization of starch in germinating seeds of soybean. α-Amylase is one of the enzymes synthesized de novo in the later stages of germination (Swain and Dekker, 1969) and the initiation of α-amylase gene expression in wheat scutella was independent of de novo GA biosynthesis, whereas that in the aleurone was largely dependent on embryo-produced GA₃ (Lenton et al., 1994). Whether β-amylase is activated or synthesized is unknown. However, a debranching enzyme amylopectin α-(1→6)-glucosidase appears to be activated from a zymogen (Mayer and Shain, 1968). Mature soybean seeds contain high levels of β-amylase and low levels of phosphorylase (Birk and Waldman, 1965; Adams et al., 1980 a, b). However, it appears to have no essential role in carbohydrate metabolism of germinating soybean seeds (Hildebrand and Hymonowitz, 1981). β-amylase activity was low in lentil cotyledons and the axis tissue contained an active β-amylase (Fernandez-Tarrago and Nicholas, 1976). Probably
oligosaccharides formed by amylolysis in lentil cotyledons were translocated to axial tissue and degraded there as in the case of peas (Swain and Dekker, 1966b).

The nature and fate of the products of starch mobilization have received little attention. Only in peas a few studies have been made. A transient accumulation of water soluble carbohydrates (oligosaccharides and dextrins) in the cotyledons during germination has been reported (Bain and Mercer, 1966; Abbott and Matheson, 1972). There are, however, several reports that neither free sugars nor dextrins accumulate in the cotyledons at any time in germinating peas (Juliano and Varner, 1969; Marbach and Mayer, 1976; Garcia-Luis and Guardiola, 1978; Monneri et al. 1986). Presence of sucrose was demonstrated in the cotyledons of germinating peas (Swain and Dekker, 1966b; Abbott and Matheson, 1972) and soybean (Brown and Huber, 1988). Accumulation of sucrose formed from the hydrolysis of stachyose and raffinose was reported to occur in the initial stages of germination (Lee and Shallenberger, 1969; Monneri et al., 1986). Raffinose family oligosaccharides occur both in the endosperm and embryo of legume seeds and are broken down during germination, resulting in a transient accumulation of free galactose and sucrose in *Trigonella foenum-graecum* (Reid, 1971) and *Dimorphandra mollis* (Buckridge et al., 1995). First storage carbohydrates to be degraded during germination are the raffinose family oligosaccharides (Reid, 1971; Buckeridge et al., 1992, 1995). The products of galactomannan degradation (galactose and mannose) do not accumulate either in the endosperm or in the embryo, where they are probably metabolized and used as a source of energy for the early growth of the plantlet (Buckeridge and Dietrich, 1996).

Sucrose formed during germination of *Sesbania marginata* was absorbed by the embryo where it probably serves as a supply of carbon and/or
energy. Similar results have been obtained in *Trigonella foenum-graecum* (Reid, 1971), *Ceratonia siliqua* (Seiler, 1977), and fenugrec (Sioufi et al., 1970). It has been proposed that sucrose (produced during raffinose family oligosaccharides breakdown) is the translocation sugar in legume seeds during germination (McCleary and Matheson 1974; Main et al., 1985). Sucrase activity was not detectable in pea cotyledons (Swain and Dekker, 1966b).

Notably, the pattern of starch depletion during germination differs between the various varieties of peas studied. In victory freezer (wrinkled variety) linear depletion of starch was shown from 2nd day to the 22nd day with the total sugars accumulating up to 7th day (Bain and Mercer, 1966). However, in full pod, which is another wrinkled variety, there was an initial slow degradation of starch up to 10 days and the rapid degradation was completed thereafter within 2 days. Transient accumulation of water-soluble carbohydrates was reported (Abbott and Matheson, 1972). In progress variety (wrinkled) starch mobilization was linear with time (Monneri et al., 1986) without any accumulation of sugars or dextrins though an initial accumulation of sucrose was noticed. In smooth peas (Early Alaska) starch degradation was characteristically biphasic with initial slow degradation up to 5 or 6 days and rapid degradation began thereafter (Juliano and Varner, 1969; Monneri et al., 1986).

Monneri et al., (1986) compared a smooth pea variety with wrinkled variety and suggested that a common pathway may be operative in both the cultivars during germination. Same sugars were found in the cotyledons of both cultivars maltose, maltotriose and linear maltodextrins were not present and trace amounts of glucose were detected suggesting degradation of starch by phosphorylase after an initial attack by α-amylases. However, uncertainty still exists as to whether these differences are unique features of individual cultivars of simply artifacts of techniques (Bewley and Black, 1978; 1994).
Interestingly, new starch is formed even when it is being mobilized in the same cells (Smith and Flinn, 1967; Harris, 1976; Briarty and Pearce, 1982). In legumes transitory starch is formed with in amyloplasts whereas starch granules in mature seeds lack a surrounding plastid membrane (Halmer, 1985). Most of the enzymes involved in the breakdown and interconversion of the carbohydrates become active during germination, most by de novo synthesis, some by activation or release (Mayer and Poljakoff-Mayber, 1982). Enzymatic protein synthesis was observed during transformation of starch into sugars in germinating seeds is suggested on the basis of regression curve between total sugar and soluble protein (Das et al., 1992/94).

**Protein Metabolism**

The storage proteins are hydrolysed to yield free aminoacids and amides which are utilized from the synthesis of new functional and structural proteins as well as sources of energy for the developing seedling. Several kinds of proteolytic enzymes such as endopeptidases (sulphydryl, acidic, metallo and serine proteinases) and exopeptidases (amino and carboxypeptidases) are involved in the degradation of storage proteins in cotyledons of germinating legume seeds (Ryan and Walker-Simmons, 1981; Shutov and Vaintraub, 1987; Rawlings and Barrett, 1993; Barrette, 1994; Bewley and Black, 1994; and Muntz, 1996). Less information is available on the occurrence and properties of various proteinases and hence the mode of enzymatic breakdown of storage proteins during legume seed germination is not yet clearly understood. The variability among the proteolytic enzymes studied is generally attributed to the species, nature of storage proteins, the part of the seed studied and the exact stage of germination (Haffaker and Peterson, 1974; Ashton, 1976; Mayer and Marbach, 1981; Bewley and Black, 1978; Ryan and Walker-Simmons, 1981; Shutov and Vaintraub, 1987). The main stages of their degradation take place within protein...
bodies and vacuoles that are formed after their fusion in germinating seeds (Ashton, 1976; Pernollet, 1978; Weber and Neumann 1980; Shutov and Vaintraub, 1987). However, in the embryo axis, as well as in the storage tissues like endosperm or cotyledons, time-course pattern and mechanism of storage protein degradation as well as its contribution to nitrogen supply for the developing embryo and its regulatory interaction with the major protein degradation processes in the proper storage tissues has not been much investigated (Muntz, 1996). The beginning of measurable storage protein degradation can be detected at days 2-3 after the start of imbibition depending on the species under investigation (Kavkin, 1982; Muntz et al., 1985; Bewley and Black, 1994). Further, storage protein breakdown proceeds much more rapidly in the cotyledons of germinating seeds of *Vigna radiata* (Baumgartner and Chrispeels, 1979), *Phaseolus vulgaris* (Neilson and Liener, 1984; Boylan and Sussex, 1987), *Pisum sativum* (Basha and Beevers, 1975), *Vicia faba* (Lichtenfeld et al., 1979, 1981), and *Glycine max* (Wilson et al., 1986). The onset of degradation is not directly related to water uptake but is determined by other subsequent processes. This conclusion is in line with the evidence indicating the role of embryo axis in protein breakdown (Kavkin, 1982; Muntz et al., 1985) and lack of autocatalytic function in protein bodies of ungerminated seeds (Harris and Chrispeels, 1975; Baumgartner and Chrispeels, 1976; Boulter, 1981; Kavkin, 1982; Muntz et al., 1985; Bewley and Black, 1994).

Seed proteinases hydrolyse their own storage proteins isolated from ungerminated seeds. They are not found in dry seeds and appear after the onset of germination and their activity increases during the breakdown of storage protein, the exhaustive hydrolysis of which results in the formation of short peptides and aminoacids. Evidently these enzymes have a common function i.e., they initiate storage protein mobilization and participate in their further hydrolysis (Shutov and Vaintraub, 1987). Acid proteinase is present in the protein
bodies of cotton seeds (Yatsu and Jacks, 1968), *Vigna species* (Prisco *et al.*, 1975) and horse gram (Karunagaran, 1990). Papain-like cysteine proteases represent the major storage degrading enzymes which appear during germination, for example in the cotyledons *Phaseolus vulgaris* (Boylan and Sussex, 1987), *Vigna mungo* (Akasofu *et al.*, 1989, 1990), in *Vicia sativa* (Becker *et al.*, 1995b). Hemp seeds contain an acid proteinase unaffected by known inhibitors of - SH or serine enzymes (St Angelo *et al.*, 1969; St Angelo and Ory, 1970). Acid - SH proteinase I and II are characterized and found to be dominant in many germinating seeds (Basha and Beevers, 1975; Baumgartner and Chrispeels, 1977; Tully and Beevers, 1978; Cornel and Plakton, 1994). In various seeds endopeptidase and carboxypeptidase activities increase while aminopeptidase decreases during germination (Chrispeels and Boulter, 1975; Crump and Murray, 1979; Feller, 1979). High activities of leucine aminopeptidase and alanine amino peptidase have been demonstrated in dry cotyledons which increased slightly on the first day and declined during later stages germination of *Vigna mungo* seeds (Mitsuhashi *et al.*, 1984). Dipeptidase activity was demonstrated in squash cotyledons (Sze and Ashton, 1971). Dry seeds of mung bean (Baumgartner and Chrispeels, 1976) and castor bean (Tully and Beevers, 1978) did not contain detectable activity of proteinases. The germinating seeds of *Phaseolus vulgaris* showed an increase in enzyme activity correlating with a decrease in protein nitrogen though no large accumulation of aminoacids occurred (Yomo and Srinivasan, 1973). Similar increase in activity coinciding well with a sharp decrease in various protein fractions was noticed in peas (Basha and Beevers, 1975). An early feature of protein utilization in peanuts and soybeans is thought to be the provision of ammonia to the developing seedling by the action of deaminases (Catsimpoolas, *et al.*, 1968; Daussant *et al.*, 1969).
The fact that reserve proteins and acid proteinases are located in the protein bodies of germinating seeds is a strong evidence of participation of acid proteases in the hydrolysis of proteins. Its pH optimum in a slightly acid range is also characteristic and indicates that it is probably localized in the protein bodies and therefore takes part in the mobilization of the seed protein. (Shutov and Vaintraub, 1987). Thus the insoluble reserve proteins are hydrolysed to soluble peptides by the cytosol where they are hydrolysed to amino acids by the relatively abundant alkaline and neutral peptidases. The amino acids formed may be utilized for the de novo synthesis of enzymes needed for the next stage of extensive mobilization of reserves. The abundance of ribosomes associated with endoplasmic reticulum and the increase in protein synthesis in the cotyledons of legumes early after imbibition point to enhanced de novo synthesis of enzymes. The enzymes may then be packed into vesicles which can migrate to the protein bodies to fuse with them eventually releasing the enzymes inside (Bewley and Black, 1978; 1994).

In mung beans despite the presence of several hydrolytic enzymes with in the protein bodies there is no evidence of autolysis of storage proteins for several days after imbibition. Then there is rapid hydrolysis of storage protein which coincides with the de novo synthesis of an endopeptidase (Chrispeels et al., 1976). The endopeptidase known as Vicillin peptidohydrolase is synthesized in the cytoplasm and then transported into the protein bodies. The endopeptidase appears to the limiting enzyme for proteolysis and active reserve breakdown commences only after the do novo synthesis. *Phaseolus vulgaris* have low activity of proteinases but a marked burst occurs five days after the start of imbibition which is suggested to be due to enzyme activation rather than de novo synthesis (Yomo and Srinivasan, 1973). The major enzyme degrading the proteins is of the endopeptidase type and there is a close
relationship in timing and location between enhanced enzyme activity and protein mobilization in germinating cow pea (Harris et al., 1975; Wilson, 1986; Shutov and Vaintraub, 1987; Vierstra, 1993). The involvement of serine endopeptidase or metallo endopeptidase in storage protein mobilization is reported in some species (Mitsuhashi et al., 1986; Belozerski et al., 1990; Elpidina et al., 1991; Qi et al., 1992). Eventually, protein bodies undergo autolysis and disappear giving rise to vacuoles which fuse into a single large vacuole (Ashton, 1976; Pernollet, 1978).

Inhibitors of trypsin and chymotrypsin are present in seeds (Vogel et al., 1968; Hobday et al., 1973). Whether they regulate protolytic activity in early stages of germination remains to be confirmed (Mayer and Poljakoff Mayber, 1982). The endogenous resistance of stored pulse crops to insect and pests could be improved by manipulating primary gene products such as proteinase inhibitors in the pulse crops.

In many legumes, protein synthesis during germination takes place after the activation of masked, long-lived RNA (Mayer and Shain, 1974). In germinating cotton seeds RNA synthesis and ribosome formation are known to occur. At the same time pre-existing in mRNA is utilized for polysome formation and for the de novo synthesis of a protease (Ihle and Dure, 1969, 1972). In castor beans, however, the dry seeds already contain ribosomal RNA and the heavy ribosomal fraction increases during germination. The activity of aminoacyl tRNA synthetase increases during the germination of Phaseolus vulgaris (Anderson and Fowden, 1969). No details are available about the individual enzymes involved in protein synthesis and their changes during germination (Mayer and Poljakoff Mayber, 1982).
Lipid metabolism

The lipids are generally present in special organelles referred to as spherosomes. The spherosomes contain or acquire part or all of the enzymes required for the breakdown of lipids to fatty acids and glycerol. The role of mobilization and utilization of reserve lipids are apparently of significance in oil-bearing leguminous seeds rather than in starch-storing seeds. Lipid degradation in germinating oil seeds involves the participation of oil bodies, mitochondria and glyoxysomes. The demands of cellular metabolism in starch storing leguminous seeds during germination are fulfilled without such a finer development of existing and additional organelles. It is thus reasonable to expect that the mobilization of the already low fat reserves in such seeds is not likely to have any major contribution to provide energy for germination.

The first step of lipolysis is carried out by lipases. Normally breakdown products of hydrolysis of lipids accumulate in the seeds and are present in small amounts. *Arachis* cotyledons contain enzyme systems that convert glycerol to glycerol phosphate which is then converted to triose phosphate. This can then be either converted to pyruvic acid or to sugars. Lipolysis during germination is usually followed by β-oxidation, glyoxylate cycle and reversal of glycolysis mainly to yield sucrose from hydrolysed fatty acids to be transported to the growing axis. An acid lipase associated with oil bodies is most active over the first two days after imbibition in castor bean endosperm although the storage triglycerides are not utilized at this time. Strangely the enzyme is capable of hydrolysing tri, di and monoglycerides. Another enzyme, alkaline lipase is found in the membrane of the glyoxysome and shows high activity when the triglycerides are being mobilized although the enzyme is specific only for monoglycerides (Muto and Beevers, 1974). In addition, a neutral lipase increases in germinating castor beans (Yamada, 1957). The close juxta
position of oil body and glyoxysome as shown by electron micrograph suggest the possibility of combined action of these enzymes but the exact mechanism of degradation remains highly speculative (Bewley and Black, 1978; 1994).

In sunflower seeds, negligible changes in lipid content and lipid activity occur during the first 24 hours of germination, which is probably due to the immature mitochondrial system at that stage. Between 2-5 days lipase activity increases correlating with a rapid decrease in total and non-polar lipids. Free fatty acids do not change indicating an efficient utilization (Bhatia et al., 1978). Whether the rise in activity of lipase following imbibition is due to de novo synthesis or activation of existing lipases reported in the dry seeds of a few species has not been established (Bewley and Black, 1978).

The products of triglyceride degradation, fatty acids and glycerol may be utilized for further fat and membrane production, particularly in persistent cotyledons as found in *Cucurbita pepo*. Glyoxysome formation is often linked to the onset of lipid utilization and they disappear after the completion of reserve mobilization. Major proportion of breakdown products are converted to hexose and finally to sucrose (Mayer and Poljakoff-Mayber, 1982).

**Regulation of Food Mobilization**

Once a seed has germinated certain developmental sequences are initiated within the food storage tissues to ensure that the reserves are mobilized to provide the essential soluble products for seedling growth. In dicotyledonous plants, the information available on the regulation of food reserve mobilization in germinating seeds is rather unsatisfactory, incomplete, confusing and less well understood (Guardiola and Sutcliffe, 1971; Munoz et al., 1990) and many conflicting results exist in the literature concerning the role of the embryonic axis in the breakdown of seed reserves (Bewley and Black, 1978; 1994; Slack
et al., 1977; Thomas, 1977; Davies and Slack, 1981; Shutov and Vaintraub, 1987; Muntz, 1996). In certain cereal grains the embryo is known to control the mobilization of reserves through the production of gibberellins and the aleurone layer responds by synthesizing and/or secreting hydrolases into the endosperm to degrade the storage materials (Bewley and Black, 1978; 1994; Yomo and Varner, 1971). In the light of this discovery much attention has been paid to investigating whether a similar mechanism operates between embryonic axis and cotyledons of germinating legume seeds. Two hypothesis have been put forth to explain the role of axis in the regulation process (Davies and Slack, 1981, Halmer and Bewley, 1982; Bewley and Black, 1994). First, the growing axis may act as sink to draw away the products of degradation which may inhibit further development of enzyme and/or inhibit their activities (Davies and Chapman, 1979 a, b; Davies and Slack, 1981; Chapman and Davies, 1983; Bewley and Black, 1994; Nandi et al., 1995). Second, the growing axis may produce the plant growth substances which stimulate the synthesis of hydrolytic enzymes needed for food reserve mobilization in the cotyledons (Locker and Ilan, 1975; Ilan and Gepstein, 1980/81; de Klerk, 1986; Karunagaran and Ramakrishna Rao, 1990, 1991; Nandi et al., 1995; Vitale and Bollini, 1995). For these, a common approach is to mimic the effect of embryonic axis has been to study the response of enzyme activity and the rate of reserve breakdown to exogenously applied plant growth regulators. Alternatively, several workers studied whether there is any continual operation of a source-sink relationship between the storage organs (source) and axis (sink) during germination and early seedling growth.

Control Process in the mobilization of stored carbohydrate reserves

Chemical analysis and determination of enzymatic activities have shown that the amylolytic pathway is the major mechanism for the break-
down of reserve starch in endosperm tissues of germinating rice seeds (Murata et al., 1968). This finding is in good agreement with results reported by several workers on the formation of $\alpha$-amylase in germinating cereal seeds i.e., barley and rice, induced by gibberellic acid (Yomo, 1960; Briggs, 1963; Paleg, 1960; Varner, 1964; Varner and Chandra, 1964; Jacobson and Beach, 1985). A common feature of developing seeds is that they do not contain active reserve-mobilizing enzymes; normally these are produced or are activated only when seeds have germinated (Garcia-Mayas et al., 1990; Bewley and Black, 1994).

The pattern of starch mobilization on a cell to cell basis differs between legume species. In peas, broad beans and peanuts degradation moves in a wave from the one face of the cotyledons inwards; in mung beans the pattern is the reverse beginning near the inner face; whereas in green beans starch is depleted first in the central tissue of cotyledons (Bewley and Black, 1978). The implication is that in terms of tissue located the mobilization is under genetic pattern control, and does not bear a fixed relationship for the sake of argument to the outer perhaps better aerated tissue or the vascular elements which convey material between cotyledons and the embryonic axis. Starch mobilization either precede or follow protein mobilization (Halmer, 1985).

Growth and reserve mobilization in dicotyledons appear to be efficiently synchronized process and this suggest that the embryo or embryonic axis has a direct influence on reserve breakdown in these seeds. In the light of discovery on the embryonic control of mobilization in cereals much attention has been paid to investigating whether a similar mechanism operates between the axis and cotyledons of germinating legume seeds. The common approach has been to compare the timings and levels of $\alpha$-amylase or total amylase activity between germinating seeds and seeds “de-axised” either before or soon after sowing. The picture that emerges is very confusing with seemingly
contradictory results (Halmer, 1985; Bewley and Black, 1994). In various pea
cultivars, for example, removal of the axis entirely prevented (Sprent, 1968),
reduced four fold (Varner et al., 1963), reduced two fold (Morohashi, 1982),
reduced slightly but significantly (Locker and Ilan, 1975) or increased slightly
(Yomo and Varner, 1973; Parys et al., 1983) amylase production. In green beans,
amylase production in isolated cotyledons was reduced two fold (Van Onckelen
et al., 1977), delayed by half day (Gepstein and Ilan, 1970) or promoted three
fold (Yomo and Srinivasan, 1973) compared to intact seeds. Axis removal did
not change $\alpha$-amylase levels in pea nuts (Allfrey and Northcote, 1977), but
reduced (Minamikawa, 1979) or promoted three fold (Koshiba and Minamikawa,
1983) in mung bean. It should be borne in mind that in many of these studies
$\alpha$- and $\beta$-amylases were neither distinguished, nor was starch degradation
measured. There appears to be no dependence of legume cotyledons on the
germinating axis for the production of substantial quantities of amylase and
axis removal only serves to modify the timing and degree of enzyme production.
In contrast, the rate of mobilization of reserves often is reduced by the removal
of the axis (Davies and Slack, 1981). The most likely explanation to be offered
for this is that in isolated cotyledons the natural sink for mobilized reserves is
absent, and enzyme activities are regulated by feedback control. Such a type
of enzyme/product feedback control has been demonstrated for protein
breakdown in cucumber cotyledons (Davies and Chapman, 1980; Davies and
Slack, 1981). There is good evidence that starch hydrolysis in peas depends
upon the development of an axial sink from the work of Garcia - Luis and
Guardiola (1975; 1978). Their observations included a stimulation of amylolytic
activity in attached cotyledons in response to $GA_3$ and not in detached
cotyledons and the failure of $GA_3$ to replace the axis effect in restoring the
lesser rate of starch degradation found in detached cotyledons. These authors
found that a change in the rate of hydrolysis and transport from the cotyledons
in response to GA$_3$ was accompanied by a parallel change in the capacity of
the axis to grow and accumulate nutrients favouring no direct role for GA$_3$
which is acting through the development of an axial sink for the products of
starch breakdown. An inverse relationship has been strongly implicated between
the rate of amylase synthesis and the amount of free sugars in pea cotyledons.
The existence of transitory starch formed during starch mobilization in some
legumes suggests that mobilization may be regulated by a balance between
degradation and synthesis. Perhaps, once granule breakdown begins in a
particular cell it cannot be stopped, but if excess sugar and hexose phosphate
are formed, it is channelled into the production of new starch in amyloplast.
Remobilization of this starch with in the plastid could then be regulated by
other mechanisms (Halmer, 1985). In black gram seeds, the development of
amylolytic and proteolytic activities is severely retarded in detached cotyledons
compared to attached cotyledons (Morohashi, 1982). While the reducing sugar
content increased in attached cotyledons, it increases, decreases or remains
unaltered when the radicle is removed with or without the hypocotyl after 2
days. The rate of amylase production in all cases is less in attached cotyledons
showing no correlation with sugar level. Under the same conditions inverse
relationship could not be established for protease and its end products.
Morohashi (1982) suggested that feed-back regulation of hydrolases is unlikely
in leguminous seeds. But the levels of $\alpha$-amylase activity and content were
reduced by high concentrations of glucose and sucrose and this effect was
caused mostly by osmotic stress and partly by end-product repression in Vigna
mungo seeds (Taneyama et al., 1995).

Gepstain and Ilan (1981) have doubted whether amylase production
in green bean cotyledons is regulated by internal osmotic pressure prompted
by the fact that the rate of decline of both, osmotic potential and amylase
production are less in detached cotyledons than in intact seeds. However,
differences in the rate of enzyme production are seen one day before the changes in osmotic potential. In peas also this osmotic hypothesis has been rejected by Morohashi and Ueno (1980).

Although, the experiments with legume seeds have provided only weak evidence of the existence of a hormonal form of control by the axis over the cotyledons, several workers have tested the response of detached cotyledons to exogenously applied plant growth regulators. In pea cotyledons, amylase is produced at a greater rate in response to 1 mM GA$_3$ or kinitin or 10 mM Zeatin, but not to 100 mM GA$_3$ (Varner et al., 1963; Sprent, 1968; Locker and Ilan, 1975). In peas, however, the appearance of the GA$_3$ in the cotyledons is not apparently dependent on the presence of the axis, the levels do not always correlate with increases in $\alpha$-amylase activity (Dale, 1969). Alternatively 1 mM ABA inhibits $\alpha$- and $\beta$-amylase production in peas and ABA like inhibitor activity declines after germination (Yomo and Varner, 1973). In green bean cotyledons, where removal of the axis delays amylase production by a half to two days, the lag can be abolished by 10 mM zeatin or 100 mM kinitin. Also $\alpha$-amylase production in intact seeds appears to be associated with high endogenous cytokinin-like activity (Gepstein and Ilan, 1970, 1979; Van Onckelen et al., 1977). There is the possibility in experiments with detached cotyledons that exogenously applied growth regulators may induce artefacts, e.g. by promoting cotyledon expansion as cytokinins are known to do, and thus creating new internal sinks for reserve breakdown products (Bewley and Black, 1978, 1994).

**Regulation of Protein Degradation**

Proteolytic cleavage plays an important role in storage protein deposition and reactivation in seeds (Muntz, 1996). During seed germination, hydrolysis of seed storage proteins provides amino acids for protein synthesis.
in the growing seedling. Protein biosynthesis and degradation represent important factors in the regulation of nitrogen sink/source relationships which are controlled by developmental as well as environmental factors, such as the storage and reactivation of vegetative storage proteins in soybean (Staswick, 1990, 1994) or storage proteins in seeds (Muntz, 1994).

The degradation of storage proteins is a controlled process with structures of proteins undergoing modification during germination (Basha and Beevers, 1976). The role of different proteases in the exact sequence of reserve protein breakdown and in the control mechanism is not very clear. It is also not certain whether the mechanisms proposed have universal applicability covering all legumes since the extensively studied systems are few.

Proteases in barley grains are synthesized primarily in aleurone grain, and the synthesis is regulated by GA₃ and ABA (Rogers et al., 1985; Koehler and Ho 1990b; Watanabe et al., 1991, Cejudo et al., 1992; Martila et al., 1993). By contrast, most dicot seeds such as non-endospermic legume seeds contain reserve proteins in the cotyledons, which are part of the embryo. Upon germination, the proteins are degraded and mobilized to the embryonic axis to support its growth (Bewley and Black, 1994; Vitale and Bollini, 1995). However, the role of the embryo or embryonic axis in the control of food mobilization in seeds of dicotyledonous plants is less well understood. Studies on proteolytic enzymes have provided more consistent results and indicate that higher activities develop in cotyledons when the embryonic axis is attached (Penner and Ashton, 1967; Yomo and Varner, 1973; Tsay and Ashton, 1974). The mobilization of storage proteins in cotyledons was shown to be depressed upon removal of the axis in a number of dicotyledonous species such as pea (Chin et al., 1972), Vigna species (Kern and Chrispeels, 1978, Minamikawa, 1979; Taneyama et al., 1996) and cucumber (Davies and Chapman, 1979,
The regulatory role of the embryonic axis in the proteolysis of storage proteins has been studied well only in monocotyledonous plants (Jones, 1985).

In dicotyledonous plants the mobilization of food reserves is much more complex. Practically in all studies the embryonic axis found to affect proteolysis of storage proteins. However, mechanisms of this effect turned out to be different. In the case of mobilization of storage proteins in sunflower cotyledons, chick pea and castor bean endosperm, the effect produced by the embryonic axis could be replaced by exogenous cytokinins (Allen et al., 1984; Pino et al., 1991) and gibberellins (Gilford et al., 1984). These hormones stimulated or induced the proteolytic activity (Penner and Ashton, 1967; Sze and Ashton, 1971; Tsay and Ashton, 1974). In excised cotyledons of pea (Yomo and Varner, 1973), French bean (Yomo and Srinivasan, 1973) and cucumber (Davies and Chapman, 1980). The inhibition of storage protein hydrolysis is related to accumulation of the hydrolysed products.

Protein breakdown in cucumber cotyledons is inhibited in the absence of axis eventhough proteolytic activity develops normally. Axis excision further results in the accumulation of end products (aminoacids) in the cotyledons suggesting the operation of a sink effect (Davies and Chapman, 1979 a, b). The overall conclusion of this work was that control of cotyledonary reserve (protein) mobilization in cucumber is mediated through the presence of an axial sink and that the mobilization enzymes are able to develop fully in the absence of axis (Chapman and Davies, 1983; Chapman and Galleschi, 1985). The exogenously applied gibberellins play an essential role in the inducible synthesis of proteases in isolated aleurone layers of cereal plants (Hooley, 1994). By contrast, exogenously applied GA\textsubscript{3} was not a prerequisite for increase in the protease activity in detached cotyledons of the seeds of V. mungo, exogenous GA\textsubscript{3} was effective only in doubling the activity in the cotyledons (Taneyama et
The endopeptidase activity in the cotyledons fell when germinating seeds well allowed to absorb a solution of amino acids at high concentrations and it was postulated that this effect might have been caused partly by osmotic stress and partly by end-product repression (Taneyama et al., 1996). Dunaevsky and Belozersky (1993) found that the ABA (10-100 mM) was without effect on modification of the 13S globulin, but suppressed the complete proteolysis of the protein by inhibiting, apparently the synthesis of the cysteine proteinase in the growing seedlings.

From the data currently available, it can be concluded that the embryonic axis has an important role to play in the control of food reserve mobilization in seeds of dicotyledonous plants (Davies and Slack, 1981; Halmer, 1985; Bewley and Black, 1978, 1994; Muntz, 1996; Yu et al., 1996).

Cytokinins, gibberellins, auxins emanating from the embryonic axis act as regulators of food reserve breakdown in certain dicotyledonous seeds though no direct control has been attributed for any growth substance with convincing experimental evidences. There is now evidence that food mobilization atleast in certain cases can be controlled by a simple source-sink relationship between the storage tissues and the growing embryo or embryonic axis. However, a single mechanism cannot be proposed for dicotyledons at present while some species exhibit a hormonal control mechanism others may operate with a sink mechanism and others both, evidently indicating the need for further work.