

STUDIES ON THE PHYSIOLOGY AND BIOCHEMISTRY OF
SEED GERMINATION OF SOME FOREST TREES OF
NORTH-EAST INDIA

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I certify that the thesis entitled "Studies on the Physiology and Biochemistry of Seed Germination of some Forest Trees of North-East India" submitted by Sri Akhila Nand Verma for the degree of Doctor of Philosophy in Botany of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by him under my supervision. He has been duly registered and the thesis presented is worthy of being considered for the award of the Ph.D. degree. The work has not been submitted for any degree of any other University.

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C O N T E N T S

	Page
Chapter I	
GENERAL INTRODUCTION - REVIEW OF LITERATURE	1 - 16
Chapter II	
EFFECT OF SEED STORAGE ON GERMINATION AND SEEDLING DEVELOPMENT IN FIELD CONDITIONS	17 - 29
Introduction	17-18
Materials and Methods	18-21
Effect of storage conditions on seed viability, germinability and rate of germination	18-19
Effect of one-year-storage of seeds on seedling growth	20
Location of germination and seedling development	20-21
Results	21-24
Discussion	24-29
Chapter III	
EFFECT OF ENVIRONMENTAL FACTORS AND GROWTH REGULATORS ON SEED GERMINATION AND SEEDLING GROWTH	30 - 52
Introduction	30-32
Materials and Methods	33-36
(i) pH	33-34
(ii) Water Stress	34
(iii) Imbibition	34
(iv) Light - photoperiod, light intensity and wave length	34-35
(v) Temperature	36
(vi) Growth Regulators	36
Results	37-42
Discussion	42-52

Chapter IV	
BIOCHEMICAL CHANGES DURING SEEDLING DEVELOPMENT	53 - 87
Introduction	53-55
Materials and Methods	56-63
Fresh weight and moisture content determinations	56
Enzyme extraction	56-57
Assay of enzymes - catalase, peroxidase, IAA-oxidase and polyphenol oxidase	57-58
Protein	58-59
Extraction of phenols - Total phenol and O-dihydroxyphenol	59-60
Extraction and estimation of nucleic acids	60-61
Electrophoresis	61-63
Results	63-76
Discussion	77-87
Chapter V	
SUMMARY	88 - 93
BIBLIOGRAPHY	94 - 131
APPENDIX	

Chapter I

GENERAL INTRODUCTION - REVIEW OF LITERATURE

The global tree resources are fast dwindling and the sheer lust for more land has obviously resulted in callousness and unprecedented apathy towards the forest wealth. The increasing population pressure has also created tremendous environmental problems in developing countries of tropics and sub-tropics. The extent of forest cover in India is about 75 million hectares which constitutes 23 per cent of the total geographical area. It has been observed by the National Committee on Environmental Planning in 1983, that deforestation occurs at the rate of at least one million hectares a year, while new plantations do not account for more than 0.5 million hectares a year (ref. Rao et al., 1985).

The North-Eastern region constitutes a total geographical area of 25.5 million hectares of which about 12.5 per cent is under forest cover. The vegetation found herein may be classified as (1) tropical-evergreen, semi-evergreen, and moist deciduous forests (2) sub-tropical pine forests (3) temperate forests and (4) alpine forests. The forest resources of the region are depleting due to indiscriminate felling of forest trees for age old practice of shifting cultivation locally termed as "Jhum", timber and fuel wood extraction, unplanned human activities like hydroelectric projects, road construction, population pressure, and ruthless exploitation of plants for trade. The impact of these factors

has resulted in serious ecological imbalances viz., soil erosion, desertification, dwindling of forest wealth, wild life and germplasm resources.

Broadly, the yield of trees can be substantially influenced by seed germination patterns. While poor germination results in low yields of seedling per unit area, the slow germination may cause a wide variation in size and acceptable quality of established seedlings. Consequently, seed germination patterns represent a major variable in the successful production of uniform seedling under controlled or natural environments (ref. Dunlap and Barnett, 1982).

Seed germination is a very complex phenomenon and has been the subject of extensive study. It is a symbol of awakening from sleep and lifelessness (Timiryazev, 1949). A much better understanding of the secret of seed germinating has been gained in the last three decades, which, in itself, has resulted in improved seed quality. Some of the relevant problems of seed germination have been discussed in detail (Barton, 1961; Mayer and Shain, 1974; Khan, 1977; Kramer and Kozlowski, 1979; Bewley and Black, 1982).

Woody plants intermittently produce large seed crops. Hence, seed longevity is extremely important for regeneration of plants in nursery beds through direct seeding (Kozlowski,

1971; Kramer and Kozłowski, 1979; Rai, 1985). Under natural conditions, remarkably high losses of seeds are traceable to lack of seed viability and seed dormancy (Roberts, 1972; Heydecker, 1973; Purohit et al., 1982; Rai, 1985). The techniques for handling the seeds of tropical and sub-tropical trees are poorly developed (Bonner, 1978; Tompsett, 1984, 1986). Some of the genera having short seed life span are Salix, Ulmus (Troup, 1921), Alnus (Boojh and Ramakrishnan, 1981a), Schima (Boojh and Ramakrishnan, 1982), Shorea (Purohit et al., 1982), Ailanthus, Artocarpus, Bischofia, Chuckrassia, Dalbergia, Michelia, Pterocarpus, Syzygium and Toona (Rai, 1985). Seeds have been broadly categorized into two types: orthodox seeds, which can be dried to about 5% moisture content without damage and then stored at low temperatures for long periods (Roberts, 1973; Ellis and Roberts, 1981) and 'recalcitrant' seeds, which cannot be dried below a relatively high moisture content without immediate damage and must be stored under moist conditions (King and Roberts, 1979). Hence, in many countries, studies are being carried out to determine the optimum storage conditions which could ensure the maximum duration of the life of seeds and successful germination. The storage of seeds require a careful consideration of the requirements like temperature, humidity etc. for the maintenance of the embryo in viable conditions (Tompsett, 1986; Verma and Tandon, 1987). Pukacka (1983) mentioned that losses

in viability of Acer platanoides seeds is related to increase in leaching of electrically conductive material. Sprackling (1976) reported that storage of Pinus gerardiana seeds at -10°C resulted in better germination whereas 4°C caused lack of viability. The higher storability at low temperatures reduces the consumption of reserve material through slow biological processes. The effects of moisture and temperature on seed storage of 40 critical and ordinary plant species, showed that air humidity has stronger effect on the loss of viability than temperature (Bulat, 1963). The normal moisture content of 33 per cent has been reported for storage of different seeds. Any further increase in the quantity of water leads to increase in the intensity of respiration and depletion of stored material in the seeds (Woodstock et al., 1984). The unfavourable storage conditions reduce seedling growth more than germination (Stone, 1957a; Kozlowski, 1971). The seed ageing, long-term storage or unfavourable storage conditions may result in various forms of abnormalities during germination (Huss, 1954; Sorensen, 1975; Chin et al., 1981) due to reduced quantity of biochemical components including growth regulators (Abdel-Magid and Osman, 1975; Shamsbery and Banerjee, 1979) or destruction of cell organelles (Chin et al., 1981).

The germination and seedling establishment of tropical and sub-tropical trees under natural conditions has received

little attention (Kinnaid, 1974; Boojh and Ramakrishnan, 1982; Shukla and Ramakrishnan, 1982; Minore, 1986). The importance of appropriate environmental conditions favourable for germination and seedling growth have been emphasized (Hinesley, 1981; Mayer and Poljakoff-Mayber, 1982; Kozlowski, 1983). Amongst the environmental factors; light, temperature, water and substrate pH influence the resumption of embryonic growth (Baker, 1950; Kamra, 1967; Ovcharov, 1969; Kozlowski and Gunn, 1972; Kramer and Kozlowski, 1979; Therios, 1982; Verma and Tandon, 1983, 1984a, b). It is well-known that acid soils are developed in wet climate under long term leaching and tend to be low in mineral nutrients, while alkaline and salty soils are usually developed in dry climate due to high evaporation and lack of leaching (Tinus, 1980). This change affects the forest regeneration (Chou and Young, 1974; Lee and Weber, 1980; Raynal et al., 1982). The moisture of the medium can be a limiting factor for the hydration of seeds and oxygen flow towards the embryo during germination (Mayer and Poljakoff-Mayber, 1982). The different temperatures are required for the seed swelling and germination, But, the temperature at which swelling takes place has a greater influence on the physiological processes in the seedlings (Ovcharov, 1969; Mayer and Poljakoff-Mayber, 1982). The stratification by means of cold soaking enhances seed germination in many species (Foot, 1967; Barnett, 1971; Ghosh et al., 1974) through increase in

gibberellic acid (GA) concentration (Sinska and Lewak, 1977; Bretzloff and Pellett, 1979), and a decrease in abscisic acid (ABA, Lee and Looney, 1978). Pital et al. (1984) reported the changes in isoenzymes of some enzymes following various periods of imbibition and germination of Pinus contorta seeds.

The significance of illumination has received as much primary importance in seed germination as temperature and soil moisture (Bevington and Hoyle, 1981; Campbell, 1982; Gross, 1985). In some species, light requirement for germination may be induced after dispersal following contact with the soil, burial or exposure to leaf filtered light (Mayer and Poljakoff-Mayber, 1982). Environmentally induced photosensitivity of seeds is often interpreted as an adaptation for ensuring the seeds to germinate in sites where probability of seedling establishment is high. It was recorded in Malesia that large seedlings of important tree species are very rare under closed canopy due to lack of sufficient light (Burgess, 1968). The light intensity, quality, and duration have strong effects on seed germination (Black and Wareing, 1955; Vaartaja, 1956; Shukla and Ramakrishnan, 1981; Campbell, 1982). The requirement of light intensity for seed germination varies for different species, for example, Picea requires 0.08 lux, Betula 1.0 lux and Pinus 5.0 lux, while seeds of few species require as much as 100 lux (Jones, 1961). Light was found to

increase the rate of mitosis (Nyman, 1961), polysome formation (Yamamoto et al., 1975), protein synthesis (Yamamoto and Sasaki, 1977) and RNA containing poly A⁺ (Yamamoto, 1982). Temperature affects the seed germination in a vast number of species and has not received considerable attention on the adaptive significance of tree species (Kozlowski and Gunn, 1972; Kramer and Kozlowski, 1979). The seeds of many species germinate well at constant temperature (Critchfield, 1957; McLemore, 1969, Barnett, 1979) while others require diurnal thermo-periodicity (Hatano and Asakawa, 1964; Dunlap and Barnett, 1982; Vázquez-Yanes and Orozco-Segovia, 1982). The requirement of temperature is species specific (Rao and Singh, 1985) and is controlled through hereditary characters (Lang, 1965).

Besides environmental factors, a large number of substances also influence seed germination and seedling growth by their inhibitory or stimulatory effects on certain aspects of metabolism (Mayer and Shain, 1974; Khan, 1977; Bewley and Black, 1982; Barendse, 1983; Kumari and Kohli, 1984; ~~Mohanna~~ et al., 1985; Sankhla et al., 1985; Van Staden et al., 1986). Plant hormones are considered to be the mediators of the physiological processes and primary germination agents (Sankhla and Huber, 1974a; Tao and Khan, 1977). The major categories of hormones associated with seed physiology are GA, ABA, cytokinins (FAP) auxins and ethylene (Ovcharov, 1969; Khan, 1977; Panel et al.,

1984). Besides, various other agents like sterols and vitamins etc. have hormone like properties and have been implicated in the physiology of some seeds (Ovcharov, 1969; Chinoy et al., 1971; Lewak, 1984). The progress in the physiological effects of plant hormones has been spectacular in recent years but probably it would have been more so, if workers in this field were less fascinated by hormone effects on extension growth and had paid equal emphasis to other aspects which are not generally considered as growth processes (Sen, 1984). The chemical treatment of seeds induce the seed germination and initiation of radicle in many species (Ovcharov, 1969; Khan, 1977; Shafiq, 1980; Shibakusa, 1980; Tinus, 1982; Mehanna et al., 1985). Trewavas and Jones (1981) mentioned the concept of specific receptors required for the action of hormones.

The auxins have been implicated in the germination of non-dormant seeds (Suszka and Tomaszewska, 1971; Tillberg, 1977). Auxin fluctuations during seed maturation and seed germination have been reported in some species (Nikolaeva, 1967; Tillberg and Pinfield, 1981). The pre-sowing treatment of apple seeds with indole-3-acetic acid (IAA, 0.01-0.001%) for 30 min improved the seed germination (Simakin, 1966). Besides this, soaking tung seeds for 12 hr in indole-butyric acid (IBA), IAA, indole-propionic acid (IPA), phenylacetic acid and

ascorbic acid (AA) improve the germination and seedling growth (Chatterjee, 1960). Many herbicides decrease the number of germinants. The inhibitory effect is caused by direct suppression of seed germination and subsequent toxicity to young seedlings or both (Sasaki and Kozlowski, 1967, 1968; Wu et al., 1971). Only two herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) are currently registered for forestry use in Canada, because of controversy over dioxin content, 2,4,5-T has been withdrawn from the effective use of many provenances (Prasad, 1983). The phytotoxic effect of herbicides on seeds and seedlings during seed control is well-known (Kozlowski and Kuntz, 1963; Prasad, 1970; Abrahamson, 1980). The use of seeds for assessing the relative toxicity of herbicides has also been reported (Prasad, 1984).

GA stimulates seed germination in many tree species (Biswas et al., 1972; Chandra and Chauhan, 1976; Shafiq, 1980; Shibakusa, 1980; Tinus, 1982; Mehanna et al., 1985). The optimum requirement of GA varies from species specificity to time as well as method of application. Kursanov et al. (1966) reported that some seeds respond to additional supply of kinins for seed ripening and germination. Treatment of green ash seeds with 1-10 mg/l 6-benzyladenine for two days stimulated the seed germination (Tinus, 1982). The exogenous application

of FAP counteracted the effects of various growth and germination inhibitors (Khan, 1967; 1971; Khan and Tolbert, 1965). It indicates that cytokinins may be a pre-requisite for germination in some species. The studies on the endogenous levels of hormone suggested a number of ways in which cytokinins could be involved during germination process (Khan, 1977).

Seeds of different plants contain different amounts of vitamins. The quantity of those vitamins in seeds of plants from one another and in the same variety grown under different conditions also varies. Therefore, it is understandable that under certain conditions, seeds accumulate the quantity of vitamins necessary for their germination while under other conditions the vitamin content is insufficient (Ovcharov, 1969). The increase in cell division at the time of transition of the seed from the stage of resting to germination apparently depends upon the vitamin content. Studies on the direct effect of vitamins on seed germination appears quite promising (Ovcharov, 1969; Chinoy and Saxena, 1978). Chinoy et al. (1971) suggested the possible role of AA in seed germination through its action as an electron donor which maintains the redox potential to participate in plant systems.

Metabolic regulation of seeds is required to ensure the distribution of germination over time and space. Phytohormones decisively interfere with the physiological process of

seed germination and may determine the ways proceeding the involvement of energy of transcriptional and translational control of enzyme formation at the ribosomal level (Tao and Khan, 1977; Taylorson and Hendricks, 1977; Barendse, 1983). Hormones are conceded to be the primary germination agents and each hormone has a specific function (Galston and Davis, 1969; Tao and Khan, 1977; Barendse, 1983). The highly selective functions of individual hormones in plant metabolism including enzyme level (Galston and Davis, 1969; Leshem, 1973; Barendse, 1983) hormonal status (Mehanna et al., 1985; Van Staden et al., 1986) macromolecular drifts (Kumari and Kohli, 1984) and control of differential gene activation or in alteration of the properties of cell membrane (Lewak, 1984) have been reported.

The factors which control the initiation of germination differ from the factors controlling enzyme activity occurring later in germination. Varner and Ho (1977) dealt with the physiological and biochemical aspects by treating the best known responses of plant hormones with regard to control of enzyme activity. Higgins and Jacobsen (1978) reported the effect of hormones on selected aspects of cellular metabolism. Letham et al. (1978), Moore (1980), Bewley and Black (1982) and Barendse (1983) have discussed the different aspects of hormonal regulation of enzyme synthesis in plants. It has been demonstrated that protein synthesis is one of the essential

steps in seed germination (Mayer and Shain, 1974). This is also characterized by the increase in activity of almost all the enzyme systems in seeds as well appearance of new enzyme systems (Mayer, 1977; Pitel et al., 1984). It is accepted that changes in enzyme levels cause developmental changes. The enzyme systems responsible for synthesis of membrane components become active early in germination (Mayer and Shain, 1974). Ching and Ching (1972) have mentioned a correlation between energy change in ponderosa pine seeds to seedling growth. A number of terminal oxidases are associated with cell membranes of seed coats (Harel and Mayer, 1963; Khan, 1977; Mayer and Poljakoff-Mayber, 1982). Seed coats of normally impermeable species contain a rather high level of phenolic compounds and the action of oxidases is oxygen dependent in them. In the absence of oxygen, phenolic compounds are not oxidized to corresponding quinones, and therefore, there is no tanning reaction of seed coat proteins (Mayer, 1977). The exogenous levels of hormones and vitamins in quiescent seeds seem to indicate their concentration adjustment during the germination process rather than being at physiologically significant balance before imbibition (Ovcharov, 1969; Khan, 1977). The effect of seed treatment with various growth regulators regulate the endogenous level of auxin through change in enzyme activity (Kulaeva, 1980; Gaspar et al., 1982). Auxin is likely to be a critical factor

affecting the synthesis, oxidation and esterification of auxin in other systems for its involvement in germination process, cell elongation and changes in structural components. Exogenous IAA logically has different effects from endogenous IAA (Khan, 1977). IAA can be oxidized by an enzyme IAA-oxidase and probably by many non-specific oxidases (Schneider and Wightman, 1974). The IAA-oxidase/oxidase has been widely studied in an attempt to understand the regulation of IAA levels in plant tissues. The oxidation of IAA means inactivation of the hormone and therefore, the control of IAA-oxidase and/or peroxidase activities may have repercussions for IAA synthesis and degradation; thus may be involved in plant growth responses to auxins (Barendse, 1983). The possible mechanism of IAA-oxidation by horse-radish peroxidase have been discussed lucidly (Nakajima and Yamazaki, 1979; Tandon, 1985).

Catalase is one of the marker enzyme of glyoxysome cycle and its activity has been studied in relation to seed germination (ref. Barendse, 1983). Kenten (1955) found that catalase could inhibit the activity of IAA-oxidase presumably by diminishing H_2O_2 levels. Evensen and Loy (1978) reported an acceleration of catalase activity by GA treatment during germination of Citrullus lanatus seed and inhibition in activity by light. An increase in catalase and lipase was also recorded by application of GA and AA in Cicer arietinum while morphaactin

retarded them (Mehta et al., 1974a). IAA and α -naphthalen-acetic acid (NAA) promoted catalase activity of Triticum vulgare seedling (Shukla and Shukla, 1975). Sawhney et al. (1979) reported an occurrence of new isoenzymes of peroxidase in GA treated plants. The increase in IAA levels in cotyledons and its decrease in the hypocotyl and radicle was observed during early development of cucumber seedlings (Elkinawy and Raa, 1973). Simultaneously, IAA-oxidase activity decreased during this period in all the organs. But IAA, IBA and triiodinebenzoic acid (TIBA) were able to increase the IAA-oxidase and peroxidase activity with progressive regeneration of Phaseolus vulgaris tissues (Brunner, 1973). It is well established that GA increases the quantity of ferulic, chlorogenic acid, and other inhibitors and reduces the activity of IAA-oxidase (Galston and Hillman, 1961). IAA-oxidase is also destroyed by peroxidase (Radionova, 1965). The presence of GA or kinetin during stratification enhances the intensity of some isoperoxidases and presence of ABA inhibited it (Tao and Khan, 1976). GA was reported to reduce the activities of phosphoenol pyruvate (PEP) carboxylase, ribulose-diphosphate (RU5P) carboxylase and maleic enzymes in leaves as well as in seedlings of Pennisetum (Huber and Sankhla, 1974a) whereas α -amylase and invertase were induced in leaves (Huber and Sankhla, 1974b). However, regulation of invertase synthesis was recorded with concomitant increase in growth with application to GA (Huber



et al., 1974). ABA which inhibits seedling growth, promotes the activities of alanine and aspartate aminotransferases, PEP-carboxylase, malate dehydrogenase and RuDP-carboxylase (Huber and Sankhla, 1974c, d; Sankhla and Huber, 1974b, c; 1975; 1979) due to concomitant decrease of glutamate dehydrogenase which resulted in inhibition of carbon dioxide fixation (Huber et al., 1977).

β -vitamins and AA have been reported to act as potent activators of electron transport system during seedling growth (Chinoy and Saxena, 1978; Kodandarmaiah and Gopala Rao, 1984). AA also increases peroxidase activity which catalyzes the production of the free radical of ascorbic acid (Mehta and Chinoy, 1978). If locations of different enzymes within the seed or sub-cellular fractions become known, their role in germination could be clearly defined. Srivastava (1964) mentioned that under influence of GA and intensive biosynthesis of soluble RNA takes place which leads to an increase in the synthesis of specific enzyme proteins. The effect of hormones on certain aspects of metabolism have been observed (Key, 1969; Tao and Khan, 1977; Barendse, 1983; Kumari and Kohli, 1984; Lewak, 1984).

Pinus kesiya Royle ex Gord. and Schima khasiana Dyer are two economically important, fast growing tree species occurring in sub-tropical pine forests and sub-tropical moist,

evergreen montane forests, respectively, of North-East India. Seed is an important propagule for regeneration in these species. Under natural conditions, high losses of seeds take place due to unfavourable environmental factors. Hence, a study was initiated to understand the behaviour of some physico-chemical factors on seed germination and seedling growth and their control mechanism on certain aspects of metabolism in P. kesiya and S. khasiana.