2. REVIEW OF LITERATURE

In general, the dormancy of seeds appears to be commonly either a consequence of structural characteristics of the seed coat or a consequence of growth regulator interactions such as inhibiting substances or promoting substances. In many cases, the growth regulatory system is most pronounced in the outer layers of the seed, especially in the seed coat, and so these two types of physiological mechanisms are sometimes interrelated.

The limitation of germination which the seed coat may impose is through the provision of chemical materials which could prevent the embryo growth. The seed coat may simply provide chemical species which create osmotic values unfavorable to growth (Koller, 1957). It may provide growth inhibitors which could limit growth or it may provide more complex biochemical systems which are related to the photosensitivity of the seeds. In all these cases the dormancy can be controlled by removal of the seed coat or simply water washings. During the sowings in soil inhibitors are less troublesome comparatively to the sowings on germination paper (Li-Sn and Thoday, 1952).

Release of solutes from seed during initial phase of hydration indicates the extent of structural reorganization of the biomembranes. Electrical conductance of seed leachate is a good indicator of the physiological status.
and emergence potential of seeds. An increase in leakage of electrolytes is associated with a decrease in germination (Simon, 1974).

Seed dormancy influences germination patterns in natural ecosystems and impacts persistence of seeds in cultivated fields. It can also alter cultural practices for crop plants by delaying germination. Studies in this area have provided evidence elucidating dormancy mechanisms in both wild and cultivated plants. Research in this area involves ecological, embryological, morpho-physiological, biochemical and molecular approaches to describe seed dormancy (Baskin and Baskin, 2000; Baskin et al, 2000; Hidayat et al, 2000).

2.1 VARIABLE DORMANCY

Wild oats (Avena fatua) produce seeds every year, but dormancy can vary among different members of the same population with some individuals remaining viable in the soil for seven years after they are produced (Naylor and Jana, 1976). Variable dormancy has also been observed in seeds of white spruce (Picea glauca Moench Vass) (Wang, 1978) and Japanese red pine (Pinus densiflora Sieb. and Zucc) (Asakawa and Hotta, 1956) that were produced by the same trees in different years. The expression of dormancy known to be under genetic control (Naylor, 1983; Edwards and El Kassaby, 1995) so variable dormancy possibly represents an evolved survival strategy to extend germination over many years and different environmental conditions. Variable dormancy ensures that no matter the present environment, at least some members of the population will survive and produce seeds for the next generation, a kind of an insurance policy against
environmental change. It is conceivable that variable dormancy is an universal phenomenon in natural populations of most plant species.

Buried seeds of small herbaceous plants and grasses exposed to natural seasonal temperature changes may exhibit annual cycles of dormancy and non-dormancy. The fresh and buried witch grass seeds that are dormant in early October become non dormant during late autumn and winter (Baskin and Baskin 1985). During spring and summer however, seeds progressively lose their ability to germinate as they gradually re-enter dormancy.

Recalcitrant seeds remain at relatively high moisture content are produced by species native to moist tropical regions. Characteristically, the seeds are non-dormant because environmental conditions such as temperature and moisture are always suitable for germination. Recalcitrant seeds usually germinate soon after maturity, and rarely can be stored beyond three months (Chin. 1990). Many orthodox seeds need dormancy release treatments to germinate but recalcitrant seeds do not.

During their maturation on the mother plant, seeds acquire the capacity of germinate but also develop a physiological blockage of this capacity called dormancy or primary dormancy. Some in the optimal conditions for germination, the dormant (D) seeds do not germinate or germinate slowly compared to non-dormant (ND) seeds. This internal transient blockage disappears by exposing seeds for a period of time to learn and dry conditions (after opening) or to cool and moist conditions (stratification) (Bewley and Black, 1994).

Mature primary dormant seed can enter into a state of secondary dormancy in response to unfavourable germination conditions and can remain
fully inhibited for long periods without loss of viability (Bewley and Black, 1994).

2.2 SEED DORMANCY AND PLANT HORMONES

The hormone theory explains seeds dormancy by the opposed properties of two types of hormones: the abscisic acid (ABA) that inhibits germination and gibberellins that stimulate it on the contrary (Wareing and Saunders 1971)

2.2.1 Abscisic acid (ABA)

ABA accumulation in developing seeds is low during the early stages is greatest during mid-development, when storage reserves are being synthesized and declines as the seed undergoes maturation drying. Prevention of germination during development may be due to the endogenous ABA content of the seed, the osmotic environment surrounding the seed, or both (Berry and Bewley 1992). Maturation drying and shedding from the parent plant are sufficient to release these constraints in non-dormant seeds. In these instances, there may be an associated decline in the ABA content of the seed and the sensitivity of the embryo to ABA is much reduced (Xu and Bewley, 1991).

The in situ ABA synthesis in the embryo is necessary for the imposition of dormancy which has been demonstrated in sunflower as well (Lepage-Degivry and Garello, 1992). Although applied ABA prevented germination of isolated developing sunflower embryos, this inhibition was overcome upon their transfer to water, only ABA synthesized within the embryo imposed a lasting dormancy (Lepage-Degivry and Garello, 1992);
The prevention of embryo radicle can be achieved by incubating mature seeds in solutions of ABA. This inhibition can occur even when ABA is introduced late during germination an hour or so before radicle extension would be expected to occur. This raises the possibilities that ABA acts to present a late event during germination such as radicle cell wall loosening. Indeed, ABA inhibits radicle extension in Brassica napus embryos, thus preventing them from completing germination (Schopfer and Plachy, 1985). Moreover, an analysis of the water relations of the embryonic axes indicates that neither the osmotic potential nor the ability to take up water is affected by the presence of ABA, but rather the cell wall loosening that is associated with radicle extension is prevented now this occurs is unknown. It should be noted that B. napus seeds are non-dormant, and inhibition of then germination can only be achieved by the application of ABA.

The presence of differential sensitivity of embryos to ABA may be important in the maintenance of mature seeds in a dormant state, although there are surprisingly few species for which this has been clearly demonstrated (Bewley and Black, 1994; Hilhorst, 1993). However, in wheat embryos, an interesting positive correlation has been shown between sensitivity to ABA and both resistance to germination during development and dormancy after maturation (Walker Simmons, 1987). This has led to a search for ABA responsive and dormancy-related genes in cereal embryos. Differences in patterns of protein synthesis between dormant and non-dormant embryos of cereals (e.g. Wild oat and wheat) have been demonstrated (Kawakami et al., 1992; Dyer, 1993; I and Forcy, 1994). The synthesis of some proteins is higher in non-dormant than in dormant embryos.
and that of others is less. However, despite differences in protein and nuclear protein synthesis profiles, no causal and effect relationship can be made between the presence or absence of a particular protein and dormancy.

Dormant seed of many species contain ABA and in sunflower embryos the continuous synthesis of ABA is required for the expression of embryonic dormancy (Le Page-Degivry and Giralde, 1992). Temporary radicle extension while this inhibitor is present, its removal leads to the completion of germination.

It has been demonstrated in various species of which Arabidopsis thaliana (Julien et al., 1999) and Nicotiana plumbaginifolia (Grappin et al., 2000) that the hormone absent and (ABA) a powerful inhibitor of germination; plays an essential role in the expression of dormancy because after sowing the dormant seeds produce ABA. However, the mechanism of the inhibition of germination by ABA stay unknown.

### 2.2.2 Gibberellic acid

Another growth substance involved in the dormancy mechanism is the gibberellic type of growth promoter. The first report on the breaking dormancy with gibberellic was reported by Franklin and Waring (1931). Experiments of Waring and Villers (1934) indicated that the growth stimulating substances may be involved in the breaking of dormancy of Flaxinus seeds.

GAs are known to ablate the requirement of seeds for various environmental conditions promote germination and to counteract the inhibitory effects of ABA frequency in combination with cytokinins (Bewley and Black, 1982, 1994). In seeds of a very few species, there is an increase in GA content in response to an external stimulus, but there is no evidence that this...
increase is important for breaking dormancy. On the other hand, sensitivity to GA may be a key factor. Karssen et al. 1989, GA-deficient mutants of tomato (cv. Albinata) and Arabidopsis (ga-3, ga-1) require an exogenous supply of GA for certain to germinate (Koostanjel and Vander Veeren 1995, Croot and Karssen 1997). Embryos of GA-deficient tomato will germinate if removed from the remaining structures. Thus, the role of GA in these seeds is probably restricted to the induction of endosperm-weakening enzymes.

GAs appear not to be involved in the control of dormancy per se but rather are important in the promotion and maintenance of germination that is they act after the ABA mediated inhibition of germination has been overcome. The activities of ABA and GA may be linked, because in the aba and toc12 mutants of Arabidopsis reduced dormancy is accompanied by a lowered requirement for GA to achieve germination (Leon-Koostanjel et al. 1995).

2.2.3 Auxins, cytokinins and ethylene

Auxins are known to be necessary for the growth of the embryonic tissue while they do increase at the time of germination or shortly before (Hemming, 1965; Kawano, 1980); there is no convincing evidence that they are directly involved in dormancy mechanisms. Kinins are also reported to break seed dormancy in few cases (Miller, 1958); but there is no evidence that these growth regulators participate in the natural dormancy phenomenon. Bogateck et al. (2004) observed that the rate of activation of ethylene biosynthesis seems to be of primary importance for the future germination and it is possible that HCN plays an important signaling role in this event leading to breaking of seed dormancy in apple. Dormancy in apple is expressed as
slow germination which results in several morphological abnormalities. These symptoms do not appear in germinating non-dormant embryos isolated from cold treated (stratified) seeds. Ethylene is one of the plant hormones which is required for removal of seed dormancy and subsequent germination. Hydrogen cyanide, occurring in apple seeds, has also been established as a dormancy affecting factor leading to elevation of dormancy symptoms (Bogatek et al., 1999).

2.3 SEED DORMANCY: SEED STRUCTURE AND EMBRYO

Embryos that are constricted by a mechanical barrier, such as the surrounding endosperm, perisperm, or megagametophyte (i.e., those that exhibit coat-enhanced dormancy), appear to require a weakening of these structure to permit radicle protrusion. This weakening involves partial enzymatic degradation of the walls (e.g., of the endosperm of tomato, lettuce, tobacco and Datura stramonium).

In germinating wild type tomato seeds, lowering of the puncture force required by the radicle to penetrate the mannan-rich endosperm cell walls has been attributed to endo-β-mannanase, an enzyme produced within the endosperm itself at about the time of radicle emergence (Groot et al., 1958). The enzyme is first produced in the micropylar region and later, after germination, in the rest of the endosperm. Each region produces different isoforms (Nonogaki and Moronashi, 1996; Tocrop et al., 1996; Vogt and Bewley, 1996). Endo-β-mannanase is also produced in the endosperm of better seeds when the seeds are released from dormancy by GA or red light, but this also occurs post-germinatively (Halmer et al., 1978).
Teak (*Tectona grandis*) is one of the several species where physical dormancy is combined with chemical inhibitors in the fruit. In addition, the fruits often need a period of after-ripening which must be carried out before the seeds respond to other pretreatment procedures (Beckel 1989). Prolonged soaking in running water for one to several days also serves both to extract inhibitors and soften the outer seed coat. This method is also applicable to teak (Keiding 1993). In India, Yadav (1992) reported that soaking in water for 6 days was found to be a suitable alternative to alternate soaking and drying in order to overcome physical dormancy in Teak (*Tectona grandis*).

The protrusion of other hemicellulases is more critical for endosperm dissolution (Dutta et al. 1994). Indeed, by contrast to the situation in germinating seeds of lettuce and tomato, seeds of *Datura ferox* exhibit increased endo-1,4-mannanase and 1,2-endomannosidase activities in the micropylar regions of the endosperm after red light stimulation and many hours before the radicle protrudes through it (Sanchez and de Migue 1997). An increase in cellulase activity in the whole seed also occurs at about the same time as red light induced radicle elongation (Sanchez et al. 1996). Thus although no cause and effect relationships have been established between the increase in activity of these hydrolytic enzymes and the completion of germination in *Datura ferox*, the possibility certainly exists. Cellulase activity is not important for the germination of tomato or lettuce seeds. In the former, cellulase activity increases after germination (Levallois et al. 1995), whereas in the latter cellulose activity is barely detectable in dormant or non-dormant seeds (Bewley et al. 1983). Tobacco weakened by β-1,3-glucanase to permit germination. Activity of this enzyme increases before emergence of the
radicle, and ABA retards both enzyme accumulation and endosperm rupture (Leuconer et al., 1988):

Secondary dormant lettuce seeds conduct <30% of the respiration and protein synthesis that occur in primary dormant seeds and use stored reserves to maintain themselves (Powell et al., 1983). Upon release from secondary dormancy, there is an increase in respiration, but this is lower than in seeds increasing from primary dormancy (Powell et al., 1984). A wide variety of chemical materials may be involved in the inhibition of seed germination, some of them as complicated as polypeptides (Elliot and Leopold, 1952) and some as simple as furchloridale.

2.4 DORMANCY VS. ENVIRONMENTAL FACTORS

Environmental controls could be mainly categorized under four heads—mechanical, light, temperature and chemical. The requirement of some seeds for low temperature has been known and utilized for centuries. In some cases, only a brief exposure to temperature near freezing is needed to break dormancy, in some extended periods needed and in few dormancy is not broken up to several years. Pecny seeds were found to accumulate large amounts of amino acids during cold stratification (Fine and Barton, 1958). Increased percentage of germination or uniform germination can often be obtained with alternating temperatures better than with any single temperature. Potassium nitrate can bring about the same effect as the temperature alteration and is not additive in its effect with the temperature alteration. High temperatures ordinarily increase the dormancy of seeds rather than improve the germination (Table, 1959). The stimulation of germination by light is ordinarily quantitative. Another type of treatment to break dormancy is
the exposure of seeds to elevated concentrations of oxygen. Wareng and Foda (1957)

Tropical species such as bakuchiol-grass may be scarified by alternate drying and soaking. The seeds are soaked in water, then left in direct sunlight for several days. This cycle may be repeated many times until evidence of coat degradation is apparent. Hard seed coats also may be degraded by partial fermentation or by exposure to light in moderate fire. The seeds are blunted after they are covered with a layer of grass. Wilson, 1984.

Few temperate forest species require scarification (Schopmeyer, 1974); does not recommend it for any British Columbia tree, but seed coats are sometimes channeled to facilitate the germination of rare seeded pines (Leader, 1980).

The seeds sown in the seed bed at correct spacing but only half covered with soil and a layer of impure grass is spread over the seedbed and set on fire. After burning, the seedbed is immediately sprinkled with water and the seeds moistened 2 cm into the soil and watered thoroughly (Scober and Agpaoa, 1975). Scorching by burning a cover of Pennisetum grass also enhanced germination of Emblica officinalis and Terminalia chebula in India (Brahmany, 1995). However, the method was limited to other treatments that water and seed scarification, and some seeds were damaged by high intensity burning or lack of layer of grass.

Nachtal and Tripathi (2004) reported that freshly harvested seeds of Corylus colurna Linn., a temperate forest tree species growing in Himalayas, do not germinate and exhibit dormancy. Routine methods of breaking dormancy were effective in helping these seeds to germinate but the nature of
inhibition seems to differ. Stratification of seeds for one month led to 38% germination that was enhanced to 58% if stratified for two months. Maximum germination in the treatment reached in 3 weeks. GA treatment of seeds resulted in 93% germination within two weeks. However, complete removal of testa not only brought out as high as 97% germination but it was accomplished in 7 days only. Partial removal of testa from micropyle end resulted in 77% germination in 15 days whereas only 32% seeds could germinate when the testa was removed partially from the post of end.

Examination of seed parts for presence of abscisic acid indicated its absence in testa and presence in cotyledon axis. Thus the seed coat seems to mediate the process of germination standing as physical barrier for escape of the inhibitor from cotyledons.

Even when the total germination percentage does not change, the germination of most tree seeds is more rapid after they have been stratified. Damaged seeds, or those of low vigour, may deteriorate during stratification (Leačen, 1966). In such cases, the seeds should be sown without chilling.


Patel et al (1999) suggested that germination testing of Pinus australis should be conducted at 15°C in a germination chamber or at 5-6°C on the lower shelf of a refrigerator. Germination decreased at higher temperatures.

Schulz et al (2002) investigated the role of dormancy temperature and light in the regulation of seed germination of four annual asteraceae from south-western Australia. The experiment achieved to identify after opening
patterns and to relate these to climatic conditions of the habitat in which the species occur. Seeds of all species were strongly dormant at maturity and maintained levels of dormancy for time periods corresponding to the duration of summer. Dry after-ripening was promoted best by temperatures lower than those prevailing in the dry season. Germination percentage was highest at average winter temperature (15°C). Three species with seeds >0.5 mg germinated better in darkness than in light whereas germination in darkness was almost inhibited in the species with the smallest seeds (0.14 mg). The course of dormancy was tested over a range of fluctuating incubation temperatures (7-30°C) showed that seeds of three species came out of dormancy first at temperatures that prevail in South Western Australia during winter (10-15°C). Seeds from one species, introduced from South Africa first lost dormancy at the slowest temperature (7°C).

Julius O Gumadho et al. (2004) examined changes in protein expression during germination of cats' whiskers (Cleome gynandra L.) under various light and temperature regimes. Germination of cats' whiskers was inhibited by light during germination at 20°C; however, seed germination was improved at 20°C in darkness. There was no photo inhibition at 30°C. Four proteins were observed to decrease in expression as germination progressed, but their expression remained during photo-inhibition.

2.5 DORMANCY OVERCOMING STRATEGY

2.5.1 Seed scarification

2.5.1.1 Hot / boiling water treatments

Hot water treatment was the most significant in reducing the hard seeds and the best results were obtained by treating seeds at 70°C for 10
minuted. Hard seed coats up to 62 percent was broken using hot water whereas the other two treatments at 35°C and 45°C did not induce permeability at all but at 65°C number of hard seeds both was more whereas higher temperatures of 65°C resulted in more number of dead seeds.

Boiling water caused the palisade layer seeds of Anacardium occidentale and separate from the inner endo mesocarp consequently cracks in the seed coat occurred at hot continuous mode allowing water to enter many sides (Brown and Booyseb, 1989). Small but significant percentages of Garcinia varius seeds became permeable after soaking in acetone and petroleum ether (Brant et al., 1971).

Clemens et al., (1977) suggested Acacia seeds by manually chopping or exposing them to hot water at different temperatures and times. Manual chopping of seeds significantly improved germination percentage; however, in some cases the germination percentages were lower with chopped seeds than with hot water treated seeds.

Doran and Endara (1987) subjected seeds of sixteen Acacia species to nine presowing treatments. They revealed that manual chopping was the best pretreatment and recommended it for germination tests of small and sensitive research lots. They also recommended 1% immersion in boiling water as a standard treatment for many hard-coated seed lots and 1% immersion in 90°C water for hard-coated seed lots sensitive to boiling water.

Hot water enhances physical dormancy - aquaporins creating tension which consequently causes cracking of these membranes by heat (Grant et al., 1971; and offsetting the osmotic effect).
most effective when seeds are submerged into the hot water, not heated together with the water.

The high temperature may damage seeds with relatively thin seed coats. In an experiment on Cassia siamea in Thailand, 1.5 min soaking in 85°C warm water at submersion at 85°C with subsequent cooling in the water for 12-16 hours gave a germination percentage of 92-98. Longer soaking at 85°C slightly increased germination percent. Soaking from 1 to 3 min in water at 85°C caused rapid reduction of viability. It was 71% after 1 min, 47%.

Hence for this species, a brief exposure to high temperature or prolonged exposure to 85°C apparently caused heat damage (Kohnoo and Helum, 1984).

In the genus *Pistacia* the seed coat represents a barrier to embryonic growth the epicarp can inhibit germination (Catalan Bachiller, 1991), while the endocarp can reduce the rate of imbibition (El-S. Elong and Roberts, 1985). Piotto (1995) while working on the influence of scarification and prechilling on germination of seeds of *Pistacia lernowskii* observed that the analysis of the variance of germination revealed no differences that can be attributed to the treatments applied to the seeds but indicated significant differences among the various treatments in relation to the mean time to complete germination. All the scarification treatments applied were able to increase the speed of germination with respect to that of control and also reported that prechilling of seeds could act like a natural scarification gradually losing seed coats.

In an experiment on *Prosopis juliflora* and *P. flexuosa* damage in the form of dead seeds and abnormal seedlings occurred after the pre-treatment in 90°C water and only 20-30% of the seeds produced normal seedling (Catalan
and Macchiavelii, 1991. However, in an experiment by Lopez and Arantes (1998), where seeds submerged into boiling water and left to cool in the water, heat damage was neither observed in these two Prosopis species nor in P. chilensis and P. tamarugo. All the species tested in their experiment had a high germination after treatment with boiling water.

The importance of the aril in improving physical dormancy is also known in eg. A. febrifuga and S. sarmatrices (Pukkala and Kannan 1990). In T. emoda, a non-legume with physical dormancy it has been found that the aril has a strong influence on dormancy. Removal of aril was sufficient to break dormancy in the majority of seeds, while the remaining seeds needed an additional scarification (Masanga and Muguhembe 1993).

Oven drying at 100°C for 10 minutes followed by cold-water immersion was found an effective pretreatment for A. mangium in Sabah (Bowen and Euselio 1981, Quoted in Acjors and Srivastava 1995). 53% of the seeds germinated after this treatment as compared to 3% for untreated and 92% for 30 sec boiling water pretreatment; 5 minutes oven drying was apparently too short (67% germination, 80% inhibition), while 15 min or longer at 100°C apparently damaged the seed more than 95% inhibited but germination rate fell from 80 to 50% after oven heating for 15 and 60 min, respectively.

Heat damage was also observed for C. sopheriana (Todokarrlie et al, 1983); although the seeds of this species still maintained high viability (75%) after 2 minutes boiling, longer boiling and any dry heat treatment rapidly reduced viability. In C. fistula a quick dip in boiling water killed 50% of the seed and 88% were killed after 5 minutes boiling (Babotley and Kandya, 1988). Boiling water was lethal to 8 out of 20 tested species.
Ethiopia vs Acanthosicyos roxa: Acacia senegal, Cassia Fistula, Decapetalate and C. spathacea (Cleary 1996a) all other species showed some what improved germination after a brief submersion in boiling water. Heat damage at 100°C was detrimental to Pantanema butantana and Alchornea preissii, while 60-80°C greatly improved germination (Sajeevukumar et al. 1995). However, in a study by Kannan et al. (1995), reduced germination capacity was observed by boiling water pretreatment.

2.5.1.2 Acid and mechanical scarification

Where physical dormancy is caused by a thick pericarp, a long soaking treatment in sulphuric acid is often necessary. Vasista and Son (1988) investigated the effect of up to 60 min soaking of drupes of Tribuva poitonia and found that germination increased proportionally with duration of soaking. However, for Ternatia benjica Brindwa and Chakraborty (1994) found that 10-12 minutes dipping in concentrated sulphuric acid was the most suitable pretreatment, which almost doubled the percentage of germination as compared to untreated control.

Acid pretreatment may in some instances be applied prior to (Warm: moist stratification as the seeds are initially treated with acid which scarifies the coat, but the treatment is stopped well before acid has penetrated the pericarp or seed coat. After careful washing the seeds are exposed to moist stratification and dormancy has been overcome. This procedure reduces the time required for moist stratification and reduced the risk of damage by prolonged acid treatment (Gordon and Rowe 1992).

Braiman et al. (1995) observed that pre-treatment either with concentrated sulfuric acid or cowdung strongly stimulated germination recorded
as rate and percentage of Sapindus micranthus 19.84% and S. rufifolius 19.75%. 21 and 33 days after sowing. Without pre-treatments germination was zero. For water soaking gave a better germination 19.76%. Soaking is recommended as an effective and cheap treatment.

Veendo et al. (1995) treated the seeds of Ceylon (Glycyrrhiza glabra) by mechanical scarification using sandblast for 15 minutes and scarification with H₂SO₄ 1:100 for 5 or 10 minutes, 50% for 10 or 30 minutes, or 25% for 30 minutes, hot water 100°C or dry heat at 140°C for 1 hr. Germination tests were conducted at 20°C in the dark and seedling vigour was assessed.

Seed dormancy in G. glabra is due to the impermeable hard seed coat. It could be overcome by pretreating the seeds with H₂SO₄ 100% per cent for 5 minutes giving the best result. Temperature and gibberellic acid treatments were ineffective.

Nadana et al. (1996) exposed the seeds of Sapindus to 60°C for periods ranging from 0 to 24 hours. The enhancement of germination was dependent on temperature and period of exposure. Incubation at 50°C for 48 hours resulted in 89.2% germination.

The scarification of seeds were also carried out with different concentrations of inorganic acids such as sulfuric, nitric, and hydrochloric acids. Concentrated or diluted sulfuric acid gave the best results in terms of germination percentage followed by nitric and hydrochloric acids.

Rehman et al. (1999) reported improved germination in A. phillippenyi seeds scarified mechanially by choosing the seed coat and chemically by immersion in 95% sulfuric acid. Soaking in water at 70°C was the most effective method with soaking times between 0 and 100 min. safety
overcoming hard seed coat dormancy and permitting maximum germination. The imbibition and germination of unscored seeds were respectively 45% and 55% of manually scarified seeds, indicating that the balch tested comprised with both dormant and non-dormant seeds. It was observed that all manually scarified seeds imbied in water. After 9.35 min, soaking imbibition increased with increasing water temperature and ranged from 61% at 25°C to 95% at 100°C with all temperature in this range significantly increasing imbibition compared with untreated seeds. The difference in imbibition rates between temperatures decreased with increasing soaking time. Immersion in sulphuric acid for 3 min or less was insufficient to significantly increasing imbibition and 100% imbibition was achieved only by soaking for 300 mins.

Ghahre and Torsicz (2000) performed experiments to determine the effect of various scarification treatments at four temperature regimes on germination of Liquorice seeds. At 5°C none of the chemically and mechanically scarified and non-scarified seeds germinated. At 15, 25 and 35°C, mechanical scarification increased seed germination to 94.98%. However, at these temperatures, germination rate of mechanically scarified seeds were lowest among all treatments. Chemical scarification also increased germination percentage significantly to 96.95% upon soaking for 45 minutes. At 15 and 25°C, seed germination percentage and germination rate increased as the soaking time in sulphuric acid increased from 5-30-45 or 60 minutes. However, at 35°C there was no difference in germination percentage between soaking times of 5 and 60 minutes. It appears that soaking for 45 minutes provides full germination at 25 and 35°C.
Veena et al. (2001) reported that the effect of various scarification treatments was significantly different on breaking hard seeds in Abutilon. In control out of 100 seeds, only eight were able to germinate revealing 8% per cent hard seededness. Similarly, the heat scarification at 60°C for 3 days and alternate temperature (15°C-30°C) did not help in eliminating hard seededness in Abutilon. Sulfuric acid treatment using 25%, 50%, 100% acid with varying time periods could not improve the germinability to significant levels. 25 per cent acid for 30 minutes gave 42 per cent germination; in fact 100 per cent acid treatment for 5 minutes gave 80 per cent germination but also resulted in increased number of dead seeds. Thus, acid treatment is not suitable for improving germination in this plant. Mechanical scarification with sand and paper for 5 minutes also improved germination up to 16 per cent as against 8 per cent in control but this treatment was also lethal to embryo increasing the number of dead seeds. Co-application of GA₃ at different concentrations viz., 500 ppm and 1000 ppm did not help much in eliminating the hardseededness in Abutilon indicum.

Sheela Verma et al. (2001) reported that acid treatment (in concentrated H₂SO₄ for 5 minutes) was effective in breaking glycyrhiza glabra dormancy (83% germination) while in P. coriifolia scarification of the seed coat with sand paper resulted in 90% germination. Intraembryonic test confirmed the presence of 90% viable embryos in C. bonalli, un-stained and partially stained embryos in P. serpentina seeds revealed the presence of non-viable embryos resulting in poor germination. Treatment of W. somnifera seeds with 100 ppm gibberelic acid resulted in improved percentage germination, speed of germination, emergence and coefficient of velocity of
germination was achieved in only one generation time compared with control seeds.

Rathnavalli et al. (2002) subjected the boiled detached seeds of Cassia angustifolia to various seed dormancy breaking methods viz. hot water soaking, H2SO4 scarification, sodium soaking and ethylene and GA3 soaking. Seed germination tests were carried out by using the between paper method at 30±2°C. It was found that H2SO4 scarification for 10 min gave highest germination percentage (78%). The highest seedling growth index (5.52±.97) was obtained by soaking the seeds in 50 ppm for 16 h with 64.5% germination. Hot water soaking for 24 h could be used for large-scale plantation being a convenient and low-cost method, which gave 62% germination.

Rathnavalli et al. (2002) scarified fresh seeds of Cassia angustifolia with 50, 100 or 200 ml of 1% H2SO4 acid. The seeds were scarified by soaking seeds mechanically scarified by mixing the seeds with sand at a 1:1 to 1:3 ratio or treated with hot water 50 or 70°C. All the treatments were carried out for 3, 10, 15 or 20 minutes. Among the seed treatments, mechanical scarification gave the highest seed germination and lowest percentage of abnormal seedlings and dead seeds.

Veena Gupta (2003) evaluated the efficacy of scarification, cold stratification and growth regulators in dormancy breaking in 39 medicinal and aromatic plants from India. The treatments were given to seeds prior to germination. A seed germination of upto 20-85% was obtained with sand paper scarification for Anogeissus latifolia, Abies pindrow, Cardiospermum halicacabum, Cassia occidentalis and Withania somnifera acid scarification for Abies pindrow, Anogeissus latifolia, Platanus occidentalis, Heptneres isora and Indigofera tinctoria. Cutting-piercing the seed coat for Acacia
cannons, Acacia mangium, Caesalpinioideae, Mimosaceae, and Rutidoideae. Pre-treatment of seeds for *Argemone mexicana*, *Randa dauciflora*, and *Rauvolfia serpentina* GA, *Garcinia acid* treatment for *Costus species* and *Embothrium* with pre-chilling of seeds for *Asparagus officinalis*, and *Buddleia cordata* with *Centaurium ruthenicum*, *Plantago lanceolata* and *Saussurea* spp. and 0.2% KNO₃ treatment for costus species and *Ocimum sanctum* (Ocimum tenuiflorum).

Acid treatment is commonly used for American Acacias and other legumes (Doran et al., 1983; Beawari and Mohamed, 1985). It must be considered one of the most effective pretreatments for hard seed, especially those with very hard coats like *A. nilotica* and *Lambrchia alba*. For thin-coated species, there is a risk of damaging seeds by over-treatment and less severe methods are normally preferred. Acid treatment also greatly improved germination of non-leguminous *Jasminum officinale* from 0 to >70% (Laurent and Charsham, 1987).

Khasa (1992) found that pretreatment of *Terminalia superba* seeds with concentrated sulphuric acid (55-98%) over 15-60 min. greatly improved germination while any exposure boiling water killed the seeds. It was suggested that the acid having a higher viscosity than water did not penetrate cells in the pericarp and hence did not come into physical contact with the embryos.

### 2.5.2 Chemical treatments

Barton (1947) found that the seeds of *Cassia* and other members of the Caesalpiniaceae and *Acacia* reegei (Mimosoideae) became permeable after they were soaked in absolute ethyl alcohol, but those of Papilionoideae...
did not. Soaking in ethyl alcohol and acetone stimulated 10-20% germination in seeds of Acacia auricula and 25-60% in those of A. tortilis at the point of water entry was the flume (Brown and Booyser, 1989). Other solvents including acetone, carbon tetrachloride, chloroform, ethylene chloride and ethyl alcohol have also been reported to increase the seed permeability.

Thiourea has a stimulating effect on breaking dormancy possibly by deactivating the effect of inhibitor e.g. ABA. Hartmann and Kester (1982) it has proved effective in overcoming photo dormancy in a number of light sensitive seeds (Mayer and Poljakoff-Mayber, 1982). Among several compounds studied, thiourea proved to be the most effective germination stimulant for Ziziphus mauritiana. 24 hr. Soaking in a 1% solution enhanced total germination percentage from 41% control to 76% at 30°C, which was considered the optimum temperature. In addition it alleviated the deleterious effects of sub-optimal temperatures both in terms of total germination and vigour.

McNeil and Duran (1982) examined the effects of various concentrations of KNO₃ ranging from 0 to 10 M, GA₃ from 0 to 10⁻² M or GA₄ from 0 to 10⁻⁴ M and temperature ranging from 10 to 30°C with or without light on germination of Plantago ovata seeds. Seed treatment with higher concentrations of GA₃, GA₄, or KNO₃ alone did not have any effect on overcoming dormancy however, its effect increased when applied with GA₃ and low temperatures. Low and high temperatures reduced the rate of germination. The optimum temperature being 14°C. Application of plant growth regulators (especially gibberellic acid and
cytokines have been shown to enhance germination of hard woods but have limited effectiveness for conifer species (Routledge, 1987).

Here and Son (1995) observed the highest percentage germination after storage for 1 and 5 months for 24 and 28°C, respectively, following treatment with nutrients + GA (GA 100 ppm + microshakes 1% + K₂HPO₄ 0.00 ppm), compared with 26.45% and 47.35% germination for untreated controls in bael (Aegle marmelos). The nutrients + GA treatment also promote seedling growth (leaf number, height, root length and seedling FW). High percentage germination values were also observed following treatment with K₂HPO₄ (1%), KNO₃ (0.163%) or GA (200 ppm). The number of days required for germination to commence and the number of days required to complete germination were reduced by the GA (200 ppm) and the nutrient + GA treatments.

KNO₃ has a strong effect on both germination percentage and vigour on and pre-coated Aegle marmelos seeds (Dear et al., 1989). At 1% concentration germination increased from 37% in control to 79% and at 2% concentration it increased to 85%. In Casuarina equsitiforma germination increased from 46% in the KNO₃ for 38 hours. Both higher and lower concentration and shorter duration of soaking showed a lower germination in that experiment (Madden et al., 1990). In the study on Ziziphus mauritiana, KNO₃ was less effective than GA and to some extent the germination parameters except root length (Murthy and Reddy, 1988).

Pawska et al. (1997) determined the effect of presoakation seed treatments on the germination and vigour of ziziphus (F. officinalis P. emblica) seeds. Treatments included gibberellic acid (GA₃) at 50 and 100
ppm soaking in water for 24 h and hot water soaking at 60°C for 5 minutes.

GA at 50 and 100 ppm increased the percentage seed germination. The tallest plants were obtained following seed treatments with 100 ppm GA and soaking for 24 h.

Muniposh et al. (2005) tested the effects of various seed pretreatments on the enhancement of seed germination and seedling vigour in F. officinalis. Maximum germination (62%) was obtained after treatment of seeds with 100 ppm kinetin. This treatment has also augmented seedling length and vigour index. Other seed treatments enhancing germination and seedling vigour of seeds were 1/4 KNO3 soaking in water and 100 ppm IBA, IBA (100 ppm; 1/4 KCl; 1/4 CaC2; and 1/4 NaN3, PO4 all reduced germination below the control (untreated level of 64%) and hence germinated less well and produced less vigorous seedlings and only the kinetin treatment improved germination (50 vs 42%) in the control.

Soman et al. (1999) performed the experiment to trace the influence of packing materials and storage temperature on the shelf life (storage life) of amla (Physalis emodi) seeds. The seeds were extracted from the fruit, cleaned and graded and packed in a cloth bag and 100 gauge polyethylene bag and stored at 5°C under ambient conditions at Coimbatore, Tamil Nadu, India until 24 months. Observations made at 6 months interval revealed that the seeds of amla are orthodox in nature and could be stored well at 5°C until 24 months with 67% germination by packing them in 700 gauge aluminium bag.

Kabir et al. (1999) also found that pre-soaking seed soaking in sodium nitrate 1% solution for 24 h significantly reduced the number of days
taken for germination 18 days), enhanced the germination percentage (92.15), germination rate index (78.74) and vigour index (670.42) compared to other pre-sowing seed treatments in Ashwagandha. The germination percentage was significantly lower when seeds were sown as such (29.73); followed by seed soaked in water (27.93). Without any soaking treatment (seed sown as such) took more number of days for germination (13.67) followed by seeds soaked in water (12). The seedling vigour revealed that seeds soaked with nitrate of sodium and potassium at 1% for 24 h produced more vigorous seedlings, higher dry matter accumulation, root length and dry root yield as compared to untreated and water soaked seeds.

Nayak and Sen (1999) treated bael seeds with different chemicals: concentrated H₂SO₄ for 10 or 20 minutes, H₂SO₄ for 10 minutes + 1.0% thiourea, 100 ppm GA₃, H₂SO₄ for 10 minutes + 100 ppm GA₃, 0.5% or 1% thiourea, soaking in water for 24 h and mechanical scarification by sand paper in Bael (Aegle marmelos). Among the various treatments water soaking resulted in the highest percentage germination (80%), which was closely followed by conc. H₂SO₄ treatment for 20 minutes (78%) it was least with conc. H₂SO₄ for 10 minutes + 1% thiourea (70%). Although water soaking resulted in the highest percentage of ultimate germination, initiation and completion of germination took longer than treatment with conc. H₂SO₄.

Naiou et al. (2000) studied the effect of GA₃, IBA or IAA each at 250, 500, 1000, 1500 or 2000 ppm on soapnut seed germination in laboratory experiments. Seeds were soaked at room temperature for 10-15 h. GA₃ was more effective in improving seed germination in soapnut than either IBA or
IAA. The effectiveness increased with concentration using 1500 ppm and with duration of soaking.

Kalliman et al. (2001) observed that seed soaking in 150 ppm sodium nitrate for 72 h significantly reduced the number of days taken for germination (9 days) increased the germination percentage (32.16%) and vigour index (670.72) compared with other pre-sowing seed treatments of Ashwagandha. Soaking of seeds in sodium nitrate (15 ppm) resulted in the highest root and shoot lengths at harvest (15.99 and 30.10 cm, respectively). Soaking of seeds in sodium nitrate recorded the highest dry root y old of 131.03 kg/ha followed by soaking seeds in potassium nitrate (10.1%) and calcium nitrate (10.5%) which recorded 114.35 and 110.92 kg/ha, respectively.

Ramamukul and Arhu (2001) reported that fresh seeds treated with Azospirillum + Phosphobacteria + 0.5% KNO3 for 6 h recorded the highest germination percentage (62.88%) and one-year old seeds treated with Azospirillum + Phosphobacteria + 200 ppm GA3 for 6 h showed higher germination (49.17%) which was significantly superior than all other treatments in non-a sterilized condition. The lowest germination percentage was obtained with untreated control with 18.28% and 18.10% germination for fresh and one-year old seeds, respectively. In fresh and one-year old seeds the same treatment was found to induce higher shoot length, root length, dry matter production and vigour index.

Survevanshi et al. (2001) reported that the highest germination (62.1%) of Ashwagandha seeds was obtained at 25°C while the highest germination of Dawara seeds was recorded at 30°C. The seeds of Soma musli Sabja and wild brinjal showed poor germination due to dormancy. Soaking of seeds in
water for 24 h and incubation at 25°C gave the highest germination in S. aromatica. Treatment of seeds with 25% sulfuric acid for 10 minutes and incubation at 30°C was found to be suitable for the germination of 75% of S. aromatica seeds. Soaking seeds in 0.5 M HNO₃ for 10 minutes followed by 10% FeSO₄ solution soaked for 24 h with alternate temperature of 20°C/30°C for 24 h.

Kaplan and Reddy (2001) observed that the pre-sowing seed treatments significantly increased all these parameters compared with the control in Ashwagandha. Seed soaked in 1% sodium nitrate recorded the greatest plant height (107 cm) followed by those soaked in 0.5% ammonium nitrate (29.7 cm) and 0.5% calcium nitrate (29.7 cm). Soaking of seeds in 10% sodium nitrate resulted in the longest root (13.6 cm) followed by 0.5% zinc nitrate (13.5 cm) and 1.0% potassium nitrate (12.5 cm). Seeds soaked in 1% sodium nitrate recorded the highest dry root yield (0.31 kg/ha) followed by those (0.25 kg/ha) and 0.5% calcium nitrate (0.22 kg/ha).

Naidu et al. (2001) applied ammonium nitrate and potassium nitrate for Soapnut (Sapindus terebinthina) seeds in concentrations of 200, 400, 600 and 800 ppm and potassium nitrate were applied in concentrations of 150, 300, 450 and 600 ppm. The seeds soaked with ammonium nitrate solution improved germinator percentage seeds treated for 80 h with 500 ppm of ammonium nitrate solution showed maximum germination percentage (65.7%) and soaking seeds with 800 ppm for different periods caused reduction in the germination percentage than the other concentrations. Inhibition of seeds for 50 h with 600 ppm of potassium nitrate caused the highest percentage germination (67%).
Thiourea of 450 ppm for duration of 50 h showed maximum germination percentage of 72%.

Rajamanickam et al. (2002) recorded thecallness of seed germination and germination percentage in fresh (30 days and 30.33%) and 1-year-old seeds (6.64 days and 45.66%) soaked in 0.5% KNO₃ for 6 h and in 200 ppm GA₃ for 8 h respectively in a non-a. The rate of germination was highest in fresh seeds soaked in 1% thiourea for 8 hours (70%) and in 1-year-old seeds soaked in cold water for 24 hours.

Utertuka and Boada (2004) reported that among the 14 pre-sowing treatments, KNO₃ (150 mM) and NaHCO₃ (30 mM) significantly stimulated seed germination and reduced mean germination time over control under both laboratory and nursery conditions. In Angelica gauca (Apiaceae) endemic to Himalayas (an endangered medicinal plant), seeds of which show poor germination. However, lower concentration of GA₃ (25 μM) was effective, though only marginally non-significant. KNO₃ and NaHCO₃ significantly enhanced plant height, whereas root length recorded significantly greater values for KNO₃ and GA₃.

Paul C. Bithka et al. (2004) reported that for both Arabidopsis and barley, seed dormancy can be broken by imbibition with nitrate or nitrite. When Arabidopsis seeds or barley grain are imbied with the Na donor, dormancy is lost and germination occurs. In one typical experiment, less than 15% of Arabidopsis seeds germinated when they were imbied with water and germinated in the light. When seeds were imbied with SNP, germination on day 8 increased to 49% with 50 μM SNP and 99% with 100 μM SNP. The
solution of SNP produce a gaseous compound that breaks dormancy. When the dormant Arabidopsis seeds are imbibed in water and placed in a closed container with an exposed solution of SNP dormancy is broken and germination occurs. Arabidopsis seeds that showed 20% germination when imbibed in water had 5.5% germination when imbibed with cPT10. Similar data were obtained for barley; cPT10 did not inhibit germination of Arabidopsis seeds or barley grain that had lost dormancy through stratification or after ripening. Experiments with ABA and GA indicate that ABA is able to overcome the effects of SNP and maintain dormancy, but that SNP and GA act synergistically to promote germination.