CHAPTER I

INTRODUCTION

INTRODUCTION

The independence of the next generation of a plant begins with the seed formation. The seed, in fact, contains the remnants of the parental generation and promises of the future generation. The seed containing the new plant in miniature, is equipped with structural and physiological devices to fit it for its role as a dispersal unit and is well-provided with food reserves to sustain the young plant until a self-sufficient, autotrophic organism can be established. The carefully planned events during the formation of seeds, the events that follow during development, the structural adaptations to withstand the adversities of nature and the innate control mechanisms operating during germination make the seed an unique phase in the life of the plant. Seed formation and development has received an enormous amount of attention from anatomical, morphological and biochemical stand point (Joshi et al, 1967; Millerd et al, 1971; Millerd and Whitfeld, 1973; Dure, 1975).

Storage of food reserves

Seeds characteristically contain large amounts of food reserves. These reserves are for the most part, but not exclusively, laid down as discrete, intracellular bodies and include lipid, protein, carbohydrate, organic phosphate and various inorganic compounds.

The seeds differ considerably in their content of food reserves. Carbohydrates, mainly starch predominate in the cereals and Graminaceous plants, though protein and lipids are also present. A large proportion of seeds store lipids as their main reserve, but again, substantial amounts of other reserves, especially proteins are present too. High levels of protein, together with even higher amounts of starch but little lipid, are found in a third group of seeds which includes many legumes such as peas and beans.

Reserve materials may be stored in the embryo or in extra embryonic tissues or both. In many species, for example legumes, reserve materials are stored in bulky cotyledons. Unusually the embryonic axis itself is the major storage region as in the Brazil nut. Most of the reserves of cereals and other grasses are located in the endosperm.

Carbohydrates

The principal, most wide spread storage carbohydrate of seeds is starch, which is laid down in discrete subcellular bodies, the starch grains. Rarely sugars are the predominant storage carbohydrate. This is the case in Acer saccharum which is reported to contain sucrose and other sugars but no starch (Crocker and Barton, 1957). Many legumes (e.g. Pisum sativum) have sucrose, raffinose and stachyose in their embryonic axis. Hemicelluloses are often found as reserves

in seeds. These hemicelluloses are laid down in the cell walls. Galactomannans are present as main reserve carbohydrate especially in the endosperm of leguminoseae (McCleary and Matheson, 1974). Glucomannans occur as reserve material in certain other seeds.

Lipids

The predominant storage lipids of seeds are the neutral fats or oils, depending on the relative amount of saturated or unsaturated fatty acids occurring in the triglycerides. These triglyceride reserves are laid down in the form of discrete subcellular organelles, the oil bodies.

Proteins

The proteins in the seeds are of two types : metabolic proteins, both enzymatic and structural, which are concerned in normal cellular activities including the synthesis of the second type, the storage proteins. Storage proteins occur within the cell in discrete protein bodies and vary according to the species. Thus, in wheat, at least four different proteins occur, glutelins, prolamins, globulins and albumins. Glutelins and prolamins form the major component of the protein. In dicotyledonous plants, prolamins seem to be almost absent. Glutelins are sometimes absent and sometimes constitute upto 50% of the total proteins in the dicotyledonous seeds. Albumins and globulins in these seeds are usually

well-defined. The globulins from various seeds are divided into two fractions, legumin and vicilin. Both the proteins have been shown to be made up of subunits.

Some of the proteins in several species of seeds act as lectins which are glycoproteins. About 2.5 percent of protein in jack bean is the lectin concanavalin-A and 1.5 percent of soybean seed protein is an agglutinin (Callow, 1975).

Storage proteins of legumes - legumin and vicilin show a similar amino acid composition (Pusztai and Watt, 1970; Bailey and Boulter, 1972; Ericson and Chrispeels, 1973; Derbyshire et al, 1976). An important common characteristic is the comparatively high level of the amides aspargine and glutamine and the amino acid arginine. Vicilin, however, lacks or has a very low content of cysteine.

The prolamins of cereals are high in proline and amides-glutamine and aspargine, and have low contents of tryptophan, lysine and methionine. The globulins of certain cereals e.g. barley, are similar in composition to other seed globulins i.e. they contain high amount of arginine (Beevers, 1976).

Other nitrogenous seed reserves

Many non-protein nitrogenous substances such as alkaloids, free amino acids and amides occur in seeds. Some of the free amino acids are the ones which are also present in proteins,

including glutamine and aspargine, but in addition some non-protein amino acids are found in certain species. Among these are γ-methylene glutamic acid, ϒ-amino butyric acid, lathyrine, canavanine, hydroxytryptophan, dihydroxyphenyl-alanine and many others. Some of these are restricted to a single species, while others are of very wide spread distribution among many species (Bell, 1966; Fowden, 1970).

Phytin

Phytin, the insoluble mixed potassium, magnesium and calcium salt of phytic acid is the major storage form of phosphate and macronutrient mineral elements in seeds. It is invariably present within a globoid in protein bodies (Lui and Altschul, 1967; Ory and Henningsen, 1969; Suvorov et al, 1970; Hofsten, 1973; Tanaka et al, 1973; Lott, 1975; Van Staden and Comins, 1976). In cereal grains it is associated with the aleurone grains in the aleurone layer and is more or less absent from the protein bodies of the starchy endosperm.

Factors controlling accumulation of reserve materials during development of seed

Reserve materials in the seeds are deposited in a number of sequential stages, namely the formation of reserve organs and the entrance of assimilates into them, the conversion of transportable forms of assimilates into the reserve material and the creation of conditions which facilitates their temporary metabolic passivity.

Accumulation of starch in wheat grain is not much regulated by the supply of sucrose from the mother plant but by the process operating within the grain itself. (Jenner and Rathjen, 1972; 1975; Jenner, 1974). Similarly the decline in the starch accumulation at the onset of ripening is due to the fall in the capacity of the grain to utilize the assimilate and not due to reduction or restriction in its supply to the grain (Jenner and Rathjen, 1975). Genetic and environmental factors can cause considerable variation in the fatty acid content in seeds (Appelqvist, 1975). Alterations in temperature at the time of seed development may also cause considerable changes in fatty acid proportions (Canvin, 1965).

Presence of gibberellic acid, kinetin, IAA and abscisic acid in developing and mature seeds suggests that these hormones may be involved in control processes during development and maturation. Recently Bewley and Black (1978) have suggested that the role of these hormones can be catagorised in four groups.

(1) They accumulate in preparation for their subsequent participation in the control of germination and early seedling growth.

(2) They control fruit growth and development, this is clearly the case in many fleshy fruits (Nitsch, 1971), but is exemplified also by Pisum sativum in which the growth rate

of the ovary wall closely relates to changes in hormonal content of the seeds (Eeuwens and Schwabe, 1975).

(3) Movement of dry matter (carbohydrates and nitrogenous substances) into the seed may be regulated by the hormones contained therein.

(4) The growth and development of the seeds themselves may be under hormonal control. The diauxic growth curve of developing seeds of the field pea accompanies changes in the extractable cytokinin content (Burrows and Carr, 1970). Peaks in cytokinin level coincide with times of maximal development of endosperm and the two periods of rapid growth of the embryo. In Pisum sativum, a fairly close correlation exists between the phases of maximum growth of the seed and the levels of auxin and gibberellin in endosperm and embryo (Eeuwens and Schwabe, 1975). Moreover, the decline in the growth rate of the seed accompanies the build up of abscisic acid.

Accumulation of proteins during development

Storage proteins, together with reserves of carbohydrates or lipids, are synthesized during seed development. In legume seeds the cotyledons form the bulk of the seed and synthesize most of the protein. In the developing cotyledon there are two phases of growth, an initial one of intensive cell division, followed by a longer period of growth by cell

expansion (Pate, 1975). During the early stages of cell expansion in non-endospermic legumes (e.g. peas and beans) the endosperm is occluded. In broad bean, field and garden pea seeds the majority of the stored reserves are laid down during the cell expansion phase, although both starch deposition and reserve protein synthesis in these seeds commence prior to the cessation of cell division. Major proportion of protein is synthesized during expansion growth (Briarty et al, 1969; Millerd et al, 1971; Smith, 1973; Millerd and Spencer, 1974).

Studies on developing field pea (Flinn and Pate, 1968) have shown that 23 percent of the total nitrogen of the seeds can be furnished from metabolites stored in the pod, endosperm and seed coats. The principle amino acids - serine, glycine and alanine are derived from photosynthesis, and these along with the amides aspargine and glutamine make up the bulk of the amino acids transported to the seed (Lewis and Pate, 1973). The synthesis of other amino acids found in storage proteins occur within the developing seeds themselves. Presumably the amino group of the amides from the phloem sap serves as the nitrogenous component for the amino acids, and the carbon skeletons are furnished by the translocated carbohydrates. A similar pattern of carbon and nitrogen availability has been reported for the broad bean (Kipps and Boulter, 1974).

Millerd and Spencer (1974) showed that vicilin synthesis in the garden pea precedes that of legumin and synthesis of the albumin fraction commences even earlier (Beevers and Poulson, 1972).

Protein synthesis during expansion phase of growth is associated with a simultaneous and continuous increase in the DNA and RNA content of the cotyledons (Millerd and Spencer, 1974). Synthesis of RNA is not unexpected because the expanding cells are actively involved in the large scale production of protein, both storage and metabolic.

Control of protein synthesis in developing seeds

Nutrition can exert a dramatic effect on both the amount of particular storage proteins synthesized and on the subunit composition. For example, when Lupinus angustifolius was grown under conditions of sulphur deficiency, there was an almost total absence of conglutin α and γ. The subunit composition of conglutin β was dramatically altered and bands which were normally minor components were now present in major amounts (Millerd, 1975).

Several studies have been carried out to correlate the levels of growth hormones and seed development (Skene and Carr, 1961; Ogawa, 1963; Hashimoto and Rappaport, 1966; Burros and Carr, 1970) but their significance, if any, in protein biosynthesis is unknown.

At the molecular level, the potential opportunities for controls and limitations are indeed inspiring. At present nothing is known about the lifetimes of mRNAs for storage proteins, if these were short, it would suggest that regulation at the transcriptional level could be important. The variability of subunit composition of storage proteins, especially in relation to environmental influences such as nutrition, suggests an involvement of translational control.

Germination of seed

Seed germination can be regarded as the consecutive number of steps which causes a dormant seed with a low water content, to show a rise in its general metabolic activity and to initiate the formation of seedling from the embryo. The process of seed germination is identified as the protrusion of some part of the embryo from the seed coat. In many seeds, radicle pierces the seed coat first and therefore, germination is equated with root protrusion. However, in some seeds, it is shoot which protrudes first, for example Salsola (Mayer and Poljakoff-Mayber, 1975). The piercing of the seed coat by the embryo could be caused by cell division and/or cell elongation.

The radicle elongation appears to occur in two phases : an initial phase of slowly accelerating elongation, followed

by a more rapid one (Bewley and Black, 1978). No dry weight changes occur during the first phase but mobilization of reserves and translocation of catabolites to the axis is characteristic of the second phase. Such a biphasic mode of radicle elongation has been reported for barley and peas (Brown, 1972) and Vicia seeds (Rogan and Simon, 1975).

Factors controlling germination

There are many environmental factors which exert marked effects upon germination. Most important of these external influences are water, temperature, gases and light. The profound effects of the environment on germination behaviour is brought about in two ways. The environment prevailing during seed formation as well as the location of seeds on the parent plant affect subsequent germination behaviour (Belderok, 1965; Come, 1970; Datta et al, 1970, 1972; Austin, 1972). However, in no case information is available about the mechanism by which germination behaviour and dormancy are determined by conditions prevailing during seed formation and ripening. Same consideration also applies to the direct effect of the environment during germination on germination behaviour. Such effects have been profusely documented by several investigators (Koller et al, 1962; Come, 1970; Mayer and Poljakoff-Mayber, 1975).

The seed coats have a profound influence on the ability of many seeds to germinate (Wareing, 1969; Come, 1970; Wareing and Saunders, 1971). The coats may regulate germination by establishing a permeability barrier and interfering with the processes such as uptake of water required for imbibition and subsequent radicle protrusion, gaseous exchange, particularly oxygen uptake required for respiration and other oxidative processes and outward diffusion of endogenous germination inhibitors. Seed coats may also offer mechanical resistance to the growth of the embryo.

Various internal factors, which influence germination are the completion of the dormant period, the age of the seed, the amount of growth regulating substances present and the amount of stored food available.

Role of hormones and growth substances in control of germination

A large number of findings demonstrating the influence of a factor emanating from the embryo on the development of enzymic activities in the cotyledons or endosperm and vice versa, have led some workers to conclude that this control is hormonal in nature. Protein metabolism in pea seed cotyledons has been shown to be controlled by an axial component (Chin et al, 1972). The development of proteolytic activity in squash cotyledons is controlled by a substance which originates

in axial tissue and is transported to the cotyledons (Penner and Ashton, 1966; Wiley and Ashton, 1967). The presence of axis could be substituted for by the external application of cytokinins (Penner and Ashton, 1967b), and it was concluded that the axis possibly may secrete a cytokinin that regulates the formation of proteolytic enzymes in the cotyledon. The same type of axial regulation was also demonstrated for the synthesis of isocitrate lyase in squash cotyledons (Penner and Ashton, 1967a). Cytokinins which may originate in the axis appeared to control amylase activity in Phaseolus vulgaris cotyledons (Gepstain and Ilan, 1970). The induction of lipase activity in the storage tissues of germinating wheat grains was found to be dependent on some factors originating from the embryo. The metabolism of triglycerides in this tissue is apparently under hormonal control (Eastwood et al, 1969; Laidman and Tavener, 1971). Thus the regulation of various enzymatic activities is under the influence of the embryonic axis, and in many cases the exogenous application of hormones may reflect the natural control of germination (Khan, 1971).

The role of gibberellic acid in the formation and secretion of a great number of enzymes is the most thoroughly documented case of an endogenously produced hormone which controls seed metabolism (Varner and Chandra, 1964; Varner et al, 1965; Taiz and Jones, 1970).

IAA can, under very special conditions, stimulate germination but normally it has little or no effect. For example, in lettuce seeds IAA raised the germination from 4-10% in dark to 20-30% but in light IAA had no effect. Similarly, at 20°C germination was increased from 27 to 47% but at 26°C IAA had no effect (Poljakoff-Mayber, 1958).

The physiology and relation of endogenous abscisins to seed dormancy and germination have been reviewed extensively (Addicott and Lyon, 1969; Wareing and Saunders, 1971). Abscisins exist in a large number of seeds and tissues surrounding seeds. Many seeds will not germinate in the presence of abscisins (Aspinall et al, 1967; Sumner and Lyon, 1967; Taylor and Smith, 1967; Dey and Sircar, 1968; Dorffling, 1970). Exogenously applied abscisins inhibit or interfere with the production of certain species of RNA in isolated peas and Fraxinus embryos (Villers, 1968; Khan and Anojulu, 1970), although in isolated bean embryos this effect was shown to occur well after elongation commenced. Karssen (1968) suggested that abscisic acid prevents embryo elongation and hence prevents protrusion through the surrounding layers. However, as in other cases, the continuous presence of abscisic acid appeared to be required. Interaction between gibberellic acid and abscisic acid have also been suggested in Acer pseudoplantanus (Pinfield and Stobart, 1972). In a

more general way promotor inhibitor ratios have been involved in the control of germination and dormancy breaking in Ambrosia (Willemsen and Rice, 1972).

Ethylene is another growth regulator produced by seeds (Stewart and Freebairn, 1969; Takayanagi and Harrington,1971). Ethylene production commences shortly after the start of imbibition and precedes radicle emergence (Mayer and Shain, 1974). Some workers assign the main role of this endogenous ethylene production to the breaking of dormancy (Ketring and Morgan, 1969), while others claim that it may play a role in the initial steps of germination (Abeles and Lonski, 1969; Stewart and Freebairn, 1969) or in enhancing the growth rate after germination (Takayanagi and Harrington, 1971). Ketring and Morgan (1970, 1971) observed that compounds such as gibberellic acid, cytokinins and to a lesser extent coumarin increased both ethylene production and seed germination.

Another compound which may be regarded as a hormonal regulator of germination is coumarin. Some of the work on the effect of coumarin on germination and its metabolism during the process has been reviewed by Van Sumere et al. 1972).

Mobilization of reserve material during germination

The metabolic activity of seed begins during imbibition and increase during the subsequent germination. Storage

materials are broken down and a part of the breakdown products are transported from the cotyledons or endosperm to the developing axis of the embryo. This series of events are well documented and some recent reviews of these sequences have appeared (Ching, 1972; Mayer and Shain, 1974; Mayer and Poljakoff-Mayber, 1975; Ashton, 1976).

The changes which occur in the composition of fats and free fatty acids during germination have been studied in various seeds (Boatman and Crombie, 1958; Hardman and Crombie, 1958). The breakdown of lipids is essentially a three step process : The hydrolysis of triglycerides, β-oxidation of the released fatty acids and channeling of the 2-carbon unit into oxidative processes - or conversion to other compounds via the glyoxylate cycle. The details of these processes are summarized in a number of reviews (Mayer and Shain, 1974; Mayer and Poljakoff-Mayber, 1975; Khan, 1977; Bewley and Black, 1978).

Carbohydrates are broken down during the early stages of germination. Mobilization of starch reserves in intact cotyledons commences after the radicle has started to elongate (Bewley and Black, 1978). The transport of carbohydrates from storage organs to the growing part of the embryo is evident during germination. This differs from seed to seed according to the nature of storage material and the structure of the seed (Koller et al, 1962).

The sugars appearing during germination can apparently be divided into two groups, those which are found as readily available substrates for respiration other than that derived from hydrolysis of the major stored reserves i.e. sucrose, raffinose and stachyose and those which are found to be the results of starch breakdown i.e. glucose, and maltose (McLeod et al, 1953; McLeod, 1957). These workers have shown that raffinose present in barley embryo is very rapidly metabolized under aerobic conditions during the first 24 hrs of germination. Similar results have been obtained with rice (Ito and Takenaga, 1958).

The enzymes involved in breakdown of starch in cereals have been reviewed by Marshall (1972). Although various amylases are no doubt involved in starch degradation, it is likely that phosphorylases are also capable of and involved in degradation of starch. The presence of starch phosphorylases in many seeds is well-established (Stumpf, 1952). Juliano and Varner (1969) have reported increase in activities of α- and β-amylases and phosphorylases during pea seed germination. Mobilization of hemicelluloses of Pisum, Phaseolus and Lupinus species has been reported although the products of hydrolysis are largely unknown (Bewley and Black, 1978). Galactomannan utilization in a number of leguminous seeds has been followed by McCleary and Matheson (1974,1976).

Galactomannans are completely broken down during the germination of seeds of Trigonella (Reid, 1971). Products of galactomannan break down are absorbed by cotyledons and after their conversion to sucrose, are transported to growing axis.

Protein, which is a frequent reserve material in seeds is broken down during germination with a concomitant rise in amino acids and amides followed by de novo protein synthesis in the growing part of the embryo (Oota et al, 1953). The first observable change during germination is therefore, often a change in the ratio of proteins to soluble nitrogen as shown in lettuce (Koller et al, 1962), pea seeds (Beevers, 1968), Phaseolus vulgaris (Yomo and Srinivasen, 1973) and pumpkin seeds (Reilly et al, 1978). Prior to major mobilization, the stored legumin and vicilin of pea seeds undergo some alterations in composition, although this does not appear to involve deamination or decarboxylation (Basha and Beevers, 1975). In contrast, an early feature of protein utilization in peanuts and soybeans is thought to be the provision of ammonia to the developing seedling by the removal of amide groups from cotyledon storage proteins by the action of deaminases (Catsimpoolas et al, 1968; Daussant et al, 1969). Removal of the sugar components from pea vicilin and legumin appears to occur after cleavage of the peptide links has commenced and the glycosyl units are then released as complete oligosaccharides (Basha and Beevers, 1976).

Studies on breakdown of reserve proteins of pea seeds (Basha and Beevers, 1975, 1976) have shown that at first the breakdown was slow and depletion of globulins exceeded that of albumins. Moreover, legumin was degraded a little more rapidly than vicilin. In Cicer arietinum the globulins present in cotyledons are degraded, while the concentration of albumin remains constant. However, changes in the nature of albumins also occur (Radionova, 1957, 1960). Similarly in Phaseolus vulgaris, globulins and basic proteins are hydrolyzed rapidly during germination, while albumin remain unchanged (Juo and Stotzky, 1970). Lichtenfeld et al (1979) have shown that in germinating seeds of Vicia faba legumin degradation commenced previous to the breakdown of vicilin.

The presence of proteases and peptidases have been shown in many seeds (Poljakoff-Mayber, 1953; Tazawa and Hirokawa, 1956; Kudryashova, 1960; Shain and Mayer, 1965; Ashton and Dahmen, 1967a,b; Wiley and Ashton, 1967; Beevers, 1968; Garg and Virupaksha, 1970; Mikola and Kolehmainen, 1972; Mikola, 1976; Tully and Beevers, 1978; Lichtenfeld et al, 1979). In mung bean cotyledons endopeptidase activity increases very sharply during germination (Chrispeels and Boulter, 1975) and the enzyme was formed by de novo synthesis (Chrispeels et al, 1976). It is suggested that the development of endopeptidase activity is a prerequisite for massive protein breakdown. The significance of endopeptidases in protein breakdown of the

maize endosperm has been shown by Harvey and Oaks (1974a,b). Both glutelin and zein were broekn down and the endopeptidase was found to be synthesized during germination. Of course, other peptidases such as carboxypeptidases are present and are of importance in germinating seeds (Mikola and Kolehmainen, 1972). Mikola (1976) proposes that in Arachis and perhaps all leguminous seeds endopeptidases are important in protein breakdown, while in starchy seeds carboxypeptidases are important.

Proteinase inhibitors are present in the seeds of many plant species. Their exact function in the germination process is still debatable. They may act by regulating proteolytic enzyme activity during germination or they may be simply a relic from the period of seed development, when they may have prevented the decomposition of newly formed storage proteins (Horiguchi and Kitagishi, 1971).

Large amount of phytin present in seeds may be regarded as a store of inorganic phosphate which is liberated as germination proceeds. Phytase hydrolyzes phytin to release phosphate and myoinositol. It has been shown that the enzymes required for phytin formation are also present in dry seed and synthesis occur during seed germination (Lahiri-Majumder et al, 1972).

Studies by Sugiura and Sunobe (1962) on Vigna seeds have shown that phytase activity increases in the cotyledons from the time of imbibition, and reaches a peak of activity on 3rd day but no significant phytase activity can be detected in the seedling axis. The enzyme is widely distributed in other dicotyledonous plants and the activity increases following germination (Mayer and Poojakoff-Mayber, 1975). Myo-inositol derived from phytin may be of importance to the growing seedling, since it is a known precursor of all pentosyl and uranosyl sugar units normally associated with pectin and certain other polysaccharides present in the cell wall (Roberts et al, 1968).

In addition to phytase, seeds contain many phosphatases, and their activity also rises during germination (Mayer and Poljakoff-Mayber, 1975).

Differences in patterns of mobilization of proteins and amino acids from cotyledons and axis.

Isolated axes of peas are not dependent on the reserve material of the cotyledons during the initial stages of radicle elongation (Bain and Mercer, 1966). Reserves of carbohydrate, protein and fat in the radicle itself must be sufficient for these early events. But after these early events have passed, the further development of root and shoot systems depends upon the contributions from the cotyledons. A number of workers (Goksöyer et al, 1953; Oota et al, 1953;

Lawrence et al, 1959; Ingle et al, 1964; Wiley and Ashton, 1967) have reported changes in protein and non-protein nitrogen in axial tissue and storage tissue as seed germination proceeded. In cotyledons of Cucurbita maxima protein nitrogen decreases, whereas non-protein nitrogen increases with germination. But in axial tissue both protein as well as non-protein nitrogen increase with germination (Wiley and Ashton, 1967).

Larson and Beevers (1965) have studied the changes in protein and amino acid content in the cotyledons and axis of dark grown Alaska pea seedlings. Protein content of cotyledons decreases whereas that of axis increases with germination. Dry pea cotyledons contain few free amino acids but their content increases following germination, at the time when the protein reserves are being mobilized. Some of these amino acids are reutilized in protein synthesis in situ, and protein turnover has also been demonstrated in cotyledons even when they are senescing. However, the majority of the amino acids are translocated via the phloem, to the growing axis, and the amino acid pool in the cotyledons is gradually depleted. This is accompanied by an increase in the level of free amino acid pool in the growing shoot.

Splittstoesser (1969a) has studied the incorporation of labelled arginine in cotyledon as well as axial proteins. The amounts of arginine incorporated into cotyledon proteins

show that synthesis and turnover were occurring at a rapid rate, whereas the label in protein was stable in the axis tissue.

Importance of arginine during germination

The presence of arginine in large quantities in storage proteins of seeds is thought to be important because of its high nitrogen content due to guanidino group. Arginine, being more nitrogen rich than most other amino acids will on breakdown, liberate relatively more nitrogen that can be recycled to form other nitrogen containing compounds including amino acids and proteins during germination (Jones and Boulter, 1968).

There is now much evidence that the Krebs-Henseleit urea cycle (Fig. 1) is operative in plants also. All the intermediates and the enzymes which cause their interconversions have been found in seed plants. In wheat seedling extracts enzymes are active which catalyze arginine synthesis from citrulline and aspartic acid in the presence of ATP (Fotyma et al, 1961). In bean seedlings citrulline is synthesized from ornithine and carbamyl phosphate in both intact and homogenized leaves and in cotyledons (Kleczkowski, 1957, 1958). The acetone powder of green pea seeds contained an enzyme forming citrulline from ornithine and carbamyl phosphate (Kleczkowski, 1958). In various parts of green pea seedlings fed with ornithine, a significant synthesis of citrulline and arginine has been observed (Kleczkowski and Reifer, 1959).

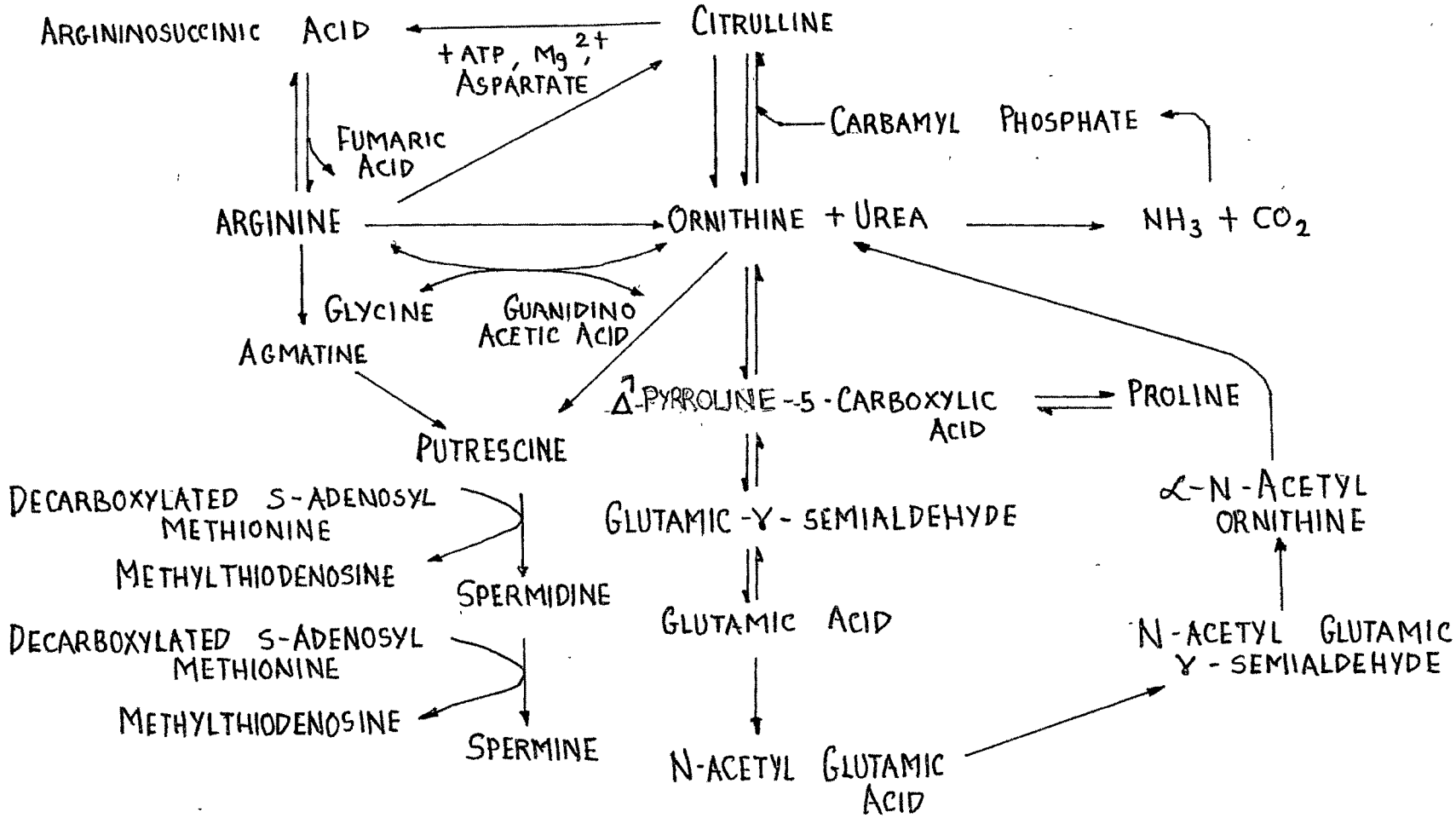

FIGURE-1 METABOLISM OF ARGININE

The addition of carbamyl phosphate did not influence the synthesis of these compounds suggesting an active carbamyl phosphate synthetase in undamaged organs of these plants. Sterile shoots of the green plant without cotyledons and seedlings of perennial ryegrass (Lolium perenne) catalyze the complete process of urea cycle (Reifer and Buraczewska, 1958; 1959). The complete metabolic sequence of urea cycle has been demonstrated in water melon seedlings (Kasting and Delwiche, 1958). Shargool and Cossins (1968, 1969) have demonstrated the presence of argininosuccinate synthetase and argininosuccinate lyase in the cotyledons of germinating pea seeds. From their studies on Vicia faba seeds, Kollöffel and Van Dijke (1975) have suggested that urea cycle is operative in these seeds. Citharel and Citharel (1977) have shown that all the reactions of urea cycle are operative in the conifer aleppo pine. Roubelakis and Kliewer (1978a;b;c) have demonstrated the presence of enzymes of Krebs-Henseleit cycle in Vitis vinifera.

Physiological role of urea cycle in plants

In animals, the urea cycle is directed to the production of urea, but in plants different intermediates may be taken out of the cycle at different stages to be metabolized via other pathways. For example, ornithine may be converted to proline and glutamic acid and urea by the action of urease to ammonia, which may be taken up by glutamic acid to form glutamine (Elliott, 1955) or the ammonia may be taken up by

α-oxo acid to form glutamic acid and possibly other amino acids (Kagan et al, 1966). Arginine itself acts as a storage amino acid for nitrogen and may be incorporated into storage proteins. Thus, although urea cycle operates in the plants, it may not be such a "self contained" cycle as in animals, for the intermediates of the cycle may form a link between other different pathways and be constantly siphoned off.

Changes in arginine levels and urea cycle enzymes during germination of seeds.

Few details are available on changes in arginine levels during germination of seeds. Jones and Boulter (1968) estimated the arginine content of germinating seeds of Vicia faba, Canavalia ensiformis, Phaseolus vulgaris and Pisum sativum. There was a decrease in the total arginine content of seeds and seedlings over the first 14 days of germination, but an increase in the free form of arginine over that period in all the species, except Vicia faba, where there was a decrease in the free form also particularly between the 7th and 14th day. In Canavalia ensiformis and Vicia faba the drop in arginine content continued over the 14 days studied, while in the other species the arginine concentration started to increase again.

In pumpkin cotyledons (Splittstoesser, 1969b) concentration of free arginine of light and dark grown plants increased

as germination progressed. In the light grown plants, the maximum arginine concentration was reached 9 days after germination, whereas in the dark grown plants the same was obtained 15 days after germination. The cotyledons of dark grown plants contained considerably more arginine at all stages of germination. There was no significant amount of free arginine in the axis tissue of seedlings grown in either light or dark.

Przybylska (1959) described free arginine in bitter and non-bitter seeds of blue and white lupine. The arginine content of low alkaloid lupines was several times higher than in bitter ones. High arginine accumulation was found in sulfur deficient peanuts (Brzozowska and Hanower, 1967) suggesting an effect of mineral deficiency concurrent with arginine utilization. Observation on sulphur deficient flax plants (Coleman, 1958) and phosphorus deficient gourd plants (Chernyavskaya and Tueva, 1972) also support this suggestion.

All the enzymes of urea cycle have not been studied extensively during germination of seeds. The first enzyme of the cycle carbamyl phosphate synthetase has been shown to be present in 10-15 day old pea seedlings (O'Neal and Naylor, 1968). However, the pattern of changes in the activity of this enzyme during germination was not reported.

It has been suggested that in the cotyledons of germinating seeds which are actively degrading arginine, the activity of enzymes concerned with arginine biosynthesis are low (Kollöffel and Stroband, 1973; Kollöffel and Van Dijke, 1975). Thus in cotyledons of germinating Vicia faba seeds, ornithine carbamyl transferase (Kollöffel and Stroband, 1973), argininosuccinate synthetase and argininosuccinate lyase (Kollöffel and Van Dijke, 1975) decrease with the period of germination.

However, in germinating Sorghum vulgare seeds, maximum ornithine carbamyl transferase activity was obtained after 5 days of germination and then it remained constant over the period (upto 11 days) studied (Citharel and Dembele, 1972).

Both argininosuccinate synthetase and argininosuccinate lyase have been detected in germinating pea seedlings (Shargool and Cossins, 1968; Shargool, 1971). The importance of arginine in plant metabolism and the relationship of argininosuccinate lyase to arginine synthesis is especially evident during germination, for at the earliest stages of seedling development, there is a considerable production of arginine in the cotyledons of germinating peas (Lawrence and Grant, 1963; Larson and Beevers, 1965). In fact, this amino acid is the predominant free amino acid in 1 day old cotyledons, but its level steadily declines after this early

stage of germination (Lawrence and Grant, 1963). These changes correlate with the level of argininosuccinate lyase activity, which reaches its maximum at the same time and declines thereafter (Shargool and Cossins, 1968).

The last enzyme of the urea cycle, arginase, which is involved in arginine breakdown has been studied in detail during germination of seeds. Studies by Morawska et al (1963) on arginase activity in dry and germinating seeds and in young seedlings of 30 plant species belonging to 12 families showed that germinating seeds have invariably much higher arginase activity than dormant seeds. No arginase activity could be detected in sunflower and poppy seeds. Young spinach seedlings showed the highest arginase activity of all the plants investigated. They suggested that arginase activity may differ widely in seeds of plants belonging to the same family. Wielgat et al (1965) studied the activity of arginase in green peas and wheat. The enzyme activity increased during the period of germination and at the time of formation of generative organs.

In case of groundnuts, arginase activity appeared in young plants, especially in the cotyledons and stems and increased when number of leaves increased from two to three, then slowly decreased during growth (Brzozowska and Hanower, 1967).

An increase, both in soluble and particle bound form of arginase has been observed during germination of field bean (Dolichos lab lab) seedlings (Vaidyanathan and Giri, 1953). Arginase activity increased regularly during early stages of plantule development of Pinus halepensis, and then decreased (Citharel and Citharel, 1975).

Weak arginase activity was present in dry seedlings of Vicia faba and it increased during imbibition (Genevieve et al, 1967). Arginase was shown to be present in roots, shoots and cotyledons during first 6 days of germination (Jones and Boulter, 1968). But in both dry and soaked seeds arginase was higher in the radicles than in the cotyledons. This was reversed in 6 day old seedlings (Genevieve and Genevieve, 1967). Splittstoesser (1969b) showed that arginase activity in cotyledons of germinating pumpkin seeds increased with the period of germination and arginase was not detectable in the axis tissue of the seedlings grown in either light or dark.

In contrast to the general observation that arginase activity increases during seed germination, some of the gymnosperm seeds studied by Guitton (1957) showed decrease in arginase activity with germination.

Formation of arginine

Arginine formation from citrulline requires aspartate as well as ATP (Ratner, 1954, 1955, 1962) and the reaction

occurs in two steps involving the intermediate formation of a compound identified as argininosuccinic acid (Ratner, 1962; Davison and Elliott, 1952; Walker, 1953). Enzymatic cleavage of latter compound gives arginine and fumaric acid.

Very few reports on the presence of the condensing enzyme (argininosuccinate synthetase) in plants and seeds of higher plants have appeared (Shargool and Cossins, 1969; Shargool, 1973; Kollöffel and Van Dijke, 1975; Citharel and Citharel, 1977; Roubelakis and Kliewer, 1978b). It has not been possible to study this enzyme in plants because of the difficulties encountered in separating it from argininosuccinate lyase. However, the enzyme has been purified from germinating pea seeds successfully (Shargool, 1971).

Kleczkowski and Jolanta (1968) observed that in homogenates of wheat seedlings arginine was synthesized from L-carbamylaspartate and L-ornithine. This synthesis required neither ATP nor Mg^{++}. In this system the original argininosuccinate formation was probably catalyzed by a specific enzyme system, since the purified preparation catalyzing arginine formation from citrulline and aspartate did not catalyze this reaction. Acetone powder lost its activity for the new pathway after 10 days of storage, but for the classical pathway of arginine synthesis the same acetone powder retained full activity for 6-8 weeks.

Cleavage of argininosuccinate to arginine and fumaric acid is catalyzed by the enzyme argininosuccinate lyase. The presence of this enzyme has been reported in pea seeds (Davison and Elliott, 1952; Reifer and Buraczewska, 1958), wheat seedlings, rye grass and green peas (Buraczewski et al, 1960; Kleczkowski and Reifer, 1959), soybean cells grown in suspension culture (Shargool, 1973) and seeds of Vicia faba (Kollöffel and Van Dijke, 1975).

Formation and utilization of ornithine

Ornithine can be formed either from arginine or from glutamate. The biosynthesis of ornithine from glutamate proceeds via N-acetyl intermediates (Fig. 1). Studies on the biosynthesis of ornithine from glutamate in higher plants by Mckay and Shargool (1977) have shown that N-acetyl ornithine is a much better acetyl group donor to glutamate than is acetyl CoA and as in the case of green alga Chlamydomonas (Staub and Denes, 1966; Farago and Denes, 1967) N-acetyl-glutamokinase is the allosteric enzyme of the pathway, and its activity is regulated by the intracellular levels of arginine present.

Ornithine can be utilized for the formation of glutamate or proline or be recycled via citrulline for arginine formation. It may also be utilized for the synthesis of polyamines. The conversion of ornithine to glutamate and proline involves a

series of steps begining with transamination, which is brought about by ornithine keto acid aminotransferase (OKAT). (L-ornithine-2-oxoglutarate δ-aminotransferase). The equilibrium for this reaction was shown to be very far towards the right, presumably because of the spontaneous conversion of glutamic-γ-semialdehyde to the cyclic form. Δ^1-pyrroline-5-carboxylate (Meister, 1954; Strecker, 1960a,b), the reduction of which leads to the formation of proline.

A similar pathway for the formation of proline is apparently present in higher plants. After feeding ornithine -^{14}C to seedlings of several species good incorporation of the label into proline has been observed (Coleman and Hegarty, 1957; Brown and Fowden, 1966). The reduction of glutamic acid -^{14}C to labelled proline has been followed in some detail in feeding experiments with whole tobacco leaves (Mizusaki et al, 1964) and leaf discs (Noguchi et al, 1968). Transaminases which utilize L-ornithine as a substrate have been detected in pea, wheat (Kleczkowski and Kretovich, 1960), mung bean (Bone, 1959), sun flower (Smith, 1962), Datura stramonium tissue culture (Jindra and Staba, 1968),groundnut (Mazelis and Fowden, 1969), Papaver somniferum (Kovacs, 1970), Cucurbita pepo (Mazelis and Lu, 1971), Cucurbita maxima (Splittstoesser and Fowden, 1973) and in leaves of mulberry (Agadzhanyan and Davtyan, 1974).

Pyridoxal phosphate has invariably been shown to be the essential cofactor for OKAT (Vogel and Kopac, 1960; Katunuma et al, 1964; Wekell and Brown, 1973). Although the enzyme from Cucurbita pepo required pyridoxal phosphate for activity (Lu and Mazelis, 1975), the enzyme from groundnut cotyledons (Mazelis and Fowden, 1969) and Cucurbita maxima cotyledons (Splittstoesser and Fowden, 1973) was not stimulated by pyridoxal phosphate.

Studies by Mazelis and Fowden (1969) on proline biosynthesis in germinating groundnut cotyledons have shown that in the course of germinating groundnut (Arachis hypogea L.) seedlings, a large increase was observed in the amount of proline in the free amino acid pool. After 3 days at 30°C in the dark, proline was the dominant constituent. Extracts of acetone powders of the cotyledons contained an enzyme system which converted ornithine into proline. The specific activity of OKAT was shown to increase during germination and the increase continued upto the 6th day of germination. The acetone powder extracts of the 2 day old mung bean cotyledons, leaves of Polygonatum multiflorum, mature marrow fruits and cauliflower buds also exhibited OKAT activity.

Studies on Cucurbita maxima cotyledons (Splittstoesser and Fowden, 1973) showed that the enzyme activity increased during germination in both the particulate and the soluble fractions. The peak of activity occurred at 5 days of

germination in the soluble fraction and at 6 days of germination in the particulate fraction and the maximum activity in the particulate fraction was considerably greater than that in the soluble fraction. At 14 days both fractions contained similar levels of activity.

OKAT is the only enzyme demonstrated that catalyzes the reversible step in the metabolic pathway interconverting arginine, proline and glutamate (Volpe et al, 1969; Wekell and Brown, 1973; Kalita et al, 1976). Due to its ability to interconvert proline, glutamate and arginine in vivo, it has been postulated (Hill and Chambers, 1967; Lu and Mazelis, 1975) that OKAT plays a key role in metabolic processes and it may be involved in regulatory mechanisms.

However, recently Mestichelli et al (1979) have shown that the metabolic conversion of ornithine into proline in 3 plant species (Nicotiana tabaccum , Datura stramonium and Lupinus angustifolius) takes place with maintenance of δ-H but with the loss of α-H. This indicates a route via α-keto-δ-aminovaleric acid (δ-amino-2-oxopentanoic acid) and disproves the accepted route via glutamic-γ-semialdehyde.

The presence of the enzyme ornithine carbamyl transferase (OCT) catalysing the conversion of ornithine to citrulline has been reported in various plant tissues such as barley and white clover (Coleman and Hegarty, 1957), water melon seedlings (Kasting and Delwiche, 1958), mung bean (Bone, 1959), pond

pine roots and root tissue cultures of both pond and sand pine (Barnes and Naylor, 1959), green peas and wheat (Wielgat, et al 1965), Sorghum vulgare seedlings (Citharel and Dembele, 1972), Vicia faba (Kollöffel and Straband, 1973), senescing apple leaf tissue (Spencer and Titus, 1974), sugar cane cell suspension (Glenn and Maretzki, 1977) and Vitis vinifera (Roubelakis and Kliewer, 1978a).

The enzyme from mung bean (Bone, 1959) and Neurospora (Bernhardt and Davis, 1972; Weiss and Davis, 1973) is present in mitochondria and that from pea seedlings (Wielgat and Kleczkowski, 1970) and Vicia faba seeds (Kollöffel and Stroband, 1973) is shown to be localized in cytoplasmic fraction.

Two forms of this enzyme are distinguished in Pisum sativum (Waly and Abdelal, 1974), sugar cane cell suspensions (Glenn and Maretzki, 1977) and apple leaf tissue (Spencer and Titus, 1974).

In plants, there is some evidence for the two separate ornithine pools. Based on the labelling studies and on the presence of OKAT in plants, Oaks and Bidwell (1970) suggested the presence of two ornithine pools in plant materials : a pool contributing carbon to arginine and one pool contributing carbon to proline or glutamate. The presence of two such ornithine pools was shown more conclusively in studies with

wild type and auxotrophic mutants of Neurospora (Vogel and Bonner, 1954; Vogel and Kopac, 1959; Davis and Mora, 1968). The possible existence of two ornithine pools in plants (Naylor, 1959; Jones and Boulter, 1968; Oaks and Bidwell, 1970) together with the separation of two OCTS (Waly and Abdelal, 1974; Spencer and Titus, 1974; Glenn and Maretzki, 1977) suggest that in plants the enzymes play different roles.

Wielgat and Kleczkowski (1973) reported that purified preparation of OCT from pea seedlings catalyze carbamylation of putrescine. Studies by Wielgat and Kleczkowski (1971) showed that in normal plants, higher than 0.5 percent putrescine was found to be highly toxic and caused damage and death of the plants. N-carbamyl putrescine even at higher concentrations was not toxic for the plants. Based on these studies, they suggested that the only role which putrescine carbamylation can play in plant cells is the detoxication of the cell medium from large amounts of putrescine, which is highly toxic for plants. In this assumption the mechanism of putrescine carbamylation should be "Oscillatory". When a high concentration of putrescine is accumulated in the cell the mechanism of carbamylation starts. Successively after removal of the excess of putrescine through its conversion to non-toxic N-carbamyl putrescine, the reverse mechanism comes into play. N-carbamyl putrescine is again converted to putrescine, which is normally metabolized to γ-amino butyric

acid. Shevyakowa (1966) also showed that in Vicia faba putrescine is oxidatively deaminated to γ-amino butyric acid. However, Yoshida and Mitake (1966) have shown that in Nicotiana tabaccum ^{14}C-N-carbamyl putrescine is a good precursor for the synthesis of the pyrrolidine ring of nicotine.

The pea seedling OCT, beside the carbamylation of ornithine and putrescine, catalyzes additionally, but to a smaller extent, the carbamylation of agmatine, spermidine and spermine (Kleczkowski and Wielgat, 1968) as well as lysine (Kleczkowski, 1965; Jasiorowska and Kleczkowski, 1970).

From the foregoing it is therefore apparent that arginine metabolism plays an important role during development and germination of seeds. Though, several studies have been conducted on the presence of the enzymes involved in the synthesis and utilization of arginine in plants, no data is available on the nature of controls operating in this pathway. The enzymes arginase, OCT and OKAT appear to be of special significance from the point of metabolic regulation as they link arginine to glutamate and proline as well as to polyamines. Further, no study seems to have been conducted comparing the pattern of these enzymes in the cotyledon and embryonal axis where the same enzyme apparently displays different role and may possibly have a differential control. It was, therefore,

of interest to study these enzymes during development and germination of seeds with a view to locate the controls, if any. Groundnut (*Arachis hypogea* L.) seeds were selected for the present study since they are known to have a high reserve protein content with a high proportion of arginine.