REVIEW OF LITERATURE ON SOYBEAN SEED GERMINATION

1 INTRODUCTION

Pulses comprise a group of crop of family Fabaceae which help in maintaining and improving soil fertility. These crops are generally included in crop rotation programme with other cereal crops and have helped to keep soil productive. Soybean (*Glycine max* L.) occupy an important place in the dietary of the people of different nations and it is an excellent source of oil and protein. It is presently the world's most important grain legume in terms of total production and international trade. Soybean also serves as excellent forage crop for cattle.

Soybean having following names :

English	-	Soybean, Soya, Soy
Spanish	-	Soja, Soya, Planta gramineae
Dutch	-	Sojaboon
French	-	Soja
German	-	Sojabohne
Portuguese	-	Reijao, Soja

Botanical name - *Glycine max* (L.) Merrill.

The USA is the world's larger exporter and together with China and Brazil accounts for over 90% of the world production of soybean. Highest domestic consumption is in Asia, where it has been basic food for centuries. Due to its

PLATE I. Soybean (*Glycine max.* (L.) Merrill.) var. MACS-13 seeds

extensive utilization it is rightly reputed as 'Cow of China'. In India also soybean is becoming quite popular among farmers. Review of literature of soybean indicates that no systematic information is collected on different aspects of seed germination in this crop. Hence it is thought worthwhile to compile review of literature on different aspects of soybean seed germination.

1.1. SOYBEAN SEED MORPHOLOGY, ANATOMY AND CHEMICAL COMPOSITION

Mature soybean seed is (Plate 1) more or less spherical in shape with a thin testa or seedcoat and a small hilum. One hundred seed weight varies considerably in the range 5-40 g depending on variety. Seed colour shows wide variation and it can be yellow, red, brown, black, slightly mottled or occasionally bicoloured which depends on variety. In a few strains the cotyledon and seedcoat remain green even on maturity. Owen (1927) showed occurrence of two types of green seeds one due to complementary action of two recessive genes (d_1d_2) and other controlled by cytoplasmic factors, since green seeds give undesirable appearance to various soyabean products such varieties are not preferred. Pale yellow is the most commercially acceptable colour for soybeans intended for human consumption and oil production.

The hilum is linear to elliptical in shape and is located on ventral face of the seed. Colour of hilum varies

from colourless, buff, light brown, black to darkblack and could be considered as characteristic of a variety. However, intensity of hilum colour occasionally varies in a variety (Bhatnagar, 1994). The micropyle is a small hole in the seed-coat formed during seed development and is located at one end of the hilum. The hypocotyl radicle axis located above the micropyle ~~are~~ is sometime visible through the seedcoat. The bulk of the seed just below the seedcoat is occupied by two fleshy cotyledons and embryo. The embryo consist of short radicle, hypocotyl axis, and well developed plumule. The position of radicle and delicacy of testa makes the seed vulnerable to mechanical injury from any surface during harvest, threshing, processing and storage etc. (Bhatnagar, 1994).

The seedcoat is made of 8-10 layer of cells. The outer layer palisade cells is the epidermis, the hypodermis is the next layer of loosely packed cells, and finally the inner parenchyma tissue which is 6-8 layers of thin walled flattened cells (Dzikowski, 1936). Studies of McDonald et al. (1988) utilizing scanning electron microscope have documented that hard seeds (which tend not to hydrate) are a result of small, elongated seedcoat pores and a high density of waxy material embeded in the seedcoat epidermis. The seed colour is localized in the palisade cells of the epidermis (Egley et al. 1983). In addition to the palisade layer, the

soybean seed coat also consists of a layer of hourglass cells, and several layer of parenchymatous cells (Calero et al 1981; Pereira and Andrews 1985). It is believed that during slow imbibition the hourglass and parenchyma cells contribute little to the over all survival of the seeds (Duke et al. 1986). Duke et al. (1986) suggested that the epidermis of the seed coat is the primary barrier to water. The dark coloured seedcoat ~~are~~ is more impermeable to water (Tulley et al., 1981).

In view of Delouchete et al. (1973) and Justice and Bass (1978) physiologically soybean seeds should be considered delicate and this leads to their poor stability.

Chemical Composition of Seed :

The average chemical composition of soybean seed is as follows (Table No. 1)

Table 1. Chemical Composition of Soybean Seed

Constituents	Range of variation %	Range of Indian Soybean %
Moisture	5.02-09.42	8.1
Protein	29.06-50.30	43.2
Fat	13.50-24.20	19.5
Fibre	2.84-06.27	3.7
Carbohydrates	14.07-23.88	20.9

Thus the protein and oil make up about 60% of the bean, and about one third carbohydrates, including polysaccharides, stachyose 3.8%,raffinose 1.1% and sucrose 5.0%. Phosphatides, sterols, minerals and other constituents are also present as minor constituents (Cartter and Hopper,

1942). The seed composition is found to vary according to the type of cultivation and also soil and climatic conditions. As a rule when protein content is high, the oil content is poor. Recently Sugimoto et al. (1989) found that phosphoenol pyruvate carboxylase level in Soybean seeds is highly correlated to its protein and lipid contents. A variation ranging from 13.2 to 23.2% in oil, and 32.4 to 50.2% in protein, is also documented (Cartter, 1940; Dies, 1942). Soybean is excellent source of proteins. A significant variation in the protein content exist from cultivar to cultivar. The protein content ranges from 35-44%. However, some strains have as high as 50% protein (Fehr, 1978). Most of proteins in soybeans are of high molecular weight; about 80% proteins having molecular weights of 10,000 or higher. Based on sedimentation coefficient, the water soluble proteins of soybean are resolved into fractions with an approximate sedimentation coefficient of 2S, 7S, 11S, and 15S. These fractions are not homogenous. The protein component that have been isolated and characterized have molecular weight ranging from 8000 to 480,000 and many of them are enzymes that occur in small amounts. The two proteins 7S and 11S globulins, constitute major protein of soybean. The 7S fraction, ß-conglycinin which belong to the 7S protein fraction is a major soybean protein comprising 25-30% of the total proteins. It is

composed of several isomeric forms containing 3 subunits (α, α' and ß) in different ratios (Yamauchi et al., 1981). In contrast, another major soya protein glycinin or 11S globulin is a single protein which consists of both acidic and basic polypeptides (Nielson 1984). The 11S fraction consists of a single protein, the 11S globulin, although there is evidence for genetic polymorphism of this protein (Wolf and Sly, 1967). Using the powerful resolution obtained with two dimensional gel electrophoresis and in vitro labelling method for detecting methionine containing proteins, George and De-lumen (1991) recently identified a new methionine rich protein (MRP) in soybean seed. The MRP has a methionine content of 12.1%, constituted 0.6% of the total protein, and has a molecular weight 10,800. This value is exceptionally high considering that the average overall methionine content of soybean seed protein is 1.4%. This is the first report of an exceptionally methionine rich protein in soybean seed. Immunohistochemical studies of soybean cotyledons performed by Tang et al. (1993) indicated higher levels for both the glycinins and beta-conglycinin in the abaxial as compared to adaxial region of cotyledon. Krobler and Cartler (1962) found that oil, sugars, and other nonprotein components were affected most by changes in the protein content, when the protein content is increased there is a significant decrease in the nonprotein constituents

such as oil, sugars and pentosans. Black seeded types, commonly cultivated in the hills of northern India, contain higher percentage of protein and lower percentage of oil than the yellow or chocolate seeded ones (Sayer, 1933).

The quality of protein is determined by its amino acid content. Smith (1959) made ~~an~~ a useful compilation of the amino acid composition of a large number of plant seeds. The amino acid content of the more common Indian pulses is given below. It is interesting to note that soybean which ranks second to pea as far as arginine is concerned but exceeds, lentil and gram in this respect. With respect to methionine content, it surpasses even pea, but is a close second to gram. The amino acid composition of soybean flour is recorded in Table No. 2

Table 2. The Amino Acid Composition of Soybean Flour

Amino acid	Content (g/16gN)
Lysine	6.4
Methionine	1.1
Cystine	1.4
Tryptophan	1.4
Threonine	3.9
Isoleucine	4.6
Leucine	7.8
Phenylalanine	5.0
Valine	4.6
Arginine	7.3
Histidine	2.6
Tyrosine	3.8
Serine	5.5
Glutamic acid	11.8
Glycine	4.3
Alanine	4.3
Proline	5.5
Ammonia	1.9

Source : Vaidechi and Kadam (1989)

Recently Lee and Karunanithy (1990) noticed that the soybean seeds have following aminoacids (Table No. 3)

Table 3. The Amino Acid Composition of Soybean Seed

Essential amino acids :	Amino acid (mg g^{-1} dry weight)
Threonine	9.23
Valine	8.43
Methionine	4.89
Isoleucine	10.0
Leucine	13.6
Phenylalanine	6.76
Histidine	5.27
Lysine	14.2
Arginine	20.2
Non-essential amino acids :	
Aspartate	35.20
Serine	9.10
Glutamate	52.10
Glycine	8.28
Cystine	2.53
Alanine	8.74
Tyrosine	3.46
Proline	15.20

The lipids in soybean seeds are deposited in spherosomes which were detected with electron microscopy (Saio and Wantanbe, 1968). The spherosomes in soybean cotyledons are interspersed between protein bodies and are about 0.2-0.5 µm in diameter. The total lipid content in soybean ranges from 8-23%. The lipid content is found to be influenced by temperature. Soybean produced under controlled temperature conditions showed oil contents of 23.2%, 20.8%

and 19.5% at temperature 85°F, 77°F and 70°F respectively (Wolf and Cowan, 1975). Soybean oil contains 88.1% neutral lipids, 9.8 phospholipid and 1.6% glycolipids (Salunkhe et al., 1982). Neutral lipids primarily consist of tryglycerides, accompanied by smaller proportion of free fatty acids, sterols and sterol esters. The main components in neutral lipids phospholipids, and glycolipids are palmitic, oleic, linoleic and linolenic acids (Mahadevappa and Raina, 1978). The oil normally contains approximately 10% linolenic, 55% oleic, and 30% linoleic acid, with upto 50% variation in a specific component. Composition of soybean seed lipids is depicted in Table 4.

Table 4. Composition of Lipids in Soybean Seed (compiled from data of Daubert, 1950)

	Total	% dry weight of soybean
Saturated fatty acids	11-13	
Myristic		0.1-0.4
Palmitic		6.5-9.8
Stearic		2.4-5.5
Arachidic		0.2-0.9
Unsaturated fatty acids	88	
Oleic		11-60
Linoleic		25-63
Tetradecenoic		trace
Hexadecenoic		trace
Average Iodine value	102-150	

Other constituents
Carotens (chiefly β)
Chlorophyll
Phytosterolins
Tocopherols
Phosphatides

Howell and Collins (1957) demonstrated that the difference in temperature caused major difference in linolenic and linoleic acid contents of soybean oil. Carbohydrates form a significant portion of legume seeds. It is indicated by Bewley and Black (1978) that the soybean cotyledons contain about 26% starch. But, most of the studies on soybean carbohydrates are mainly restricted to sugars. The predominant sugars of soybean are sucrose, raffinose, stachyose and verbascose (Smith and Circle, 1978).

Soybean seeds also contain appreciable quantities of vitamins, and they are a good source of water soluble vitamins. The vitamins present in the seeds are depicted in table no. 5.

Table 5. The Composition of Vitamins in Soybean Seeds

Vitamin	Content
ß-Carotene (µg/g)	0.2-2.4
Thiamine (µg/g)	11.2-17.5
Riboflavin (µg/g)	2.3
Niacin (µg/g)	20.0-25.9
Pantothenic acid (µg/g)	12.0
Pyridoxine (µg/g)	6.4
Biotin (µg/g)	0.6
Folic acid (µg/g)	2.3
Choline (mg/g)	3.4
Ascorbic acid (mg/g)	0.2

Source : Liner (1978).

The soybean seeds have about 4.5% ash and this is due to presence of different mineral elements as indicated below Table No. 6.

Table 6 The Mineral Elements in Soybean Seeds

Mineral constituents	% air dry weight of seeds.
Phosphorous	0.63
Potassium	1.67
Calcium	0.26
Magnesium	0.22
Sulphur	0.41
Chlorine	0.024
Iodine	trace
Sodium	0.34
Manganese	0.0028
Zinc	0.0022
Aluminium	0.0007
Copper	0.0012
Iron	0.0097

(Compiled from data at Morse, 1950).

The phosphorus content in soybean is about 0.69%. It is mainly deposited in the form of phytate. The phytate phosphorus accounts for about 80% of the total phosphorus and is present as a complex salt of minerals or complexed with proteins. Phytic acid binds with minerals in physiological pH range. The occurrence of phytate is soybean is well documented. The data of phytic acid content in various products of soybean are shown in table No.7.

Table 7 The Phytic Acid Contents in Soybean Seeds

Sample	Phytate percentage
Soybean raw	1.4
Soybean soaked	1.43
Soybean boiled	1.23

1.2. FACTOR INFLUENCING SOYBEAN SEED GERMINATION

a. Viability and Vigour

The longevity of soybeans, relative to other crop seeds is low. Under storage conditions encountered in Ontario, rapid decrease in viability was found to occur when soybeans were stored for more than three years (Laughland and Laughland, 1939). Viability was maintained for 5 years under Colorado conditions after which time Robertson et al. (1943) found soybeans decreased rapidly in germination. Under the same conditions the viability of small grains diminished only slightly in 10 years. When stored in bins in Illinois, soybeans near the surface were found by Burlison et al. (1940) to decrease appreciably in germination within 1 year from harvest. Within two years germinability of soybean 4 feet below the surface also had decreased. Relatively rapid loss of viability at the surface was attributed to greater absorption of moisture. Under the conditions of high temperatures and humidity encountered in Puerto Rico, viability of soybeans stored for more than few month was consistently low (Stoddard, 1945). Soybean seeds with the impermeable seed coat trait retained viability better than permeable seeds under delayed harvested field conditions (Potts et al., 1978) and poor storage conditions (Maxey et al. 1980; Minor and Paschal 1982).

Hill et al. (1986) indicated that variation in the

impermeability response of seeds within an impermeable seed line was associated with seed size. Smaller seeds expressed the impermeability response in a higher frequency than larger seeds. Duke et al. (1986) also reported that soybean seed testa epidermis is extremely important in protecting the seed from injury by rapid and slow hydration and in maintaining seed viability. According to Tilden and West (1985) the vigor of soybean seeds was increased by priming. Metabolic repair probably included other subcellular components as well as plasma membrane.

Ferguson et al. (1990) found that seed vigor (as measured by accelerated aging) declined significantly after 7 and 10 months storage. Mitochondrial stage 3 (ST_3) respiration rates of mitochondria from axes decreased throughout the storage and (RCR) respiratory control ratio preceded measurable declines in whole seed vigor and correlated with increase in the conductivity of axes leachate.

Seed deterioration, which was indicator of loss of viability is caused by a wide variety of injurious treatments, eg X-ray irradiation, mechanical damage, desiccation, heat and adverse storage conditions and leads to change in respiratory metabolism, such as lower rates of oxygen uptake, higher respiratory quotients or both (Wood stock and Grabe 1967). Wood stock and Taylorson (1981)

suggested that, during aging, an imbalance between tricarboxylic acid cycle and glycolytic activities present during early imbibition to some degree even in vigorous unchanged soybean seeds, becomes more pronounced and leads to accumulation of ethanol and acetaldehyde. Parish and Leopold (1978) concluded that the basic cause of deterioration in soybean is the inability of the cell membranes to reform and function upon rehydration. Priestly and Leopold (1979) could find no evidence of lipid oxidation during aging in soybeans. According to Oathout (1928) the soybeans which did not at any time appreciably exceed normal water content maintained their vitality for than two years regardless of conditions under which they are stored. Miles et al. (1988) reported that freshly harvested immature soybean seeds did not need a desiccation period prior to expression of maximum viability and normal seedling development. According to McDonald et al. (1988) soybean seed imbibition is a critical stage in crop establishment. Their studies showed that the embryonic axis hydrated more than the cotyledons and further indicated that deteriorated seeds hydrate in a manner similar to nondeteriorated seeds in liquid water but that differences in the axis are detectable when hydrated in a high humidity environment. The effects of testa epidermal crack on imbibition induced soybean (*Glycine max*[L.] Merr.) seed damage, as measured by

the leakage of intracelluler constituents and subsequent seedling survival were examined by Duke et al. (1986). Testa epidermal cracking increased with increasing seed surface area and this was caused by periods of dry seed hydration as short as 1 min followed by air drying. As compared to slow imbibition in germination paper, rapid imbibition of seeds caused by submersion in distilled water for 6 hr. at 25°C greatly increased the leakage of intracellular substances during imbibition and greatly decreased the survival of seeds with intact testae, testa epidermal cracks, and without testae. Seeds that were aerated during rapid hydration germinated as poorly and teached solutes as proflusely as seeds not aerated. This indicated that anoxia is not a significant factor in rapid hydration effects on soybean seeds.

Environmental stress during seed production can affect subsequent quality. Stress occurring after physiological maturity, but before harvest has been reported to cause reductions in soybean seed germination and vigor (Sediyama et al., 1972). Rassini and Lin (1981) reported reduction in soybean seed vigor as drought increased during growth stage R_3 to R_5. Yaklich (1984) reported a similar decrease in soybean seed vigor as measured by the accelerated aging test, following drought stress during the seed filling period. However, there was no effect on

seedling emergence from sand or soil. Dornbas et al. (1989) also reported that drought stress during the seed filling period in soybean caused significant reduction in germination axis, dry weight and increase in membrane damage in greenhouse study. In contrast, Vieira et al. (1991) reported yield reduction of 35-41% when drought stress was imposed during seed filling in two greenhouse experiments, but found no effect on germination or vigor of resultant seeds. Smiciklas et al. (1989) reported a significant reduction in seed germination (from 96 to 85%) when plants were exposed to drought stress in the field during growth stage R_5; however, drought stress during growth stages R_2, R_4 or R_6 had no effect on germination. They suggested that seed Ca^{++} concentration was related to the decrease in germination. Seed Ca concentration for unstressed seeds was higher (1648 $\mu g\ g^{-1}$) than for stressed seeds (343 $\mu g\ g^{-1}$). Vieira et al. (1992) suggested that drought stress would have no effect on seed germination or vigor, unless the stress is severe enough to produce shriveded, flat undeveloped seeds.

Koslanund and Delouche (1987) observed that reduced germination in soybean was associated with low initial moisture (4-8%), large seed size, light coloured seed coat, low imbibition temperature (5°C) and abundant water supply and with interaction between these factors.

High moisture content and high temperatures both have been shown to decrease longevity of soybean seed (Ramstad and Geddes, 1942); Toole and Toole, 1946). Soybean with 13.5% moisture maintained at 30°C failed to germinate after 5 months whereas full viability was maintained after 10 years of storage at -10°C. Soybeans maintained at 20°C with 18 percent moisture lost all viability from 5 to 9 months, whereas with 8 to 9% moisture, 90% germination resulted after 5 years of storage. Weiss (1949) has reported that soybeans with 13.5% moisture maintained at 30°C failed to germinate after 5 months, whereas full viability was obtained after 10 years of storage at 10°C. Further, if soybean were stored at 20°C with 8-9 percent moisture, 90 percent germination was obtained even after 5 years of storage. In the experiments of Tomes et al. (1981), soybean seeds of differing vigour levels were aged over a range of times (48-96 h.), temperature (37-45°C) and moisture content on wire mesh screens placed in plastic boxes containing 50 ml water. Seeds aged for 72 h. at 37°C showed little decline in germination. Maximum deterioration occurred at temperature > 41°C, with medium low vigour seeds being most severely affected. Temperature above 43°C killed most seed regardless of vigour level. Seed aged at the recommended temperature of 41°C for 48 h showed little deterioration at all vigour levels.

Papp and Szabo (1979) reported that higher percentage viability was achieved between 20-30°C in all soybean classes. At lower temperature germination was slow but seedling vigour excelled. Most rapid emergence occurred at 35°C but was followed by poor vigour and uneven growth. Ideal temperature in the storage room for maintaining seed viability in soybean for 8-9 months was found to be 20-27°C at relative humidity of 50% (Delouche 1974). Singh and Singh (1992) stored soybean cv pb seeds (4 months old, 10% initial moisture content) in sealed tins or gunny bags or stored in sealed tins after drying to 7.5, 5.5 or 4.3% moisture and studied germination. At 10% moisture, seeds could be stored in sealed container for only 6 months before losing viability, while at 4.3% moisture content, 90% germination was retained after 28 month storage at room temperature. At 10% moisture content seeds could be stored for 12 months in gunny bags. According to Roberts (1972) following equation is applicable to seed viability of various crop species including soybean

$$K_E - C_W \log m - C_H t - C_Q t^2$$

$$V = K_i - P/10$$

where, v = percentage viability after any storage period P, m = moisture content, t = storage temperature, K_E, C_W, C_H and C_Q = constant.

Feng and Egli (1994) recently studied the effect

of the premature light environment on germination of immature soybean seeds by exposing plants to various light regimes. There was a significant negative relation between germination (radicle protrusion through the testa) in culture and average solar radiation for 5 days before culture for control seed from greenhouse and field experiments. They placed plants in reduced radiation or complete darkness for 12-13 days before culture and found that this treatment increased germination. This response could be reversed by placing the plants in light again before culturing. Longer dark treatments were required in the field to get similar responses. The light reduction treatments reduced soluble sugar levels and increased the osmotic potential of the embroynic axes. *In vitro* germination was associated with changes in axis osmotic potential that were caused by the light treatments. The light treatment was probably mediated by changes in photosynthesis. These workers suggested that seed water status is involved in regulating the shift from development to germination.

Rathore et al. (1982) noticed that in early stage of emergence, oxygen diffusion rate (ODR) was the main limiting factor and at late stage crust strength. Small seeded soybean cultivars were less adversely affected by increasing crust strength and after germination at all

stages had higher emergence counts than large seeded ones. Seedling mortality in large seeded cultivars was > 50% compared with about 30% in small seeded ones. The viability of soybean seeds is also probably lost due to chemical factors. Sathiamoorthy (1990) identified germination inhibitors in nonviable soybean seeds as vanillic acid and P - Coumaric acid. Saxena et al. (1988) observed that soybean seed could be stored for 20 months at 10°C without germination falling below 60%. Prolonged storage increased in the percentage of abnormal seedlings but this was partially reversed by GA_3 treatment. Sing et al. (1981) found that soybean seed treatment with 0.1-10 mg Co or 1-3 mg Mo/l improved the viability and seedling vigour of soybean. Mo at 1 mg and Co at 0.1 mg/l were most effective in increasing germination percentage and decreasing electrical conductivity of seed leachate of deteriorated soybean seeds. According to Knypl and Knyp (1993), 0.01-1.0% poly (acrylic acid ethyl acrylate vinyl acetate) (PAEVA). Solution per seed also increased field emergence of soybeans. When seed vigour was reduced or increased by exposure to low or high relative humidity for 4 d, PAEVA increased germination of low vigour seed but initially reduced that of high vigour seeds of soybean. PAEVA reduced water uptake by seeds and decreased evaporation from the soil. Pardeshi et al. (1989) noticed that soybean seed

viability was increased with application of Mancozeb, Bavistin, Thiram, Vitavax and Captan Fungicides. Carbenddazim was most effective among these fungicides.

b. **Temperature**:

The temperature of soil is determined by balance between incoming and outgoing radiation at the soil surface, and by the way that this energy is redistributed throughout at the soil as heat. According to Gordon (1973) rate of germination is closely correlated with temperature in the laboratory, as is rate of emergence with mean soil temperature in the field.

It was Sachs(1860) who first proposed the idea that for every plant species upper (maximal) and lower (minimal) temperature limits exist outside of which germination of seeds cannot take place. This was the outcome of his experiments on the effect of constant temperature on seed germination of several cultivated crops. The term optimal temperature was used to describe intermediate temperature at which best germination was obtained. These minimal, optimal, and maximal temperatures represented cardinal points and plant species show great variations with respect to these cardinal points. For soybean Inouye (1953) reported the optimum temperature for germination to be 34°C, the minimum as 2-4°C, and maximum as 42-44°C. Maximum

germination of soybeans in the shortest time occurred at a constant temperature of 30°C with no improvement in germination by altering the temperature (Delouche 1953). In several experiments the effect of temperature on hypocotyl elongation and seedling emergence of soybean has been investigated. The hypocotyl elongation of soybean cultivar of Ford was inhibited at 25°C while seedlings were normal if germinated at 15°C, 20°C and 30°C (Grabe and Metzer, 1969). Twenty five genotypes were subsequently classified as short, intermediate or long based on their ability to emerge at 25°C from depth of 10 cm and indicting genetic control over the temperature induced hypocotyl inhibition. Gilman et al. (1973) found that temperature between 21°C and 28°C inhibited hypocotyl elongation with maximum inhibition at 25°C. Hatfield and Egli (1974) reported that the time for hypocotyl elongation to 5 cm decreased as temperature increased between 10°C and 32°C for hypocotyl elongation of soybean. High moisture 15% content and temperature 25°C increased germination of soybean seeds (Balma 1988). Covell et al. (1986) reported the values of maximum temperature for germination from 46.8°C to 55.2°C for soybean seeds. In soybean, Hadley et al. (1984) have calculated a mean base temperature of 7.8°C for rate of progress from sowing to first open flower for photoperiod - sensitive genotypes grown in inductive photoperiods shorter than their

respective critical day lengths. Summerfield and Roberts (1985b) have calculated values between 4.4°C and 10.2°C for genotypes of temperature and tropical origin respectively. Stucky (1976) studied the effect of planting depth and temperature on emergence of several soybean cultivars for double cropping systems. He concluded that most cultivars can emerge from planting depth of upto 7.5 cm and high temperature (32°C) accelerated emergence with some reduction in percentage of emergence. According to Papp and Szabo (1979) in laboratory tests of six American and six Hungarian soybean varieties, the optimum temperature was between 20 and 30°C although some varietal difference in rate of germination was evident. No variety germinated at 5°C, but at 10°C the Hungarian variates germinated faster than the American ones. Nsowah (1980) observed that seedling emergence rate were faster at 30-40°C than at < 30°C and > 40°C. Hypocotyl development rates were faster between 25°C and 35°C than at > 40°C. At 49°C, hypocotyl development was minimal after seedling emergence of soybean seeds. Murphy and Noland (1982) indicated that the temperature effect on imbibition rates was correlated with the viscocity of water. The period of imbibition by soybean seeds is found to be a period of sensitivity to stresses, especially to low temperature. (Shull, 1920; Pollock, 1969 and Leopold, 1980). Christina and Leopold (1983) speculated that damage to

soybeans during imbibition such as in case of chilling injury, may be particularly related to the initial wetting reaction rather than to the longer term imbibitional rate. Willing and Leopold (1983) also suggested that the initial wetting reaction may play a major role in chilling injury during imbibition. Low temperature may decrease the ability of the tissue components to withstand expansion during wetting events, and initial moisture content of the tissue would be expected to magnify the importance of the wetting event in the initial stage of water entry. Chabot and Leopold (1985) reported that in chilled soybean seeds, mitochondria show weakly defined envelops and cristae in contrast to the well formed internal structure in the controls. Nuclei of chilled seeds were irregular in shape. Tannic acid was used as a probe of plasmolemma integrity. Most cells in both chilled and control treatments were able to exclude tannin, indicating that chilling injury effects did not damage the plasma membrane enough to allow passage of molecules of the size. Li et al. (1991) used water uptake and solute leakage to asses the mechanism of chilling injury during imbibition in 9 cultivars of soybeans namely B_3, B_9 and B_{10} absorbed less water and had a higher germination rate. Tully et al. (1981) have studied these effects in seeds of pea (*Pisum sativum* L.) and soybean (*Glycine max* (L.). Imbibition for 2 hours at 20°C. The differences

between species could not be related to differences in the major lipid components of the seed membranes. Much of the resistance to chilling injury however, was due to seed coat. The rate of imbibition of cold water was a major factor in chilling injury. The imbibition of peas proceeded slowly, but that of soybean rapidly. Soybean seeds with black coats imbibed much slower and germinated more than non-pigmented seeds. The black coats were thicker and tougher than the non-pigmented.

The effect of temperature and soil/seed factors on seedling emergence force of soybeans (*Glycine max* (L.)) was recently studied in the USA by Goyal et al. (1980). They tabled all results from the literature in 1950-1978 on the maximum seedling emergence force of different agricultural crops. The critical time at which a seedling attained its maximum force was negatively correlated with the temperature studied and with the soil impedance/seed energy ratio. When the soil impedance, the seed moisture and the soil water content each were kept at a constant level the emergence force was maximal at 26°C. The sensitivity to low temperature (5-7°C) in the initial stages of soybean germination was recently studied by Chinese scientist (Guang-hua et al., 1981). Presoaking in water at 25°C for 4-6 hours or 35°C for 1-2 hours, prestoring at -2°C for a month, or soaking in a chemical solution such as ethephon at

10 mg/l protected the seeds from chilling injury. The minimum germination temperature was also studied in poland for 25 soybean genotypes (Szyrmer and Szczepanska, 1982).

Kim et al. (1990) studied water uptake properties and volume changes during soaking in water at 4-98°C for soybean cultivars, Paldal, Danycob, Jangback, Baegum, Jangyeob, Local 1 and Local 2 seeds. Increase in seed weight and volume were more rapid at 60-98°C than at lower temperatures and slowest at 4°C. Moisture gain at 4-60°C was linearly related with the square root of the soaking time.

c. Light

Light is one of the most important factor which regulate many life processes of plants. The importance of this factor in germinating process was first recognized by Ciser (1883) and latter work in last thirty years has revealed involvement of phytochrome system. In view of general action of light on seed germination, seeds are broadly classified into two main classes. Light requiring seeds and dark requiring seeds. The phytochrome system has been detected in soybean seeds and reversion of PFr to Pr has also been demonstrated in dark grown soybean seedlings (Hopkins and Hillman, 1965). According to Valio (1986) the early germination of naked soybean seeds or isolated embryonic axes is enhanced by darkness. Red light produced

similar effect as white providing evidance for effective wave length of the photoinhibition. Far red light acted similarly to darkness in promoting the early germination of the embryos. It was suggested that possibly light may act through phytochrome by the high irradiance system.

d. Gases

Normally most of the seeds germinate well under normal atmospheric condition (20% O_2, and 0.3% CO_2). Increase in respiratory rate is a characteristic biochemical event during seed germination and this involves exchange of O_2 and CO_2. Oxygen is essential for several oxidation processes and availability of oxygen many times becomes a limiting factor for germination. Although the oxygen requirements for germinating soybean seed have not been quantitatively defined, oxygen is found necessary for germination (Toole et al. 1956). Al-Ani et al. (1985) reported that the maximum germination and respiration rates were reached in most seeds at O_2 pressures close to that of air. Decreasing the O_2 pressure produced a gradual decrease of germination rate. The seeds could be classed in two groups, according to their response to low O_2 pressures. Group I includes Lettuce, Sunflower, Radish, Turnip, Cabhag and Soybean; at O_2 pressures close to 2 kilopascals, the germination in this group was stopped and the adenylate energy charge was lower than 0.6. Group II includes rice,

wheat, maize, sorghum and pea. The germination rate of these seeds was also gradually decreased by lowering the O_2 partial pressure germination still occurred very slowly, at 0.1 kilopascal; the adenylate energy charge remained higher than 0.6. Vantoal and Bolles (1991) suggested that the post anoxic injury of soybean roots after a short anoxic stress was associated with increased superoxide radical production capacity coupled with decreased superoxide dismutase activity. They further noticed that a period of anoxia of at least 3 hours were necessary for soybean seedlings to develop the ability to cope with post anoxic stress.

CO_2 is not necessary component in atmosphere to support germination but CO_2 concentration greater than normally found in soils, are found to stimulate germination in soybeans (Grable and Danielson, 1965).

e. <u>Water Status</u>

Soybean seeds are generally tolerant of dehydration at maturity and during the early stage of germination, but once germination proceeds beyond a critical stage, they become suceptible to dehydration injury (Senaratna and Mckersle 1983). The symptoms of dehydration injury in soybean are loss of germination, slower growth rates of isolated axes, hypocotyl and root curling and altered membrane permeability. Increased electrolyte

leakage due to dehydration treatment was observed only from isolated axes but not from cotyledons, suggesting that coty ledons are more tolerant of dehydration. According to Senaratna et al. (1985) the axes of soybean seeds suffer dehydration injury, if they are dried to 10% moisture at 36 hours of imbibition, but tolerate this stress, if dried at 6 hours of imbibition. To support soybean seed germination, the soil moisture level must be higher than that required for seeds of corn. Soil moisture tension can not be less than -6.6 bars for soybean germination to occur within 5-8 days at 25°C. (Senaratna et al., 1985). Senaratna and McKersie (1983) reported that the prevention of cell elongation by osmotic treatment in PEG (-6 bar) or imbibition in 20 micrograms per milliliter cycloheximide did not prevent the loss of dehydration tolerance. They suggested that neither cell elongation nor cytoplasmic protein synthesis was responsible for the change in sensitivity of soybean seeds to dehydration. The rate of dehydration or rate of rehydration did not alter the response to the dehydration stress. Nonami and Boyer (1990) measured the cell wall properties and cell hydraulic conductivities in dark grown soybean seedling exposed to low water potentials (-0.28 Mpa). They concluded that the cell wall plastic properties and cell membrane conductance to water were decreased in elongating stem tissues at low water

potentials and the elastic properties of the cell wall were independent of these changes. Seong et al. (1988) studied the germination of seeds of 8 soybean cultivars at 15 or 30°C with 15 ml of solution containing 0, 20, 30 or 35 gm PEG /100 g water adjusted to give water potentials of 0, -0.5, -1.1 and -1.5 Mpa had potential use in screening soybean cultivars for improved emergence under water stress.

Excessive soil moisture levels are also not conducive to germination and early root growth of soybeans. Soybean roots develop faster at soil moisture tension of -0.5 bar than at -0.9 bar (Grable and Danielson 1965). Because oxygen is required for root respiration, excess soil moisture limits available oxygen, which, in turn, slows root growth and also provide an environment that fosters the occurrance of root rot organisms such as *Phytophthora megasperma* var. Sojae (Hildebrand, 1959) during seed germination.

f. Soil Texture

Rathore et al. (1981) studied the effects of soil crusting on seedling emergence of soybean in the field by photographing a series of position taken by emerging hypocotyls. The hypocotyl was brittle and often collapsed at the stress point between the emerging hypocotyl and burried cotyledons. A number of seedlings subjected to several

impedance stresses often broke off at the bypocotyl hook when soil surface ruptured. They noticed that the emergence force of the cultivars tested was closely related to their seed weight.

g. Soil Salinity

Soil salinity is a threatening problem for crop production in numerous parts of the world. Saline environment is due to excess of salts of Sodium, Magnesium and Calcium in the medium. According to Bernstein (1974), under field conditions the germination failure is caused by exceptionally high concentration of salts in the surface 2-3 cm of the soil where the crop seeds are planted. Crop species and varieties show marked difference with respect to germination behavior and saline conditions. Mukhiya et al. germinated wheat, barley and soybean under salt stress (NaCl) in the soil. Soybean was the most sensitive and barley the most tolerant species. Paliwal et al. (1970) noticed that different soybean varities show a different response to salinity at germination stage. They noticed that varieties Bragg and G-3 could germinate in saline solutions with 6 mmhos/cm electrical conductivity while seeds of cultivar Baroda could germinate in solutions having 12 mmhos/cm electrical conductivity.

Islam (1978) made germination studies of three

soybean cultivar namely Lee-24, Improved pelican and Semmes in NaCl concentrations of upto 1000 ppm. After 9 day semmes showed 100% germination at 0, 100 and 200 ppm. NaCl while higher concentrations caused decrease, while the other 2 cultivars gave 80% germination. Lee-74 showed 100% germination and improved Pelican 90% germination 100 and 200 ppm, NaCl. In both cultivars the percentage germination decreased at above 300 ppm Nacl, was lowest at 400 and 500 ppm; rose to 100 and 98.7% respectively; at 700, 800 ppm, then decreased. Seedling height and weight in these cultivars were little affected by NaCl. Chanprasert et al. 1992) recently conducted germination studies of soybean seeds at 0, 0.2, 0.4 or 0.8% NaCl. Germination was decreased by salinity after 3 days. Srivastava et al. studied the biochemical basis of salt stress resistance in germinating seeds of soybean. Seeds after surface sterilization was germinated on filter paper moistened with salt solutions to give levels of osmotic pressure from 0.0 to 11.4 atm. After 144 h. the axes were excised, dried and subjected to chemical analysis. Protein, reducing sugars, total water-soluble carbohydrates, fatty acid methyl esters were determined. The effect of increasing concentrations on the various components was highly variable. Because of this, the authors concluded that further work was necessary before coming to any conclusions.

1.3 INFLUENCE OF PLANT GROWTH REGULATORS AND OTHER CHEMICALS ON SOYBEAN SEED GERMINATION

Mehta and Subhadradevi (1974) noticed that Gibberellic acid and Ascorbic acid ON seedling growth had promotive effect on the germination pattern of soybean seeds. They further observed that the inhibitory effect caused by maleic hydrazide on soybean seed germination was overcome by the addition of Gibberellic acid. They further noticed that activity of catalase in embryo axis and cotyledons was stimulated by GA and AA and inhibited by maleic hydrazide. Saxena et al (1988) germinated soybean seeds in 10 ppm GA_3, Kinetin and ethephon and found that their treatments increased percentage by 20-25%. GA_3 increased embryo axis length. According to Sharma et al (1987) etheral counteracted the adverse effect of waterstress on germination, vigour index, germinative relative index, protease activity and total free aminoacids. Etheral thus not only stimulated germination but also overcome the inhibition caused by moisture stress and is therefore involved in stress tolerance. Experiments of Switzer (1957) indicated that 2-4 Dichlorophenoxy acetic acid inhibited respiration in intact mitochondria of soybean seedlings.

Bay et al. (1986) found a stimulation of ascorbate

synthesis in soybean seeds due to Saponin in ginseng extract. Padmawati et al (1992) reported that the chemical mutagens DES and MH affected the germination percentage, seedling height of soybean cultivar KHSB-2 and MACS-24. Pristley and Leopold (1980) studied the effect of alcohol stress on soybean seeds. When soybean seeds were exposed to pure aliphatic alcohols, the shorter alcohols were the most damaging (methanol > ethanol > n-propanol > n-butanol); when the alcohols were applied in equal volume of water, the opposite was found (n-propanol > ethanol > methanol); Leakage of solutes from the pretreated tissue during subsequent imbibition in water was associated in each case with the loss of germination and a decline in axis growth. Damage by the pure alcohols was related to the extent of their penetration and the amount of phospholipid eluted; injury caused by alcohols in the presence of water did not exhibit these features. It was concluded that damage to seeds by alcohols was due to the elution or displacement of cellular phospholipids and possibly the partial denaturation of membrane proteins. Setty et al (1991) found that captan increased germination of soybean seeds by 10% *in vitro* and 21% *in vivo*. Manocozels was more effective with increase of 23 and 40% respectively. Barbaro and Szalonke (1980) observed effect of soybean seed dressing with fungicide T (50% Thiram), Funaben T (50% N-2-benzimidazole carbamate

methyl ester +15% thirum), the insecticide ziarnochron (55% anthroquinone) and with growth substance RW-3. They noticed that treated seeds took longer time to germinate than untreated controls, particularly at 5 and 10°C, but at these temperature seed dressing improved emergence and seedling survival. Barbaro and Kucmierz (1980) further studied effect of seed dressing with fungicide oxafun T(37.5% Carboxin + 37.5% Thiram), Phenyl mercuric acetate, Benlate, maleic-hydrazide and herbicides, Linurou and Treflan on germination performance. They noticed that dressed seed took longer time to germinate at 5 and 10°C but germinated more rapidly than untreated seeds at 20°C. Weston et al. (1986) reported that aqueous extract of auaua grass (*Agropyron repens* (L)) shoots and rhizomes inhibited seed germination and root growth of alfalfa and soybean at concentrations of less than 2-5 mg dried extract/ml.

The effect of presowing soaking treatments with growth regulators and other chemicals on germination of soybean seeds has been studied by several workers. Kwon and Guh (1987) soaked soybean cultivar Bragg seed in 10 ppm, Kinetin, IAA, Etheral (Ethephon) salicylic acid or water for 6 hours and dried in the shade for 48 hours. Seedling emergence observed within 2.3, 2.3, 2.4, 2.7, and 2.4 day corresponding germination percentage were 63, 65, 67, 60 and 69% respectively. According to Khan (1977) priming of

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soybean, CVS. Altona and Traverse, seeds for 4-8 day in 25 to 35% PEG at 15°C increased the rate of seedling emergence from soil kept at 8-10°C. The rate was further enhanced whtn 50µm GA_3, 50µm kinetin, or 20µm fusicoccin was present during priming and reported that addition of 0.2% thiram to PEG solution proved essential for restricting proliferation of seedborne fungi during the priming process. Sing and Thakur (1979) soaked soybean seeds in water 0.1 and 1 ppm cobalt chloride and 1 and 2 ppm molybdophosphoric acid and dried to the original weight. The seeds were germinated alongwith untreated seeds under 0, 0.1, 0.4 and 1 atmospheric tension for 96 hours. Treatments of seeds in water, Co and Mo, solution increased germination percentage and germination relative index compared with unsoaked seeds.

Biochemical changes during soybean seed germination

The metabolism of germinating seeds is amphibolic that is, it is both catabolic in the sense of degrading reserve compounds to provide energy and raw materials for the early growth of seedling, and anabolic in the sense of producing machinary for protein synthesis and biogenesis of various organells needed for catabolic activity as well as the true anabolic synthesis of new cells and tissues (Ching 1972). The first process in the germination sequences of events is imbibition of water, water availability is, therefore, the major environmental factor affecting

germination. The seed moisture content (dry weight basis) required for soybean seed germination is about 50% compared with 30% corn, 26% for rice, and 31% for sugar beets (Hunter and Erickson, 1952). The process of imbibition includes an initial wetting of seed tissues (Vertucci and Leopoid, 1983), and establishment of a sharp wetting front (Waggoner and Parlange, 1976) followed by a continued swelling of the seed and an associated increase in seed water potential as seed water content increases. Parrish and Leopold (1977) found that the initial stage of imbibition (within 10 minutes) in soybean seeds culminated in a wetting of the seedcoat and the release of adsorbed gases. Respiration during germination involve, 4 phases. They have been summarized by Bewley and Black (1978) as follows :

Phase I : This is characterized by a sharp rise in respiration lasting about 10 h and is attributed in part to activation and hydration of mitochrondrial enzymes associated with the citric acid cycle and electron transport chain. Respiratory quotient values at this time are slightly above 1; the major respiratory substrate is probably sucrose. The rise in respiration during this phase progreses linearly with degree of swelling of the cotyledon tissue.

Phase II : Here there is a lag in respiration between hours 10 and 25 after the start of imbibition. Hydration of the cotyledon, is now completed and all pre-existent enzymes

are activated. At this time have been observed to rise above R.Q 3.0, which is indicative of some anaerobic respiration. The lag phase may be due to limited supply of oxygen at this phase.

Phase III : A second respiratory burst characterizes this phase. During this phase the R.2 falls to about 1.0 indicating that aerobic respiration of carbohydrate is predominant.

Phase IV : The marked fall in respiration noted at this time coincides with the disintegration of storage organs following depletion of the stored reserves.

Bewley and Black (1978) noticed that seeds of some species show the respiratory lag phase during germination while other species do not show such behaviour. They mentioned that soybean belongs to the first category as it displays respiratory lag phase during germination. During the initial stage of germination extensive membrane reorganization of important cellular organells is believed to occur (Simon 1974, Leopold 1980) followed by enhanced mitochondrial development (Morohashi 1986). Respiratory activity requiring an uptake of oxygen was found to increased rapidly with the onset of soybean seed germination (Howell 1963).

Morohashi (1986) examined the patterns of mitochondrial development in reserve tissues of several species of seeds during 3 to 4 days after the start of imbibition. In starch storing seeds (cowpea, Kidney bean, Egyptian kidney bean, mung bean, black gram and soybean), mitochondrial activities (state 3 respiration rate, respiratory control ratio) increased during the first 1 to 2 days after imbibition and leveled off thereafter. The initial increase in the activities was little affected by cycloheximide and chloramphenicol. Activities of mitochondrial enzymes (succinate dehydrogenase EC1.3.99.1; NAD malate dehydrogenase, EC 1.1.1.37; cytochrome oxidase, EC 1.9.3.1) did not change much during experimental period. This suggested that mitochondrial development in starch-storing seeds like soybean is primarily due to improvement of preexisting mitochondria.

Sing et al. (1987) observed that succinate dehydrogenase (SDH) activity significantly increased upto 15 hours then gradually decreased upto 36 hours during soybean seed germination. Protein content of mitochondria increased upto 24 hours and then decreased during early stage of germination of soybean seeds.

Okuda et al. (1987) reported that after 48 hours of soybean seed germination the activities of 6 phosphogluconate, glucose, L-isocitrate dehydrogenase were

2-4 times as high those of embryonic axis from dry seeds. The activities of L-glutamate, meso-D-diaminopimelate dehydrogenase and L-isocitrate were still low. The activities of L-malate dehydrogenase and acid phosphatase increased strikingly after germination. The enzyme hexokinase (ATP:D-hexose 6-phosphotransferase, EC.2.7.1.1) plays a key role in glycolysis and other pathways of carbohydrate metabolism. Roye et al (1981) studied the hexokinase activity of germinating soybean seeds. They observed that the 1 h soaked seeds didnot show any hexokinase activity. Activity was observed from day 1 to day 12 in the germinated seeds. In this experiment the highest activity was obtained in the particulate fraction of 2 day old seedlings. The activity was fairly high on day 1. However, the activity declined from day 2 onwards. Hexokinase activity was found in the basis of seedlings viz. Cotyledons, roots and shoots which were studied from day 4 onwards when differentiation into these parts became evident, the highest activity was again found in the particulate fraction of cotyledons, followed by the shoots and roots. The activity declined with increase in the number of days of germination in all the tissues. In another studies of Roye et al. (1981), the soybean seeds were soaked in water for 1-48 h and the axes were separated from the cotyledons and the enzyme activity was determined from the

homogenized tissues. Very little particulate fraction could be obtained in this study after centrifugation at 16000 g for 30 minutes. The measurements were therefore made on the whole homogenate. It was seen that enzymes activity was present in both axes and cotyledons 6 hours after imbibition. The activity in axes was higher as compared to the cotyledons from 6h to 24h. At 48 h cotyledons showed higher activity. These workers speculated that perhaps the axes had hexokinase activity from the very beginning. Activity of enzyme catalase was found to increase in both embryo axis and cotyledons during germination and it was stimulated by GA and Ascorbic acid and inhibited by maleic hydrazide (Mehta and Subhadradevi, 1974).

A major metablic event in the germinating seed is the hydrolysis of seed protein reserves to provide the growing seedling with the nutrients necessary before photosynthesis is established. There are two major storage proteins in the soybean [Glycine max (L) merrill] seed, glycinin and ß-conglycinin, which tegether make up 70% of the seed protein reserves by dry weight. Glycinin consists of six nonidentical subunits. Each subunit has one acidic chain and one basic chain linked by single disulfide bond (Nielson, 1985). ß-conglycinin is a glycoprotein that is composed of three noncovalently associated subunits, α', α and ß (Thanb and Shibasaki, 1978), with molecular weights of

76,000, 66,000, and 47,500 respectively. During soybean seed germination and early growth, these storage proteins are degraded by proteolysis. The predominant pattern is one of limited proteolytic cleavage by protease specific for reserve protein followed by more rapid proteolysis by less specific protease (Muntz et al. 1985). Several soybean protease have been described. These includes six proteolytic enzymes from ungerminated seed separated by anion exchange chromatography (Weil et al. 1966), two carboxypeptidases from germinating soybeans (Kubota et al., 1976), two endopeptidases, one exhibiting an acidic pH optimum and the other a basic pH optimum (Bond and Bowles, 1983) and a trypsin like protease (Nishikata 1984). *In vivo* studies of Wilson et al. (1986) showed that the pattern of the degradation of ß-conglycinin degraded into 3 subunits of ß-conglycinin, the α' and α subunits are more rapidly degraded upon soybean seed imbibition.

According to Qi et al. (1992) protease C1, the protease responsible for initial degradation of the α' and α subunits of the soybean ß-conglycinin storage protein, increase during the first few days of germination and early growth. These workers speculated that protease C1 plays an important physiological role, but not an exclusive one, in the living plant. They further suggested that the action of protease C1 on ß-conglycinin mobilizes upto one third of the

α' and α subunit amino acid residues. The observation of the proteolytic products of protease C1 action as early as day 1 showed that this pathway is one of the earlist generators of amino acids for the germinating seed. Limitation of this action of protease C1, such that the 50.0 and 48.0 KD intermediates as well as the ß subunit could not be degraded, ensures that the amino acids stored in ß-conglycinin are not all released in one early burst, but rather are made available over a longer period of time. Thus due to this activity the soybean seedling is ensured both a quick, early supply of amino acids as well as supply lasting until such time as the plant becomes a photosynthetic autotroph. In soybean proteinase activity was studied by Tazakawa and Hirokawa (1956). Activity rose much more rapidly and earlier in the cotyledons than in the axis. They further observed that the highest proteinase in cotyledon was after 6 days of germination and then decreases upto 12 days of germination. On the other hand, in the axis the activity of proteinase started to increase after 6 days of germination and reached maximum after 12 days of germination. Hoagland (1987) assayed proteolytic enzyme activity with two substrate glycine-p-nitroanilidine (GPNA) and Leucine p-nitroanilidine (LPNA) and found that the enzyme increased over 72 h in light grown soybean (Glycine max) axes, while activity decreased with GPNA or nearly

constant on LPNA in dark grown seedling axes. An early feature of protein utilization in soybeans is thought to be the provision of ammonia to the developing seedling by the removal of amidogroups from cotyledon storage proteins by the action of deaminases (Catsimpoolas et al. 1968, Daussant et al. 1969). Tajima et al. (1985) noticed that a ureate degrading diamine oxidases and peroxidase system exist in soybean radicles and the reaction rate of urate degradation is controlled by the concentration of cadaverine.

Kamboj (1977) noticed that oil and soluble protein content decreased with time in the germinated seedling and there were relatively crude protein contents during germination in 4 soybean cultivars. He found that the peroxidase isoenzyme pattern of soybean seeds which failed to germinate resembled that of the mature seeds, during germinaiton, the number of bands decreased in all cultivars of soybean. The glutamine synthetase activity increased upto 6th day of germination in all cultivars. Seeds of soybean which failed to germinate did not show any change in oil, crude protein and soluble protein contents and in activities of peroxidase and glutamine synthetase. Seeds of cultivar black tur had the very low peroxidase activity and gave the highest germination percentage. On the other hand, seeds of cultivar Ankur had a very high peroxidase activity and gave the lowest germination percentage. Hailstones and Smith

(1991) observed that lipid hydroperoxide delcined significantly in seed axes over the first hour of imbibition. Lipid metabolism occupies a key place during germination of fatty seeds like soybean. Fats and Lipids are broken down at the first instance by the action of lipases and glycerol and fatty acids are liberated. The bulk of fatty acids are further broken down by oxidation reactions. (especially ß oxidation) to yield 5 carbon compounds. Directly coupled with these processes is the glyoxylate cycle which takes in the acetyl COA and in a series of enzymatic reactions links this to the glycolytic pathway which then operates in reverse to generate hexose. Glycerol also enter the glycolytic pathway. All these reactions have been shown to occur in the cotyledons and endosperm of fat containing seeds such as soybean (Mayer and Poljakoff-Mayber, 1978). Ishinaga et al. (1992) noticed that the percentage of long saturated fatty acids (21:0, 23:0 and 25:0) increased during seedling development. These fatty acids, represented > 40% of fatty acids in dolichyl fatty acyl esters in stems of soybean seedlings. Adams et al. (1981) reported that during soybean seed development α-amylase activity increased to a maximum and then declined towards maturity. They noticed that mature and germinating seeds retained relatively low activity of α-amylase. Hillbrand Hymowitz (1981) reported that difference in starch

metabolism during seed development and germination of 2 soybean genotypes with normal seed ß-amylase activity. Starch and soluble sugar profiles were essentially the same during seed development and germination. Morita and Yagi (1979) studied distribution of isoenzymes of ß-amylase in seeds of soybean (*Glycine max*), the semicultivated type (*G. gracilis*) and wild type (*G. ussvriensis*) by the isoelectric focussing method. Four isoenzymes (1-4) were found in seeds of soybean and related species. Their respective isoelectric points were 5.12, 5.25, 5.38 and 5.52. According to Silva et al. (1990) quantities of simple sugars produced were always less than the amount of sucrose and raffinose plus stachyose which disappeared. Thus during germination there was decrease oligosaccharides in soybean. Bewely and Black (1978) mentioned that in soybean both raffinose and stachyose levels decline by about 50% over the first 48 h after imbibition starts, during which time germination occurs and rootlets are formed. In view of Pazer et al. (1962) free fructose and glucose accumulated within germinating seeds of soybean were due to sucrose, raffinose, and stachyose breakdown. Endogenous α galactosidase and invertase activity were found to increase during germination time reaching the maximum concentration after 6 h of soaking followed by 72 h of germination (Cruz et al. 1986). McCleary and Matheson (1974, 1975, 1976) observed very low levels of

ß-mannanase activity in soybean seeds which according to them reflects low amount of galactomonnan in the soybean seeds.

The process of nucleic acid and protien synthesis in soybean seeds during germination has been studied by some workers. Electron microscopic studies on cotyledons of pea, soybean, *Vicia faba* and *Phaseolus vulgaris* have shown that ribosomes aggregate into polysomes over a period of several days after imbibition, and that these polysomes are often associated with endoplasmic reticulum. Six leucyl and three tyrosyl t-RNA have been extracted from two day imbibed soybean seeds. Between the 2nd and 15th day, however, two of the leucyl t-RNA increase about threefold while the levels of the others fluctuate only slightly. Of the tyrosyl t-RNA, two delcine and one rises over the same period (Bicke et al. 1970). The significance of these changes can still only be guessed but one possibility is that protein synthesis might be regulated at the translational level in cotyledons of different ages by the availability of certain aminoacyl t-RNA. In differentiated seedlings of pea and soybean differences occur in the synthetases and t-RNA species between difference organs (Anderson and Cherry 1969), Kanabus, 1971; Wright et al. 1974). In 4 day old soybean cotyledons there are three leucyl t-RNA synthetases, one of which charges two of the six leucyl t-RNA species while the

other two synthetase charge the remaining four leucyl t-RNA species. In contrast, the hypocotyls contain only the latter two synthetase, perhaps because only four leucyl t-RNA spceis occur in appreciable quantities in this organ. According to Anderson (1979) isolated soybean Cv. Kent embryonic axes metabolized C^{14}-glycine to ATP within one hour of imbibition. About 5 times as much C^{14} hypoxanthine and C^{14} inocine was converted into GTP as into ATP in embryonic axes. 2 independent pools of AMP appeared to be used in purine nucleotide synthesis of soybean axes. Simontacchi et al. (1993) monitored the content of α tocopherol (αT) in isolated soybean Cv, Hood embryonic axes upon germination. Dry, high vigour axes contained 1-2 n mol αT/axis and after an increase during the first 6h of imbibition, there was delcine to 1.0 n mol at 24 h of incubation. It was suggested that the cellular content of αT is physiologically adjusted as a response to conditions of oxidative stress. Bae et al. (1986) studied influence of ginseng saponin on ascorbate synthesis in germinating soybean seeds. They found that ascorbate synthesized during the germination was always much higher than that of control when soybean were rinsed in 10^{-4}% ginseng saponin solution for 24 h. It was confirmed that the saponin fraction stimulated the ascorbate biosynthesis during soybean seed germination.