Chapter-2

REVIEW OF LITERATURE

The research work so far carried out mainly on medicinal plants is aiming only at foliage and herbage yield. But there are only few published literatures on pharmacological aspects as well. In Aegle marmelos.Corr., an important medicinal plant, certain research work has been carried out in this line. However, in order to have a clear understanding of its seed technology, physiology and biochemistry aspects; the literature available is yet to be explored. Present work is based on these aspects of the plant species.

2.1. Germination

The seed occupies a unique position in plant science and related scientific analytic methods. It has fundamental importance as a tool in plant physiology due to its known physiological growth potential and its role in growth and development (Chinoy et al., 1969). The reaction between activation of essential
enzymes, sequential release of hormones and the energy relations of the process during seed germination are still unknown. It is here that plant physiologists, biochemists and biophysicists need to work together to analyse the metabolic function (Sen, 1984). There is voluminous data on seed germination which has been adequately dealt with in research reviews published (Mayer and Poljakoff-Mayber, 1963, 1975, 1982, 1989; Borthwick, 1965; Brown, 1965, 1972; Lang, 1965; Kozlowski, 1972; Khan, 1977; Bewley and Black, 1978, 1982, 1985, 1994; Oaks, 1983; Murray, 1984).

The degradation of storage proteins during seed germination has been studied for a long time, the starting point being the establishment of protein degradation and the detection of proteolytic enzymes in seeds (Phinney and West, 1960; and Lieerman, 1979). Since then, histochemical and cytological investigations on the characteristics of the process of protein degradation which leads
eventually to free amino acids have been carried out (Ashton, 1976; Muntz et al., 1985).

In freshly harvested seeds of Rauvolfia, heavier and better germination has been reported (Choudhary, 1963). These seeds do not require any rest period and they germinate soon after harvest, as reported by Dutta (1962). A study conducted by Verma et al. (1989) reveals that by the germination test for Isabgol (Plantago ovata) using the top of the paper method (T.P) at 25°C germination took place in seven days. Kumar et al. (1992) reports that the removal of pulp helps higher germination in Clove seeds.

The process of seed germination as defined by Bewley (1997) commences with the intake of water by the dry seed and is completed when a part of the embryo, usually the radicle extends to penetrate the structure that surrounds it. Subsequent events including the mobilization of the major storage reserves are associated with seedling growth. Imbibition of water is followed by a general activation of seed metabolism.
Increased respiration is one of the earliest biochemical events already detected in imbibed seeds. This is closely followed by the release of hydrolytic enzymes that digest and mobilize the stored reserves and renew cell division and cell enlargement in the embryonic axis (Bewley and Black, 1985).

The mobilization of the stored reserves in seeds studied in cereal grains and legumes (Bewley and Black, 1983, 1985; Mayer and Poljakoff-Mayber, 1989; Copeland and McDonald, 1995) reveals that the breakdown of carbohydrate reserves, starting with enzymes and the regulation of these enzymes, activated by hormones are well documented in literature. The importance of the effect of embryonic axis on reserve mobilization during germination has been emphasized by Pretorius et al. (1998). The soaking injury was more pronounced in the embryonic axis than in the cotyledon in Phaseolus vulgaris.
2.2. Dormancy

The state at which growth of seeds is temporarily suspended was distinguished as dormancy from quiescence. Quiescence is the condition of a seed when it is unable to germinate because the external conditions normally required are not present; and dormancy is the condition of a seed when it fails to germinate because of internal conditions, even though external conditions are suitable. Recently Bewley (1997) defined dormancy as the failure of an intact viable seed to complete germination under favourable condition.

The presence of dormancy possesses problem in the field by showing variation in germination resulting in unevenness of coat-imposed dormancy and maturity of the crop. Certain concepts and theories of seed dormancy have been propounded over the years by several researchers on these aspects (Bewley and Black, 1982,1994; Mayer and Poljakoff-Mayber, 1989; Bewley, 1997).
A major factor of seed dormancy is the presence of a hard seed coat, which was studied in many plant families. According to Quinlivan (1971), the seed dormancy in leguminosae family is due to 'hard seededness'. The impermeability of the seed coat is related to changes occurring in the fine structure of the hilum (Pohtsov, 1976). Dutta and Basu (1982) have reported that the dormancy in Cassia sophera seeds is exogenous and coat-imposed. The seeds of most leguminous plant species are dormant due to hard seed coat which are impermeable to water and gases (Kohli and Kumari, 1986). The dormancy may also be due to immature embryo and after-ripening requirements and the presence of the inhibitors.

The failure of germination in Kolinji, a medicinal plant, is due to the presence of a thick waxy layer upon the seed and not due to hardness of the seed coat. The variability in the germination/sprouting of hard seeds appears to be due to variations in the degree of permeability of moisture through the wax layer
which again possibly depends on the thickness of the waxy layer (Utaman, 1953). Atwater (1980) reports that the presence of seed dormancy in Catharanthus roseus is due to poor imbibition or partial imbibition which prevents the leaching and neutralization of contained inhibitors. Kumari and Kohli (1984) on Cassia occidentalis reports that there is inbuilt dormancy in seeds which is not due to the imbalance or absence of particular active hormone. It is also suggested that the impermeability of seed coat is due to the presence of waxy coating in the species. Thomas (1994) on Cassia sophera, reports that it is obvious that the dormancy of seeds might be exogenous and coat-imposed.

The seed coat play vital role in the species because it prevents water intake. According to Bewley (1997) seeds of some species are prevented from completing germination because the embryo is constrained by its surrounding structures and the phenomenon is known as coat-imposed dormancy, and embryos isolated from these seeds are not dormant.
The germination capacity of seeds of medicinal plants is normally low due to the presence of dormancy. A slight damage or mechanical injury to the seed coat or chemical treatments could release the seeds from dormancy. Pre-treatments such as mechanical or chemical scarification, chemical soaking, leaching with water, are also employed to break dormancy in seeds. Seed germination responses of certain wild and cultivated medicinal plants to various physical and chemical treatments aimed at breaking dormancy and improving seed germination percentage are also reviewed.

Santapan (1956) suggested that the poor germination in Rauvolfia seed was due to the fact that the embryos of freshly harvested/collected seeds were not fully developed and that some time was required after harvest for their full development. In contrast, Dutta (1963) reported that the seeds of Rauvolfia did not require any rest period and they germinated soon after harvest. The development of hard seeds is often noticed in some of the leguminous
medicinal plants. Sidhu and Caners (1977) reported the development of 'hard seededness' in *Medicago lupulina*.

Bewley and Black (1982) have cited reasons for coat-imposed dormancy. The dormancy in *Tetrapleura tetraptera* is due to the presence of hard seed coat and lack of certain growth-promoting substances in seeds is reported by Odoemena (1988). However, Bewley and Black (1982), Mayer and Poljakoff-Mayber (1989) have also mentioned the controlling factors which establishes coat imposed dormancy.

According to Chen et al. (1984), coat-imposed dormancy in 80 species of medicinal plants is due to (i) Impermeability of seed coat to water or gases (ii) Seeds with immature embryos (iii) and/or both. Bahuguna et al. (1987) reported the presence of dormancy in *Cinnamomum camphora* due to unbreakable hard seed coat.

At various times the suggestion has been made that loss of viability is due to the
accumulation of toxic substances of various sorts. According to Dey et al. (1967) the accumulation of phenolics, including coumarin and ferulic acid may contribute to the inability of non-viable rice seeds to germinate.

2.3. Water soaking

Washing *Panax gingseng* seeds in running water for 15-30 days followed by treating them either with Gibberellic acid (200 ppm), Kinetin (20 ppm) or Ethylene (500 ppm) for two days eliminates the presence of inhibitors and shortens the duration of after-ripening period from 8 months to 4 months (Chen et al., 1984). The embryonic axis damage can be reduced by exposing the seed to a limited amount of water (Presowing seed treatment), which enables all parts of the seed to become hydrated uniformly before planting (Noggle and Fritz, 1986). Cseresnyes and Baleanu (1987) recommended two hours of water soaking at room temperature for improvement of Coriander seed germination. Mandal et al. (1987) observed 80-82 per cent
germination in Coriander and Fennel seeds soaked in water for 5 and 10 hours respectively before sowing. Szabady et al. (1987) suggested that presoaking the seeds in tap water at room temperature followed by pre-treatment at 4°C for four days improved germination in Amsonia tubernaemontana. However, Basu and Sur (1988) reported that the presowing treatments in general showed beneficial effect on crop growth by inducing tolerance to stress.

2.4. Mechanical scarification

Damage or mechanical injury to the seed coat could release the seed from dormancy. The mechanical scarification also is a method employed to break dormancy in seeds. Germination improvement through various treatments to the seeds is aimed at breaking dormancy and there by it enhances germination percentage. Mechanical treatments like scratching or shaking the seeds with some abrasives or cutting the seed coats or thermal treatment with boiling water improve the
permeability of the seed (Rolston, 1978; Shirai and Kagei, 1985; Kohli and Kumari, 1986; Muir and Pitman, 1987; Singer and Pitman, 1988). Shirai and Kagei (1985) achieved an improved germination rate of 89 per cent in green house by seeds whose testa had been slightly cut with scissors, as against the normal rate of 17 per cent germination by intact, untreated seeds.

2.5. Temperature treatment

The importance of seed treatments for achieving higher germination at a high temperature has been emphasized by several workers (Lang, 1965; Koller, 1972; Heydecker, 1977; Menon and Kulkarni, 1987; Mayer and Poljakoff-Mayber, 1989). According to them, different seeds have different temperature ranges for their germination. The temperature optima during treatment of seeds varied from species to species or even in different cultivars of the same species. The optimal temperature for the germination of a seed is that at which maximum percentage of germination
is obtained in the shortest time. In *Cucumis sativa* the maximal temperature at which germination occurs may be as high as 48°C (Knapp, 1967).

Quick germination and vigorous seedling growth due to thermal treatment is ascribed to invigoration of the dormant system during the process of treatment leading to increased tissue hydration, quick distribution of nutrient reserves and better reactive atmosphere for various metabolic processes (Mehta and Chinoy, 1978). The seeds of *Solanum nigrum* progressively lose their ability to germinate within 48 hours at 50°C or within 6 hours at 55°C (Givelberg et al., 1984). In many desert seeds storage at 50°C promotes germination while storage at normal temperature lowers the rate of germination. Thus, there is usually an optimal temperature below and above which germination is delayed but not prevented (Mayer and Poljakoff-Mayber, 1989). Presence of Abscisic acid in the seed delays seed dormancy in *Cornus officinalis*; stimulating effects of low temperature on seed
germination occur after the seeds are treated with high temperature for a long time (Guan et al., 1989).

Wagenvoot and Vanopstal (1979) suggested alternate temperature and cold treatment for improving the germination of Solanum nigrum seeds. Swertia thirata seeds required pre-chilling treatment 0-3°C for 15 days for the improvement of germination (Raina et al., 1994). Bewley (1997) suggested that abscisic acid is involved in regulating the onset of seed dormancy and in maintaining the dormant state.

2.6. Chemical scarification

Bhat and Dhar (1971) investigated the nature of dormancy in Belladonna seeds and concluded that the dormancy is due to the presence of seed coat as inhibitor and suggested that treatment of seeds with H₂SO₄, ethyl alcohol and petroleum ether should overcome dormancy. There are reports of sulphuric acid scarification being an efficient means to break the coat-imposed dormancy of seeds and to

According to Singh et al. (1984) chemical scarification and water soaking enhance seed germination in *Tephrosia purpura* and *Abras precatorius*. According to Al-Helal et al. (1989) seed treatment with H₂SO₄ is more effective in breaking seed coat dormancy in *Cassia* and *Senna* than mechanical scarification or boiling water or incision of testa. Mehta and Sen (1991) reported that seeds of *Cassia italica* exhibited seed dormancy and pretreatment with Conc. H₂SO₄ and mechanical scarification improved the germination. Sharma et al. (1992) reported significant improvement in germination of the seeds of *Terminalia bellarica* when soaked in commercial H₂SO₄ for 15 minutes followed by cracking of seeds with one hammer stroke. Sulphuric acid scarification results in rapid dehydration of seed coat of *Psoralea corylifolia*
(Linn.). However breaking of exocarp or pericarp layers enable seeds to absorb water, which results in the softening of the hard seed coat in the seeds of this species (Vivekmitter et al., 1993). Scarification treatment with 90 per cent \( \text{H}_2\text{SO}_4 \) for 20 minutes is recommended as the most beneficial in breaking the seed coat dormancy of *Cassia sophera* seeds (Thomas, 1994).

According to Kalavathi (1996), the hard seeds prevailed up to 67 per cent in *Cassia* and to overcome this, treatment with commercial \( \text{H}_2\text{SO}_4 \) 100ml Kg\(^{-1}\) (v/w) for 10 minutes followed by thorough washing, significantly improved the germination without affecting seed quality.

2.7. Growth hormones

Plant-growth regulators are known to influence germination of seeds. The effect of these substances has been studied primarily with a view to improving germination under normal as well as unfavourable conditions. The externally applied plant growth substances affect various metabolic pathways as well as developmental
stages such as regulation of precocious germination. Information is available on the effect of exogenously applied compounds on the germination of the seeds. Some of the notable reports are available on this topic (Letham et al., 1978; Crozier and Hillman, 1984; Murray, 1984; Jacobsen and Beach, 1985; Chadwick and Garrod, 1986; Davies, 1987; Mayer and Poljakoff-Mayber, 1989). It is found that endogenous hormones and those applied exogenously act in similar fashion although this assumption is by no means justified (Mayer and Poljakoff-Mayber, 1989).

The beneficial effect of presowing treatment of seeds with growth regulators on seed germination and growth has been reported by a number of workers (Chinoy et al., 1969, 1970; Saxena, 1974; Vora and Patel, 1979; Sing and Saxena, 1991). Thus the presowing seed treatment with growth regulators has been a matter of interest for plant physiologists for a long time.
According to Dhar and Bhat (1978) the maximum germination in *Belladonna* is obtained by treating with GA$_3$. In an experiment on pea plant (*Pisum sativum*), Padma (1980) observed more leaves and higher dry matter production due to the presowing seed treatment with 100 ppm GA$_3$ solution as compared to the control. Horowitz and Givelberg (1982) reported that soaking the seeds in $5 \times 10^{-4}$m concentration of GA$_3$ solution improved the germination of *Solanum nigrum* both in dark and light. Suchorska and Ruminska (1983) found that the seeds of *Belladonna* and *Datura* contain high amount of Abscisic acid which inhibits germination and treating the seeds with GA resulted in higher germination.

Saxena *et al.* (1987) investigated the pretreatment effect on pea seeds with IAA, GA$_3$ and Kinetin during growth and found that the pretreatments increased plant dry weight and yield characters. *Gloriosa* seed germination had improved by soaking in 4000ppm thiourea and the early germination was achieved by soaking in GA$_3$ (Supari *et al.*, 1993). Laura *et al.* (1994)
reported improvement in the germination of newly harvested *Muntingia calabura* seeds subjected to 210 ppm of GA$_3$ treatment.

2.8. Metabolic changes during germination

The metabolic activity of seeds begins with the imbibition of water and gradually increases as germination marches on. The storage materials in the seed are broken down and parts of the broken down products are transported from the cotyledon or endosperm to the growing axis. The metabolic changes in the composition of seeds during germination have been investigated in a number of plant species (Chinoy et al., 1969; Palmiano and Juliano, 1972). Smith (1974) carried out some interesting work on the reserve hydrolysis in legumes and this study of 500 legume species has revealed that there are 8 basic patterns of hydrolysis of reserves from the cotyledons.

Studies of Smith (1974) on *Cassia* revealed that the mobilization of reserves begins on the abaxial side of the cotyledons and the patterns
of mobilization are not associated with vascular strands. The series of biochemical changes during seed germination have been documented by many authors (Mayer and Poljakoff-Mayber, 1975, 1982, 1989; Ashton, 1976; Bewley and Black, 1978).

2.8.1. Starch

According to Abrahamsen (1964) and Abrahamsen and Sudia (1966) their studies on varieties of soyabean have revealed that storage of carbohydrates is the principal primary substrates during germination. It has been reported that the starch content in the endosperm of many monocotyledonous seeds decreases with the advance in germination (Saxena et al., 1970; Vora et al., 1974a, 1975). This suggests that mobilization and utilization of starch increase with the advance in the germination process of seeds.

In legume seeds also, the starch content depletes and the amylase activity increases during the early periods of germination (Juliano
According to Panneerselvam (1987) in the tubers of *Dioscorea esculenta* and in the rhizomes of *Curcuma longa* it has been observed that the breakdown of starch in the sugars is at a very slow pace when the buds are dormant and it attains a rapid pace just before the sprouting occurs. On the contrary, in Soya beans, the starch content increases during seed imbibition and germination (Adams et al., 1980; Kamaladevi et al., 1990). Savitri and Desikachar (1990) showed an increase in starch content in Soya bean seedlings and a starch depletion in Pigeon pea seedlings in 24, 48, and 72 hours after the commencement of germination. In *Tagetes minuta*, a transient accumulation of starch synthesized from free sugars formed as a result of glycoxylate pathway from lipid reserves was reported (Drewes and Vanstaden, 1991).

Germination pattern and food reserve mobilization in winged bean showed that
comparatively very small amount of starch was present in winged bean seeds. But during germination starch content was increasing gradually. The enhancement of starch content was significant up to the 5th day of germination and later only marginal increase was observed (Nabeesa-Salim and Harikumar, 1994). According to these authors considerable amount of ethanol soluble sugar content was present in dry and imbibed seeds of winged bean.

2.8.2. Sugars

During germination of seeds the degradation of starch results in the formation of glucose and eventually sucrose is synthesized. Sucrose is formed by a complex mechanism. Mayer and Poljakoff-Mayber (1989) have depicted this as follows: Glucose is phosphorylated in the presence of ATP. Part of Glucose-6-phosphate formed is converted to fructose-6-phosphate (F-6-P) and part to glucose-1-phosphate (G-1-P). The G-1-P in the presence of uridine-triphosphate (UTP), is converted to uridine-diphosphoglucose (UDPG). Sucrose is then formed
by the condensation of UDPG and F-6-P. Finally sucrose is synthesized and the broken down products are transported to the developing embryo. It is reported that in seedling embryos, the sucrose synthase pathway and acid invertase are active and sucrose is broken down by the sucrose synthase pathway. This pathway is dependent upon UDP and pyrophosphate (PPi), which are produced by a cyclic series of reactions (Huber and Akazawa, 1986; Sung et al., 1986; Xu et al., 1986;).

When sucrose is broken down by this pathway the hexoses from sucrose can enter glycolysis. In addition, Xu et al. (1989) proposed that in the plant cell cytoplasm, alternative enzymes are present at various steps in glycolysis and gluconeogenesis for the inter-conversion of sucrose and pyruvate. They have further reported the sucrose metabolism of developing and germinating Lima bean seed with a view to characterizing the fundamental portion of cellular carbon nutrition.
In the seedlings of *Sorghum vulgare*, the total sugar content increases up to 72 hours of germination and then falls (Vora *et al.*, 1974a). The soluble sugar present in the cotyledons of different varieties of Pigeon pea increases to a maximum on the second day of germination and decreases slowly up to the fourth day followed by a rapid decline (Sharma and Pant, 1979).

2.8.3. Protein and Amino acids

Seed storage proteins, which are metabolically inactive, are usually located in protein bodies. Oota *et al.* (1953) have concluded that the proteins, which are frequent reserve material in the seed, are often broken down during germination with concomitant rise in amino acids and amides followed by de novo protein synthesis in the growing part of the embryo. Thus, the first observable change in germination is a change in protein into soluble nitrogen (Klein, 1955). He showed clearly that there was an increase in soluble nitrogen in the seeds of Lettuce which germinated, and not in
those which remained dormant. Cherry (1963) showed that during the germination of Peanut seed, over 60 per cent of the dry weight of cotyledon and 70 per cent of the protein are depleted. Varner (1965) suggested that during germination, seed proteins are hydrolysed in the endosperm or cotyledon into peptidases and amino acids, which are, translocated in the growing axis. The maximum rate of hydrolysis of storage proteins coincides with the maximum rate of growth of the seedling.

The early protein degradation might be due to the combined activity of pre-formed soluble peptidases present in dry seeds whose activity rapidly declines over the first few days after imbibition (Chrispeels and Varner, 1967) and an insoluble membrane-bound proteinase, which appears to be synthesized de novo within 48 hours of initial imbibition (Taiz and Jones, 1970). The biochemical aspects of legume seed proteins have been reviewed by Millerd (1975). Wandawi et al. (1984) from their studies have reported 25.20 per cent protein, 21.10 per cent
lipid and 18 per cent amino acids at maturity in Hibiscus sabdariffa seeds.

In this respect, major work had been carried out in pea seeds (Beevers and Splittstoesser, 1968). Generally some of the proteins and peptidases are present in dry seed while others appear during germination. The protein synthesis is activated in the imbibition phase (Marcus and Feely, 1964) and it also varies with the period of germination (Daussant et al., 1969). The protein changes were studied in detail in bean seed during germination (Juo and Stazby, 1970) and in Vicia faba (Briarty et al., 1970).

According to Harvey and Oaks (1974), the hydrolysis of endosperm protein in Zea mays could be attributed to the de novo synthesis of hydrolytic enzymes. Huang (1987) reported that protein content decreased during the germination with a corresponding increase in amino acid and proteinase activity. According to Dommes and Van de Walle (1990), during early phase of
imbibition, polysomes are synthesized and they act as new protein synthesizing complexes. New mRNAs are transcribed as germination proceeds and these encode proteins essential for the cellular metabolism during germination (Bewley and Marcus, 1990). In spite of the marked hydrolysis of protein content in winged bean during seed/seedling, many cells near the vasculature in most of the ground cells of the adaxial side shown by histochemical studies, reduction occurred in protein content was estimated by biochemical methods is very low (Kahleel, 1998).

2.9. Hydrolysing enzymes involved in germination

Perusal of literature on metabolic changes during germination, reveals that enhanced activity of catalase, cytochrome oxidase, peroxidase, phosphatase and polyphenolase occurs during seed germination phase (Vanfleet, 1952; Burris, 1953). Generally the activity of catabolic enzymes for the hydrolysis of starch, protein, hemicelluloses, poly
phosphates, lipid and other storage materials rises fairly rapidly as germination proceeds (Mayer and Poljakoff-Mayber, 1975).

Enzymes for the hydrolysis and transformation of sucrose are absent from the cotyledons but are present in the axis, to which sugar is presumably transported (Bewley and Black, 1985). Control by the embryonic axis of the breakdown of storage proteins in cotyledons of germinating seeds of Citrus lemon has been studied by Garcia-Agustin et al. (1991). During early phase of germination, i.e. at the time of radicle emergence, there is a burst of respiratory activity and both glycolytic and oxidative pentose phosphate pathways resume at this phase of germination (Botha et al., 1992; Bewley and Black, 1994).

2.9.1. Amylase

The site of origin and extent of activity of amylase in Maize seeds during germination were studied by Dure (1960). Fredenberg and Nielson (1965) studied the isozyme pattern of
amylase in germinating barley seeds which consisted of different zones of which two were β-amylase isozymes and 5-α-amylase isozymes. The increased activity of α-amylase during germination is probably due to the de novo synthesis in these cases (Filner and Varner, 1967). Similar work was done in pea seeds also (Yomo and Varner, 1973). Eugeniusz et al. (1973) explained that the amylase activity is regulated by the concentration of reducing sugars in vivo in both cotyledons and axis. Majority of the work in this field was done on α- and β-amylases during germination.

The enzyme changes were followed in other crops like Mung bean (Sheoran, 1980), seeds of soya bean (Hildebrand and Hymowitz, 1981), bean stem tissues (Bill, 1984) and Ground nut (Aruna Sharma and Sen-Gupta, 1987). The multiple forms of amylase in germinating rice were studied by Daussant et al. (1983). Wang et al. (1988) observed that the amylase activity in the cotyledons increased gradually and reached a
maximum on the 5th day, while the starch decreased and soluble sugars increased.

2.9.2. Invertase

The presence of the enzyme in germinating seeds of barley had been demonstrated by Prentice (1972), which accounted for partial break down of sucrose. Eldan and Alfred (1974) reported acid invertase activity in germinating Lettuce seeds. According to them, de novo synthesis of the enzyme takes place during germination.

2.9.3. Protease

The presence of proteases and peptidases has been shown in many seeds especially during germination. In germinating Peanut seeds, the accumulation of amino acids regulates the protease level in the cotyledons (Yomo and Varner, 1973). Yomo and Srinivasan (1973) followed the protein breakdown and formation of protease in attached and detached cotyledons of Phaseolus vulgaris and found an increase in both tissues. It is seen that protease activity
present in dry seed is enhanced during germination (Harvey and Oaks, 1974). Basha and Beevers (1978) also carried out similar studies on the development of proteolytic activity and protein degradation in Pisum sativum. During germination proteins are hydrolysed to free amino acids, which support protein synthesis in endosperm and embryo (Tully and Beevers, 1978). In germinating Beans, the proteolytic activity increased during the first 7 days of germination.

The increase was partially dependent on the embryonic axis (Gepstin and Ilan, 1980). The combined action of various proteolytic enzymes thus results in total degradation of storage proteins (Ikuko and Hiroshi, 1980). According to Aruna Sharma and Sen-Gupta (1987) the protease development system may not be regulated by a feed-back inhibition mechanism. Abe and Arai (1977) could purify and characterise the protease enzyme in germinating corn.
2.9.4. Lipase

Much work had been done in various crops as regard to lipid level and lipase activity during the germination phase. Hutton and Stumpf (1969) during their studies on the fat metabolism of Castor bean seeds reported that β-oxidation takes place 4 days after germination. The major hydrolytic enzymes concerned with the lipid metabolism during germination is the lipase. Tovener and Laidman (1972) who reported the induction of lipase activity during germination of wheat grains showed that the activity is dependent on factors emanating from the embryo.

According to Shoshi and Beevers (1974), two lipases were found in Castor bean seed endosperm namely; acid lipase in dry seed and alkaline lipase during germination. Storage tissues of all the oil seeds except Castor bean contained only lipase activity which increased during germination (Anthony and Robert, 1978).

2.9.5. Catalase

Some workers reported a direct correlation of catalase activity and seedling growth (Nanda,
1950; Chikasne, 1953; Verma and Van Huystee, 1970) while others showed an inverse correlation (Galston, 1951; Halevy, 1964; Prathapasenan et al., 1969). Yokoyama (1956) studied the relation of catalase activity to mitochondrial respiration and pointed out that catalase directly affected the oxidation and reduction of cytochrome-C oxidase system. A rise in enzyme catalase has been reported as germination progresses (Papov, 1965).

On the other hand, low moisture content due to water stress reduced the catalase activity in Wheat, Sesamum and Eleusine as and when germination advanced (Acharya, 1968).

Vora et al. (1974 b) have also observed that the low moisture level of seedlings can depress the enzyme activity. Esashi et al. (1979) have studied the involvement of catalase in the regulation of germination of Cocklebur seeds. Eising (1989) has determined the catalase synthesis and turn over during peroxisome transition in sunflower cotyledons.
2.9.6. Peroxidase

Peroxidase catalyses the oxidation of diverse hydrogen donors and peroxidase activity is implicated in many biological events in plants depending on the nature of the donor. This enzyme is known to fulfil many functions in plants. Peroxidase activity is reported to be associated with active differentiation (Halevy, 1964; Saxena, 1979). It is well established that high concentrations of auxins promotes the release of ethylene in the regulation of peroxidase activity by growth hormones in many plant species, reported by various authors (Lavee and Galston, 1968; Stuber and Levings, 1969; Birecka and Galston, 1970; Ritzert and Turin, 1970; Lee, 1972). In cotton plants, auxin-induced evolution of ethylene coincides with the enhancement of peroxidase activity (Sakai and Imaseki, 1971; Fowler and Morgan, 1972).
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