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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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 <u>et al.</u>, 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 Kozlowski, 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 cannnot 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; Shamshery 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 (Kinnaird, 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). Pitel *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; Mohamma *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 (Chattarjee, 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; Abrahmson, 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 proceding 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 occuring 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/peroxidase 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 repurcussions 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 morphactin retarded them (Mehta *et al.*, 1974a). IAA and α-naphthalenacetic 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 regneration of *Phaseolus vulgaris* tissues (Brunner, 1978). 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 (RUDP) carboxylase and maleic enzymes in leaves as well as in seedlings of *Pennisetum* (Huber and Sankhla, 1974a) whereas -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).

B-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 defind. 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*.

Chapter II

EFFECT OF SEED STORAGE ON GERMINATION AND SEEDLING DEVELOPMENT IN FIELD CONDITIONS

Introduction

Longivity of seeds is controlled by hereditary characteristics and environmental conditions. The viability of seeds is strongly affected by temperature, moisture content and oxygen availability during preservation in majority of the species (Barton, 1961; Roberts, 1981; Bewley and Black, 1982). While some information is available on the methods of storage of economically important temperate forest tree seeds (Wang, 1974), it is scanty in case of tropical and sub-tropical tree species (Bonner, 1976; Tompsett, 1984; 1986; Athaya, 1985; Rai, 1985). At the same time it is well known that seeds of many commercially important tropical trees are short-lived and difficult to store (Harrington, 1972; Bewley and Black, 1982) due to higher moisture content (Barnett, 1974; 1982; King and Roberts, 1979; Chin et al., 1981; Purohit et al., 1982; Tompsett, 1984). Therefore, these seeds require optimum conditions for protection of viability during transit. This could also control the rate of germination and, in turn, yield through increase in plant density (Kozlowski, 1971).

The effect of seed storage on seedling character of many important trees has been studied (Huss, 1954; Stone, 1957a; Kozlowski, 1971; Sorensen, 1975; Chin et al., 1981). Emergence and seedling growth of the species depend on the interaction between their hereditary potentialities and the environmental conditions. Light, temperature and humidity are so interdependent that a change in one factor alters the others (Kramer and Kozlowski, 1979; Kozlowski, 1983). The study of weather effects on seedling growth is important as it may provide an opportunity to produce large number of uniform transplants in controlled environment in a limited time.

Pinus kesiya Royle ex Gord. and *Schima khasiana* Dyer are two economically important early successional forest trees which are predominant in the sub-tropics of North-East India. A study was initiated to understand the ideal storage conditions for higher viability, germination and seedling growth in *P. kesiya* and *S. khasiana* as these aspects have received little attention.

Materials and Methods

Mature cones of *Pinus kesiya* Royle ex Gord. and fruits of *Schima khasiana* Dyer were collected, respectively, from University campus, Shillong, and Upper Shillong forests (lat. 24°35'N and Long. 91°56'E, Alt. 1500-1800 m) during the months of February-March, 1983. Seeds were extracted by air drying the cones and fruits at 25-30°C.

Effect of storage conditions on seed viability, germinability and rate of germination

Both seed lots were stored in dark at 3-5°C and at laboratory temperature (15-22°C) in open and in sealed plastic bags with and without calcium oxide (CaO). The experimental design consisted of 2 species x 2 germination pattern (viability and germinability) x 6 time intervals (bimonthly) x 6 storage conditions x 7 replicates (3 viability and 4 germinability) x 100 seeds (5 groups of 20 seeds). In another set the design comprised of 2 species x 6 storage conditions x 4 replicates x 100 seeds.

Viability of seeds was tested with 0.1% 2,3,5-triphenyltetrazolium chloride by puncturing the seed coat with the help of a needle. Seeds were surface sterilized with 0.1% mercuric chloride for 30 min and 5 min for P. kesiya and S. khasiana, respectively. The seeds were then washed repeatedly with sterilized distilled water to remove the disinfectant. 20 seeds were placed equidistant from each other in a covered plastic petridish (9 cm diam.) containing sterilized moist filter paper and germinated in dark in a germinator at 20 ± 2°C. Seeds were considered germinated when radicle protruded about 1 mm beyond the seed coat (Campbell and Sorensen, 1979). Germination was recorded from 6th-20th day at 2 day interval. The viability and germinability are expressed on percentage seed basis. The rate of germination (% seed day $^{-1}$) was calculated following Campbell and Sorensen (1979).

Effect of one-year storage of seeds on seedling growth

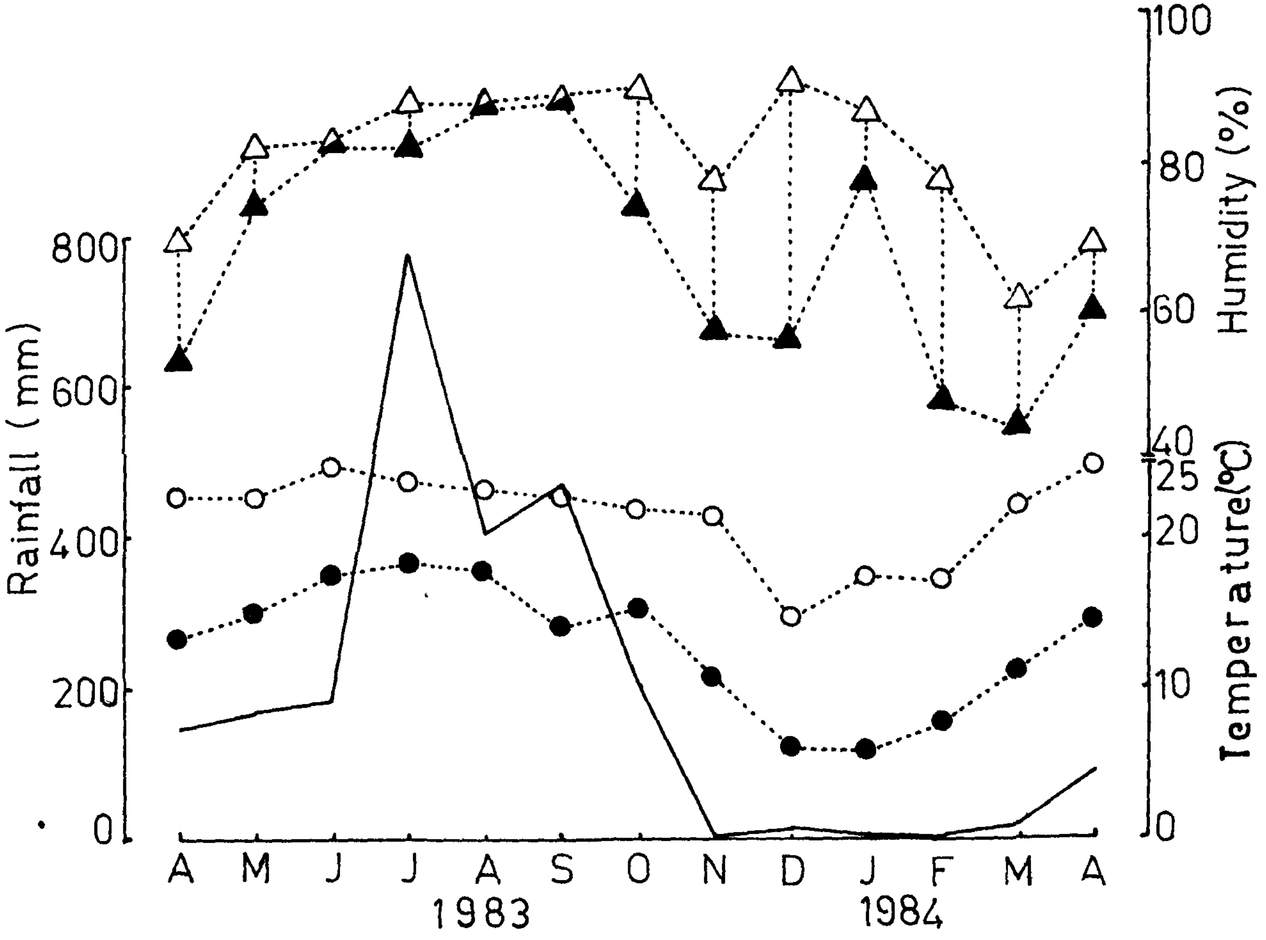
Seeds from the second set of design mentioned above were sown at 1 cm soil depth in plastic pots (30 cm diam.) in open field conditions during the month of April, 1984. Seedlings were lifted after 2 months and washed thoroughly to remove the adhering soil particles. The length of root and shoot was measured and they were oven dried at 60 ± 5°C for 48 hr for dry weight estimations. The results obtained by sowing fresh seeds during the previous year from the same provenance were used as control.

Location of germination and seedling development

Emergence of seedlings and their growth pattern in relation to some climatic factors were studied at the University Campus, Shillong (Alt. 1500 m). The temperature, relative humidity and rainfall data were collected from the meteriological centre in Shillong. The climate is divided by seasonal differences into (i) monsoonic season, with higher temperature, humidity and rainfall extending from May-October (ii) dry and humid winter season from November-February followed by (iii) warm, dry summer of March and April (Fig. 2.1).

Fifty seeds of both the species were sown in plastic pots as mentioned above during April, 1983. The duration of seedling emergence was recorded. Ten seedlings were maintained

Fig. 2.1 : Climatic data of the study site (Shillong); maximum temperature (O ... O), minimum temperature (● ... ●), average rainfall (——), and humidity (morning △, evening ▲).



Rainfall (mm)
800
600
400
200
0
100
80
60
40
Humidity (%)
25
20
10
0
Temperature(°C)
A
M
J
J
A
S
O
N
D
J
F
M
A
1983
1984

Fig. 2.1

in each pot and twenty seedlings of each species in 4 groups of 5 were excavated every month. The root was washed thoroughly and length of the root and shoot was measured. Seedlings were separated into root, shoot, needle/leaf and oven dried as mentioned above. The increases in seasonal dry weight, length and relative growth rate (RGR) on dry weight basis of seedling and seedling parts were calculated. Standard deviation was performed for statistical significance (Snedecor, 1961).

Results

Effect of storage conditions on seed viability, germinability and rate of germination

The higher viability and germinability of P. kesiya and S. khasiana were recorded in seeds preserved at 3-5°C as compared to ones at laboratory temperature (Tables 2.1 - 2.4). A gradual decrease in viability was noted under different storage conditions. P. kesiya seeds showed about 85% viability (Table 2.1) and 84% germinability (Table 2.2) after one year of storage at 3-5°C in sealed bags as against the germination capacity of 95% in fresh seeds. During 8 months of storage of S. khasiana seeds at laboratory temperature, low viabilities of 13% and 10% were recorded in open and over CaO containing bags, respectively (Table 2.3). However, about 33% viability was recorded in seeds stored in sealed bags. On

Table 2.1 : Effect of storage on the viability of *Pinus kesiya* seeds

Storage conditions	Viability (% seed) after months						
	fresh	2	4	6	8	10	12
3-5° C							
Open	95.00 ±5.00	90.00 ±7.50	90.00 ±2.50	90.00 ±2.50	85.00 ±0.00	85.00 ±5.00	82.50 ±2.50
Sealed		92.50 ±2.50	92.50 ±2.50	92.50 ±5.00	90.00 ±10.00	85.00 ±10.00	85.00 ±5.00
Over CaO		92.50 ±2.50	92.50 ±2.50	92.50 ±2.50	90.00 ±5.00	90.00 ±0.00	85.00 ±5.00
Laboratory temperature (15-22° C)							
Open		85.00 ±5.00	85.00 ±10.00	75.00 ±10.00	72.50 ±2.50	75.00 ±5.00	75.00 ±0.00
Sealed		90.00 ±7.50	87.50 ±2.50	87.50 ±7.50	80.00 ±0.00	78.50 ±3.00	75.00 ±3.00
Over CaO		81.60 ±7.60	73.30 ±3.00	72.50 ±10.00	70.00 ±0.00	75.00 ±5.00	72.50 ±3.00

± S.D.

Table 2.2 : Effect of seed storage on the germinability in *Pinus kesiya*

Storage conditions	Germination (% seed) after months						
	fresh	2	4	6	8	10	12
3-5°C							
Open	95.00 ±5.00	90.00 ±7.00	90.00 ±7.00	90.00 ±4.80	85.00 ±2.50	83.75 ±2.50	81.25 ±4.80
Sealed		92.50 ±9.50	92.50 ±6.40	91.25 ±7.50	86.25 ±6.30	86.25 ±8.50	83.75 ±11.00
Over CaO		92.50 ±6.50	92.50 ±6.30	92.50 ±6.30	82.50 ±8.60	82.50 ±4.60	80.00 ±4.00
Laboratory temperature (15-22°C)							
Open		82.50 ±6.50	82.50 ±2.50	75.00 ±5.00	75.00 ±4.00	70.00 ±2.50	72.50 ±4.30
Sealed		85.00 ±5.80	85.00 ±4.00	85.00 ±4.30	80.00 ±4.00	80.00 ±2.50	75.00 ±4.80
Over CaO		77.50 ±11.90	71.50 ±11.80	70.00 ±2.50	67.50 ±8.60	67.50 ±2.50	65.00 ±4.00

± S.D.

Table 2.3 : Effect of storage on the viability of *Schima khasiana* seeds

Storage conditions	Viability (% seed) after months						
	fresh	2	4	6	8	10	12
3-5° C							
Open	73.30 ±2.90	55.00 ±10.00	45.00 ±5.00	40.00 ±5.00	40.00 ±5.00	37.50 ±2.50	37.50 ±7.50
Sealed		60.00 ±5.00	62.50 ±7.50	57.50 ±2.50	51.70 ±2.50	50.00 ±0.00	40.00 ±0.00
Over CaO		57.50 ±2.50	45.00 ±0.00	40.00 ±7.50	40.00 ±10.00	42.50 ±2.50	40.00 ±5.00
Laboratory temperature (15-22° C)							
Open		47.50 ±10.50	40.00 ±10.00	32.50 ±7.50	12.50 ±2.50	-	-
Sealed		55.00 ±10.00	45.00 ±5.00	35.00 ±7.50	35.00 ±5.00	32.50 ±7.50	32.50 ±2.50
Over CaO		47.50 ±7.50	40.00 ±5.00	30.00 ±5.00	10.00 ±5.00	-	-

± S.D.

\- Non-viable seeds.

Table 2.4 : Effect of seed storage on the germinability in *Schima khasiana*

Storage conditions	Germination (% seed) after months						
	fresh	2	4	6	8	10	12
3-5° C							
Open	48.35 ±7.50	45.00 ±10.00	45.00 ±4.10	37.50 ±8.60	35.00 ±8.20	32.50 ±9.50	32.50 ±10.80
Sealed		46.25 ±10.00	43.75 ±4.40	41.25 ±6.30	41.25 ±2.50	42.50 ±9.50	40.00 ±5.80
Over CaO		45.00 ±10.80	45.00 ±7.30	40.00 ±4.10	38.75 ±7.50	31.25 ±8.50	28.75 ±4.80
Laboratory temperature (15-22° C)							
Open		28.75 ±7.50	1.25 ±0.50	-	-	-	-
Sealed		36.25 ±7.50	35.00 ±7.00	30.00 ±10.00	30.00 ±8.50	26.25 ±2.50	25.00 ±5.80
Over CaO		28.30 ±6.80	10.00 ±7.60	1.25 ±0.50	-	-	-

± S.D.

\- No germination.

the other hand, the seeds stored at 3-5°C in sealed bags showed about 62%, 52% and 40% viability after 4, 8 and 12 months, respectively, as compared to fresh seeds having about 73% viability. While the germinability in S. khasiana seeds was retained even after one year of storage in sealed bags at 3-5°C, it was completely lost within 4 months in open and CaO containing sealed bags stored at laboratory temperature (Table 2.4). The rate of germination in P. kesiya (Table 2.5) and S. khasiana (Table 2.6) were better when seeds were stored at 3-5°C in sealed bags as compared to other storage conditions. Seeds stored at 3-5°C in sealed bags showed non significant changes in the rate of germination throughout the year in P. kesiya. However, the rate of germination decreased significantly after 4 months in S. khasiana. On the other hand, the seeds stored at laboratory temperature in open and over CaO did not show any germination rate after 4 months.

Effect of one-year-storage of seeds on seedling growth

Storage of seeds for a year under various conditions resulted in decrease of yield in both the species (Table 2.7). The root growth was less in P. kesiya seeds stored at 3-5°C in open and sealed bags. On the other hand, reduction in both shoot and root growth was observed in seeds stored under different conditions at laboratory temperature. In case of

Table 2.5 : Effect of seed storage on the rate of germination in *Pinus kesiya*

Storage conditions	Germination rate (% seed day^{-1}) after months						
	fresh	2	4	6	8	10	12
3-5°C							
Open	43.20 ±9.60	35.30 ±6.70	35.20 ±8.30	35.00 ±4.00	35.60 ±3.25	35.80 ±3.50	27.40 ±2.80
Sealed		41.20 ±10.00	39.10 ±7.20	40.80 ±4.80	40.00 ±5.00	41.30 ±4.20	42.20 ±9.60
Over CaO		40.00 ±7.90	39.00 ±10.10	39.10 ±4.90	39.40 ±2.75	30.90 ±7.00	32.90 ±3.80
Laboratory temperature (15-22°C)							
Open		39.50 ±5.40	30.60 ±4.10	28.00 ±3.80	28.80 ±2.00	28.25 ±4.60	20.40 ±5.30
Sealed		37.00 ±4.25	34.70 ±7.80	36.30 ±4.10	31.60 ±1.80	31.60 ±5.40	21.30 ±4.40
Over CaO		36.60 ±5.00	30.80 ±8.25	29.20 ±1.20	28.50 ±3.50	27.20 ±8.90	20.50 ±5.80

± S.D.

Table 2.6 : Effect of seed storage on the rate of germination in *Schima khasiana*

Storage conditions	Germination rate (% seed day^{-1}) after months						
	fresh	2	4	6	8	10	12
3-5°C							
Open	39.60 ±5.00	33.70 ±4.60	24.80 ±8.20	24.60 ±7.25	24.90 ±2.25	25.10 ±6.20	21.80 ±6.30
Sealed		33.50 ±7.20	28.20 ±8.80	25.10 ±7.40	25.80 ±1.20	25.90 ±7.50	25.50 ±10.50
Over CaO		31.60 ±5.60	24.00 ±10.60	23.60 ±3.60	21.70 ±2.70	19.50 ±6.50	18.50 ±5.70
Laboratory temperature (15-22°C)							
Open		31.80 ±5.00	3.60 ±1.00	-	-	-	-
Sealed		30.40 ±7.40	23.20 ±2.40	22.00 ±3.20	20.20 ±1.50	18.80 ±6.50	16.80 ±4.20
Over CaO		32.60 ±6.00	19.00 ±4.30	-	-	-	-

± S.D.

- No germination.

Table 2.7 : Effect of one-year-storage of seeds on seedling growth of *Pinus kesiya* and *Schima khasiana* in field conditions

Storage conditions	*Pinus kesiya*				*Schima khasiana*			
	Length(cm)		Yield (mg)		Length(cm)		Yield (mg)	
	Root	Shoot	seed-ling^{-1}	Seed-lings pot^{-1}	Root	Shoot	seed-ling^{-1}	Seed-lings pot^{-1}
Fresh seeds	11.80 ±5.00	5.10 ±0.70	38.80 ±6.00	1125.00 ±171.00	7.00 ±3.25	4.80 ±0.70	29.60 ±3.40	580.50 ±8.25
One-year stored seeds 3-5°C								
Open	10.40 ±4.80	5.00 ±0.80	29.40 ±7.40	1117.20 ±283.80	6.80 ±4.00	3.50 ±0.70	18.50 ±1.00	128.70 ±7.80
Sealed	9.60 ±5.70	5.20 ±0.80	34.50 ±8.20	1034.00 ±247.80	7.10 ±3.80	3.70 ±0.40	25.40 ±0.30	254.40 ±3.00
Over CaO	11.75 ±4.90	5.00 ±0.60	29.60 ±5.75	858.75 ±164.20	7.20 ±2.70	3.60 ±0.60	26.00 ±5.00	208.00 ±40.00
Laboratory temperature (15-22°C)								
Open	9.50 ±5.20	4.60 ±0.60	28.40 ±3.50	728.00 ±130.00	-	-	-	-
Sealed	9.80 ±5.30	4.70 ±0.70	28.00 ±6.40	784.40 ±180.80	5.20 ±3.60	3.40 ±0.40	20.80 ±0.60	102.00 ±45.00
Over CaO	9.30 ±4.75	4.60 ±0.80	28.20 ±4.50	795.80 ±193.10	-	-	-	-

± S.D.

\- Represent lack of seedling growth.

S. khasiana root growth was less in seeds stored in open bags at 3-5°C and also in the seeds stored in sealed bags at laboratory temperature. Further, shoot elongation was less in seeds stored under different conditions. *S. khasiana* seeds stored in open and over CaO at laboratory temperature did not germinate.

Seedling emergence and growth responses to climatic conditions

Seedlings emerged in *P. kesiya* and *S. khasiana* between 20-40 and 12-25 days, respectively, under open field conditions (Table 2.8). The root and shoot elongations were more pronounced in *P. kesiya* as compared to *S. khasiana* at the time of seedling emergence. However, a reverse picture was obtained for shoot elongation after a year of growth. During the study of the seasonal variation of climate, a better dry weight production of seedling and its parts in both the species (except for root) was recorded in monsoon (Table 2.9). The root dry weight was maximum during summer. As given in Fig. 2.2, *P. kesiya* seedlings, showed maximum shoot elongation during July and August (mean temperature 23.5/17.5°C and RH 85-90%, average rainfall 600 mm - Fig. 2.1) which was almost negligible during winter (mean temperature 17.5/7.5°C and RH 60-85%; no rainfall). The maximum increase in root elongation in this species was recorded during September (mean temperature 22.5/14°C, RH 90% and average rainfall 450 mm) and was

Table 2.8 : Emergence and growth of seedlings of *Pinus kesiya* and *Schima khasiana* in field conditions

Species	Emergence period		Growth (cm or cm^2)/seedling part					
	Days of initiation of germination	Days of final germination	After emergence			After one year		
			Root	Shoot	Needle/leaf	Root	Shoot	Needle/leaf
Pinus kesiya	20	40	3.00 ±0.10	4.35 ±0.20	2.45 ±2.02	32.80 ±0.10	11.50 ±0.10	3.60 ±0.25
**Schima khasiana*	12	25	2.10 ±0.10	3.00 ±0.05	0.70 ±0.02	19.80 ±0.01	15.50 ±1.90	9.10 ±1.55

± S.D.

* leaf (cm^2).

Table 2.9 : Seasonal variations in yield of *Pinus kesiya* and *Schima khasiana* seedlings during the course of one year

Seedling part	Yield (dry wt in mg/seedling part)		
	Seasons		
	Monsoon	Winter	Summer
Pinus kesiya			
Seedling	371.50±25.70	5.10±0.50	103.40±24.00
Shoot	317.90±26.30	-	26.60± 3.80
Root	53.60± 0.60	37.60±7.20	76.80± 4.90
Needle	1.60± 0.10	-	1.35± 1.20
Schima khasiana			
Seedling	560.00±16.40	-	503.00±28.50
Shoot	465.60±25.20	-	284.00±12.60
Root	94.20±11.60	102.80±9.25	219.00±21.00
Leaf	42.50± 0.70	-	13.10± 1.50

± S.D.

\- No increase in growth.

Fig. 2.2 : Increase in root (O) and shoot (●) length during the first year development of *Pinus kesiya* (——) and *Schima khasiana* (---) seedlings.

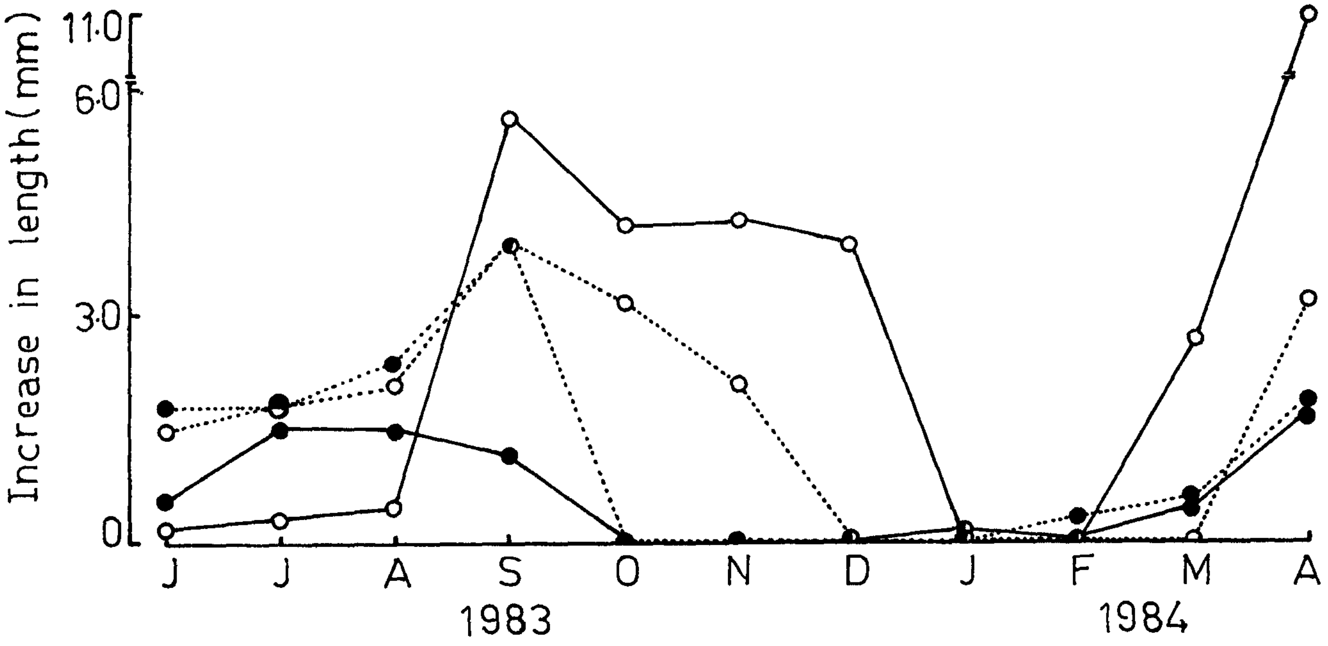


Fig. 2.2

Table 2.10 : Mean relative growth rate (mg mg^{-1} day^{-1}) of *Pinus kesiya* and *Schima khasiana* seedlings during the course of one year

	M-J	J-J	J-A	A-S	S-O	O-N	N-D	D-J	J-F	F-M	M-A
Pinus kesiya											
Seedling	0.508	0.879	1.231	8.500	0.584	-0.367	-0.141	1.908	0.544	0.605	2.768
Shoot	0.482	0.817	1.164	7.155	0.387	-0.549	-0.151	1.081	0.254	0.376	0.504
Root	0.026	0.062	0.067	1.345	0.197	0.082	0.010	0.829	0.290	0.230	2.265
Needle	0.003	0.002	0.002	0.021	0.000	-0.007	-0.001	-0.002	-0.014	0.007	0.073
Schima khasiana											
Seedling	0.774	1.383	1.763	3.463	10.735	0.155	-1.130	0.436	1.134	3.005	7.744
Shoot	0.598	1.172	1.449	3.097	8.812	-1.168	-2.226	0.353	0.541	2.512	3.996
Root	0.108	0.211	0.314	0.516	1.923	1.323	1.095	0.084	0.593	1.493	3.729
Leaf	0.092	0.120	0.175	0.280	0.790	-0.005	-0.021	-0.059	0.053	0.080	0.426

completely inhibited during January-February (mean temperature 17.5/7.5° C, RH 65-80% and no rainfall). The maximum increase in shoot length of S. khasiana was recorded during September and thereafter it was markedly decreased at the beginning of winter (Fig. 2.2). The reoccurrence of its growth was observed at the onset of summer. The root elongation was also maximum in September and it declined gradually. It was inhibited completely from December-March and thereafter it resumed the growth. The peak periods for RGR in P. kesiya and S. khasiana seedlings were found in August-September (mean temperature 22.5/15° C, RH 80-90% and rainfall 450 mm) and September-October (mean temperature 22.5/15° C, RH 80-90% and rainfall 325 mm), respectively (Table 2.10). The negative RGRs were obtained in shoot of both the species during October-December (mean temperature 15/10° C, RH 60-85% and rainfall 70 mm), in P. kesiya needle during October-February and in S. khasiana leaves during October-January. The seedlings also showed negative RGRs from October-December and November-December in P. kesiya and S. khasiana, respectively.

Discussion

The preservation of seed viability during the course of storage is greatly influenced by its hereditary characters and storage conditions. The seeds of many tropical trees deteriorate rapidly with time (Barton, 1961; Tompsett, 1984, 1986). However, viability of seeds can be prolonged by appropriate storage conditions for e.g., lower temperature and moisture levels (Barton, 1961; Roberts, 1972, 1981; Barnett, 1974; King and Roberts, 1979; Boojh and Ramakrishnan, 1981a; 1982, Chin et al., 1981; Bewley and Black, 1982; Mayer and Poljakoff-Mayber, 1982; Purohit et al., 1982; Tompsett, 1984, 1986). In general it has been suggested that storage life of orthodox seeds is doubled for 1-2% decrease in moisture content (Bewley and Black, 1982). However, in certain seeds decreasing the moisture content below 5-6% may not increase the seed storability (Roberts, 1972). Conversely, for agronomic seeds it was noted that an increase in 1% moisture content from 5-14% halved the life of seeds (Harrington, 1973). During the present investigation in P. kesiya, maximum viability, germinability and rate of germination were observed when seeds were preserved in sealed bags at 3-5°C as compared to laboratory temperature. The life span of pine seeds is reported to decrease faster at laboratory temperature than at 5°C (Barton, 1953). Allen (1957) reported the suitability of constant low temperature for conifer seed storage. Further, there was no advantage by storing the seeds at -18°C over 0°C. Seeds of Araucaria columnaris (Tompsett, 1984) and Pinus taeda (Barnett, 1974) failed to germinate when these were stored below freezing due to high moisture contents leading to ice-crystal formation, causing fatal damage to seeds (Roberts, 1972).

S. khasiana seeds showed better viability, germinability and rate of germination when stored in sealed bags at 3-5°C as compared to other storage conditions. A higher retention of viability for 7 months was also reported at 4°C in tea (Bulat, 1963) which belongs to the same family as that of Schima. The reduced viability of S. khasiana at laboratory temperature in open and over CaO may be due to reduction in moisture level. The decrease in germination capacity of Fraxinus pennsylvania, Acer saccharianum, Leriodendron, Quercus, and Citrus seeds was reported due to their desiccation (Barton, 1961; Kozlowski, 1971). The changes in physiological state of seeds on account of moisture deficit have been reported due to unfavourable storage conditions (Chin et al., 1981; Pukacka, 1983). The changes in respiratory metabolism are reported as one of the major factors for seed deterioration (Abdul-Baki, 1980; Woodstock et al., 1984). However, better retention of viability at optimum conditions of storage could be due to slow biological processes and also accumulation of substances which preserve structural formation of the cells for ensuring normal germination.

Variations in the seedling growth and yield were noticed when seeds of both P. kesiya and S. khasiana were stored under different conditions for a period of one year. The various forms of abnormal seedling growth might occur in low viability of seeds because of seed aging and long term storage under unfavourable conditions (Huss, 1954; Stone, 1957a; Kozlowski, 1971; Sorensen, 1975; Chin *et al.*, 1981). The unfavourable storage conditions are reported to result in disruption of cell wall, cell membrane and ill-defined nuclear membrane and nucleolus in *Hevea brasiliensis* (Chin *et al.*, 1981).

The early seedling emergence in *S. khasiana* as compared to *P. kesiya* seeds was recorded. The probable cause of early germination in the former is due to the high permiability of the seed-coat (Mayer and Poljakoff-Mayber, 1982). Seed size (Silvertown, 1981) and seed mass (Thomas, 1966) have important ecological consequences on seedling vigour. During the present study on seedling emergence, the higher seed mass of *P. kesiya* than *S. khasiana* could be correlated with seedling length. However, a reverse picture obtained for seedling growth after a year is due to genetic variation of seeds of both the species. Similar conclusions have been reported with regard to seed weight and dry weight of about one year old seedlings of six tree species, i.e. *Eucalyptus marginata*, *E. calophylla*, *Banksia grandis*, *Allocasuarina fraseriana*, *Persoonia longifolia* and *P. elliptica* grown in mediterranean forest of Western Australia (Abbott, 1984). The maximum dry weight production of seedling and its above parts in both *P. kesiya* and *S. khasiana* during monsoon was directly related to optimum conditions of temperature, humidity and rainfall required for growth. But root dry weight was highest during summer on account of maximum development of roots needed for extra absorption (Mason et al., 1970). Hence, it could be concluded that exponential growth of plants is time dependant and environmentally controlled (Ågren, 1985). The shoot elongation of P. kesiya and S. khasiana were maximum during July-August (temperature 23.5/17.5°C RH 85-90% and rainfall 600 mm) and September (temperature 22.5/14°C, RH 90% and rainfall 450 mm), respectively. Both shoot and root were sensitive to low temperature corresponding to 17.5/7.5°C of day and night in winter. Almost similar observations were reported earlier with regard to the completion of shoot elongation in red pine by the beginning of July (Kozlowski and Ward, 1961) and in eastern cotton-wood from late April-late September (Minckler and Woerheide, 1968). The root growth of Mugho pine seedlings in Southern Ontario continued from late April-late November (Mason et al., 1970). During the summer, daily root elongation averaged about 6.5 mm, but during autumn it was 2.9 mm (Mason et al., 1970). Besides, RGR (on dry weight basis) revealed that August-September and September-October were optimum periods for the seedling growth of P. kesiya and S. khasiana, respectively. The negative RGR of the seedling and its above parts in both the species was due to needle and leaf fall during the winter. The comparative study of increase in length and RGR of various parts exhibited that prevailing conditions in August (temperature 23.5/17.5°C, RH 85-90% and rainfall 400 mm) and September (temperature 22.5/

14°C, RH 90% and rainfall 450 mm) may be optimum for production of nursery transplants of P. kesiya and S. khasiana, respectively. The day temperatures of 22-27°C and night temperatures of 13-19°C were optimum for containerized Fraser-fir seedlings during active growth (Hinesley, 1981). The occurrence in field conditions of alternating temperatures of 23.5/17.5°C for P. kesiya and 22.5/14°C for S. khasiana resulted in higher growth under high humidity (approximately 80-90%) and moderate rainfall (average 400 mm) exhibiting the thermoperiodic behaviour of growth in both the species.

Chapter III

EFFECT OF ENVIRONMENTAL FACTORS AND GROWTH REGULATORS ON SEED GERMINATION AND SEEDLING GROWTH

Introduction

The regeneration of pioneer and commercially important tree species is very important to produce uniform seedling crops. Dry seeds are in a slow state of metabolism and are capable of withstanding extreme variations in their environment. Amongst environmental factors, substrate pH, moisture level, light and temperature play a significant role in resumption of embryonic growth during germination and juvenile stage in early seedling establishment (Ovcharov, 1969; Kozlowski, 1971; Kramer and Kozlowski, 1979; Mayer and Poljakoff-Mayber, 1982; Everitt, 1983). In general, plants can tolerate a pH range of 4.0-8.0 (Arnon and Johnson, 1942). However, high concentration of H^+ and OH^- ions can be directly toxic and can also inhibit the nutrient uptake in plants. The increase in acidity or alkalinity from the normal can decrease the forest productivity or alter the regeneration patterns (Lee and Weber, 1980; Raynal _et al._, 1982; McColl and Johnson, 1983). The moisture availability of the medium can be a limiting factor for hydration of seeds which is required to initiate intricate and inter-locked biochemical processes essential for germination and growth (Kramer and Kozlowski, 1979; Everitt, 1983). Cold soaking referred to as stratification (Rudolf, 1950; 1952), influences the germination in many species (Rudolf, 1952; Stone, 1957b; Foot, 1967; Barnett, 1971; Biswas _et al._, 1972; Shafiq, 1979; Bevington and Hoyle, 1981).. Different temperatures are required for the swelling and germination of the seeds but the temperature at which swelling takes place has a greater influence on the physiological processes in the seedling (Ovcharov, 1969; Barnett, 1980).

Light and temperature also play a great role on seedling emergence under natural conditions (Kozlowski and Gunn, 1972; Toole, 1973). Under natural conditions, light gaps control vegetation dynamics and establishment of many forest trees (Budowski, 1965; Burgess, 1968; Hartshorn, 1978; Denslow, 1980; Vázques-Yanes, 1980; Orians, 1982). The exposure to light stimulates seed germination in many trees (Jones, 1961; Toole *et al.*, 1961; Kamra, 1967, 1969; Sasaki and Asakawa,1974; Shukla and Ramakrishnan, 1981; Campbell, 1982) by increasing the growth potential of the embryo. Whereas light intensity has relatively minor effects on germination, both day length and wave length often have pronounced effects (Kramer and Kozlowski, 1979). While the majority of tree seeds require 8-12 hr light for maximum and fast germination (Olson *et al.*, 1959; Jones, 1961; Kamra, 1969; Kramer and Kozlowski, 1979). Some others require long photoperiods of 16-20 hr (Vaartaja, 1956; Wareing, 1956; Shukla and Ramakrishnan, 1981).

Seeds of many species germinate equally well over a wide range of temperature (Critchfield, 1957; Ackerman and Farrar, 1965; Kaufman and Eckard, 1977). Seeds of certain species require constant temperature (McLemore, 1966, 1969; Farmer and Bonner, 1967; Barnett, 1977, 1979; Boojh and Ramakrishnan, 1981a, 1981b, 1982; Yousheng and Sziklai, 1985) for better germination and growth. On the other hand, acceleration in germination was reported in some cases by diurnal thermo-periodicity (Hatano and Asakawa, 1964; Dunlap and Barnett, 1982; Vázquez-Yanes and Orozco-Segovia, 1982).

Besides environmental factors, growth regulators have also been implicated to promote seed germination and growth performance in a number of species (Sankhla and Sankhla, 1968; Ovcharov, 1969; Khan, 1977; Thomas, 1981; Mehanna _et al._, 1985). During the storage of seeds, the reduction in quantity of hormones and vitamins or accumulation of growth inhibitory substances have been reported. The application of growth stimulatory substances antagonize the effect of growth inhibitory substances and also intensify the metabolism during germination (Ovcharov, 1969; Khan, 1977; Lewak, 1984). In commercial crop production, where high yields and uniform produce are two main considerations, slow and variable seedlings can be a major problem (Dunlap and Barnett, 1982). Rapid uniform and complete germination are pre-requisites for achieving good plant populations from the seeds. The use of regulatory compounds to improve seedling performance has become more realistic possibility as our understanding of the hormonal control of metabolic processes has increased.

Materials and Methods

Seeds of P. kesiya and S. khasiana stored in sealed plastic bags at 3-5°C were used for the present study. Surface sterilized P. kesiya and S. khasiana seeds were placed in petridishes (as described in chapter II) and germinated at 25±2°C and 20±1°C, respectively, unless indicated otherwise. Each set of treatment consisted of four replicates and experiments were performed twice. The seed germination and seedling growth (on 20th day) were measured as given in chapter II. The days to peak value was calculated following Campbell and Sorensen (1979). Statistical analyses were performed wherever necessary (Snedecor, 1961).

For effects of pH and water stress, the unimbibed seeds were supplied with 2.0 ml of appropriate experimental solutions. These were drained off from the petridishes and replaced with fresh solutions at 2-day interval.

(i) pH

The pH of distilled water was adjusted either with 0.1 N HCl or 0.1 N NaOH solution.

P. kesiya seeds were supplied with acidic water (pH 4.8-6.8).

S. khasiana seeds were supplied with acidic and alkaline water ranging from pH 4.8-8.0.

The seeds mentioned above were germinated in dark. The controls comprised of seeds of both the species subjected to pH 7.0.

(ii) Water stress

Water stress was created with either sucrose or polyethylene glycol, 6000 (PEG) following the methods of Bonner (1968) or Michel and Kaufman (1973), respectively.

P. kesiya seeds were subjected to water potential from 0 to -15.0 bars created with sucrose and germinated at 25 lux for 16 hr day.

S. khasiana seeds were subjected to different water potentials from 0 to -5.0 bars created with either sucrose or PEG and germinated in dark.

(iii) Imbibition

Both P. kesiya and S. khasiana seeds were soaked in distilled water at different temperatures (5, 15, 25 and 35° C) for 24 hr. In addition, P. kesiya and S. khasiana seeds were imbibed for 0-96 hr at 5° C and 0-24 hr at 15° C, respectively.

For different investigations, P. kesiya and S. khasiana seeds were allowed to imbibe in dark at 5° C for 24 hr and 15° C for 6 hr, respectively.

(iv) Light

The mixed light of 4,000 lux was obtained by using two fluorescent cool day lamps (40 W) and three incandescent bulbs (100 W). The different intensities of light were obtained in a closed box at 30° base with a wide opening at another end (Verma and Tandon, 1984b).

The seeds of both the species germinated in dark, were used as controls for the following studies:

Photoperiod

P. kesiya and *S. khasiana* seeds were germinated under 8-20 hr and 6-24 hr photoperiod, respectively.

Light intensity

P. kesiya seeds were germinated at 1-10 lux light of 16 hr day length.

S. khasiana seeds were germinated at different light intensities of 1,5,10,25,50,100 and 2,000 lux for 8 hr day length.

Wavelength

Red light was obtained by filtering the mixed light with four layers of red cellophane and far-red by filtering through three layers of blue and one layer of red cellophane following the procedure of Nyman (1961).

P. kesiya seeds were irradiated for 10 min with red and 20 min with far-red light and germinated in dark.

(v) Temperature

P. kesiya seeds were germinated in dark at constant (15, 20, 25, 30 and 35° C) and diurnal (20/15, 25/15, 30/15, 35/15, 30/20, 35/20, and 35/25° C) temperatures. For diurnal temperature, the higher temperature treatment was given for 16 hr at 3 lux light and lower for 8 hr in dark.

S. khasiana seeds were germinated at various constant temperatures (10, 15, 20 and 25° C) in dark and at diurnal temperatures (15/20, 20/10, 25/10 and 25/15° C); higher temperature being provided with 25 lux of light for 8 hr followed by dark.

(vi) Growth Regulators

The influence of growth regulators was studied on one-year-stored seeds at laboratory temperature.

P. kesiya seeds were soaked in IAA (1-100 mg/l), 2,4-D (10-200 mg/l), GA (10-250 mg/l), FAP (0.5-10 mg/l) and AA (1-100 mg/l) solutions.

S. khasiana seeds were soaked in IAA (1-100 mg/l), 2,4-D (10-100 mg/l), GA (1-100 mg/l), FAP (0.05-1.0 mg/l) and AA (1-50 mg/l).

After soaking, the seeds were thoroughly washed 4-5 times with sterilized distilled water and germinated under 3 lux light for 16 hr and 25 lux for 8 hr day length in *P. kesiya* and *S. khasiana*, respectively.

Results

(i) pH

P. kesiya : Maximum percentage germination was recorded at pH 4.8 (Table 3.1). However, early germination resulted at pH 6.0 and 6.4. The growth of seedling in all the treatments was less as compared to control, though it was statistically insignificant (Fig. 3.1).

S. khasiana : The earlier and better germination was recorded in slightly acidic to neutral pH (6.4-7.2) treatments (Table 3.1). The higher elongation and dry weight were recorded in mildly acidic pH (6.0-6.8) (Fig. 3.2).

(ii) Water stress

P. kesiya : As compared to control, a water potential of -2.5 bars resulted in maximum germination (Table 3.2) and seedling growth (Fig. 3.3). Increase in water potential above this value decreased both germination and growth. The complete inhibition of growth was recorded at -10.0 and -15.0 bars water potential.

S. khasiana : The germination percentage was significantly inhibited ($p \leq 0.05$) above -1.0 bar water potential. The earlier germination was recorded at both -0.1 and -0.5 bars (Table 3.3). With increase in osmotic potential created with PEG, shoot and radicle growth were reduced compared to control.

Table 3.1 : Effect of pH on seed germination in *Pinus kesiya* and *Schima khasiana*

pH	Germination (%)	Germination rate (% seed day^{-1})	Days to peak value
Pinus kesiya			
7.0 (Control)	79.25	21.70 ± 5.80	3.30
4.8	90.30	34.50 ± 5.00	2.50
5.2	84.10	40.10 ± 2.60	2.50
5.6	82.20	41.20 ± 4.60	2.20
6.0	80.10	41.60 ± 7.00	2.00
6.4	80.50	43.20 ± 6.35	2.00
6.8	80.50	38.80 ± 6.00	2.20
L.S.D. (p=0.05)	11.80		
Schima khasiana			
7.0 (Control)	37.20	56.80 ±17.10	1.20
4.8	26.90	10.20 ± 3.70	6.10
5.2	26.90	16.40 ± 3.00	5.00
5.6	31.20	18.50 ± 3.10	4.70
6.0	31.20	21.60 ± 7.10	3.00
6.4	36.30	42.30 ±10.30	1.80
6.8	37.20	57.10 ±17.30	1.20
7.2	37.20	56.70 ±16.80	1.20
7.6	24.80	11.00 ± 6.50	3.60
8.0	20.60	9.30 ± 3.70	3.60
L.S.D. (p=0.05)	9.40		

± S.E.

Fig. 3.1 : Effect of pH on length of hypocotyl (○), radicle (△), cotyledon (□) and biomass of hypocotyl (●), radicle (▲), cotyledon (■), shoot (x) and seedling (●) of *Pinus kesiya*. Vertical bars (L.S.D. $p=0.05$).

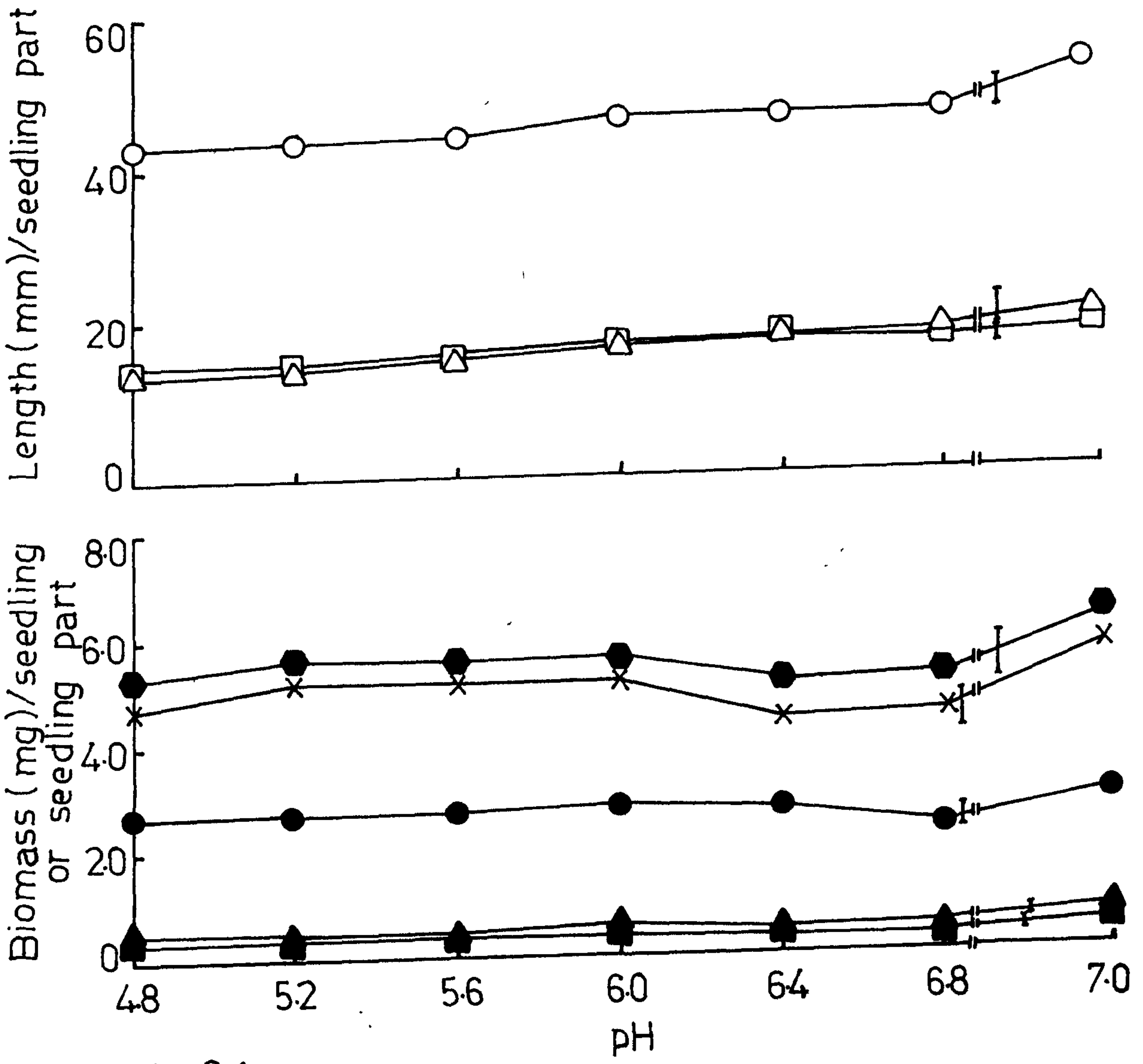

Length (mm)/seedling part
60
40
20
0
Biomass (mg)/seedling or seedling part
8·0
6·0
4·0
2·0
0
4·8
5·2
5·6
6·0
6·4
6·8
7·0
pH

Fig. 3·1

Fig. 3.2 : Effect of pH on length of hypocotyl (O) and radicle (△), cotyledon expansion (▣) and biomass of hypocotyl (x), radicle (▲), cotyledon (■), shoot (◆) and seedling (●) of *Schima khasiana*. Vertical bars (L.S.D. $p=0.05$).

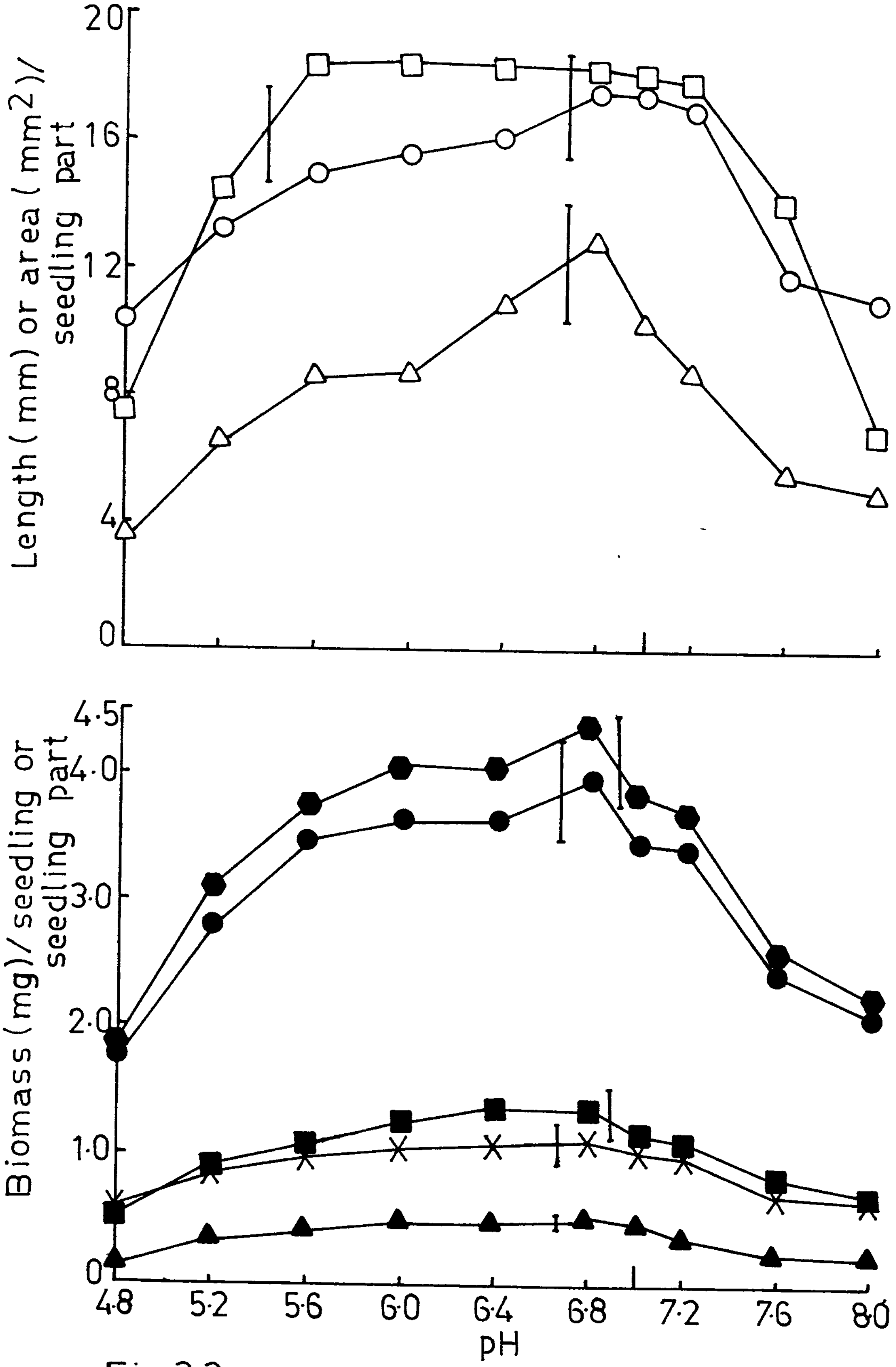

Length (mm) or area (mm^2)/ seedling part
20
16
12
8
4
0
Biomass (mg)/ seedling or seedling part
4·5
4·0
3·0
2·0
1·0
0
4·8
5·2
5·6
6·0
6·4
6·8
7·2
7·6
8·0
pH

Fig. 3·2

Table : 3.2 : Effect of water stress on seed germination in *Pinus kesiya*

Water potential (-bar)	Germination (%)	Germination rate (% seed day^{-1})	Days to peak value
0 (Control)	90.00	35.50 ± 3.90	2.60
2.50	95.00	31.60 ± 1.85	1.60
3.75	79.10	29.00 ± 1.65	3.40
5.00	79.10	20.90 ± 1.80	4.80
10.00	15.80	16.80 ± 2.45	5.20
15.00	5.50	8.50 ± 0.35	11.75
L.S.D. (p-0.05)	21.70		

± S.E.

Fig. 3.3 : Effect of water stress on length of hypocotyl (O), radicle (△), cotyledon (□) and biomass of hypocotyle (●), radicle (▲), cotyledon (■), shoot (x) and seedling (●) in *Pinus kesiya*. Verticale bars (L.S.D. $p=0.05$).

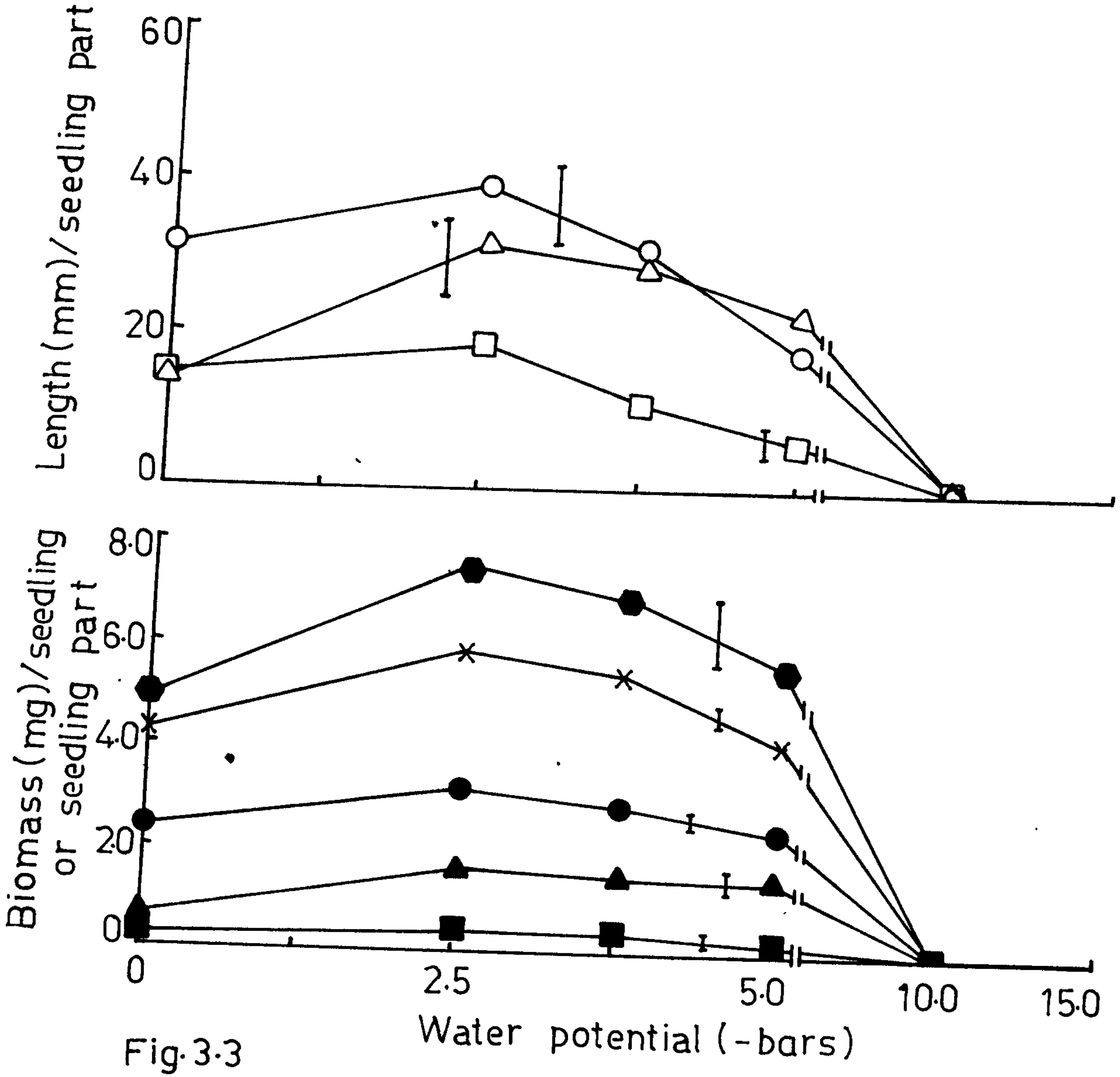

60
40
20
0
Length (mm)/seedling part
8.0
6.0
4.0
2.0
0
Biomass (mg)/seedling or seedling part
0
2.5
5.0
10.0
15.0
Water potential (-bars)

Fig. 3.3

Table 3.3 : Effect of water stress on seed germination in *Schima khasiana*

Water potential (-bar)	Germination (%)		Germination rate (% seed day^{-1})		Days to peak value	
	PEG	Sucrose	PEG	Sucrose	PEG	Sucrose
0 (Control)	40.00 ± 3.00	40.00 ± 3.00	32.80 ± 8.8	32.80 ± 8.8	2.20	2.20
0.1	42.00 ± 4.50	36.00 ± 4.60	39.60 ±10.5	43.30 ± 5.9	1.80	2.00
0.5	40.00 ± 4.00	30.00 ± 3.10	39.10 ±10.1	41.60 ± 6.8	2.00	2.00
1.0	40.00 ± 5.00	30.00 ± 5.00	39.90 ± 5.6	26.60 ± 6.8	2.20	2.70
2.5	28.00 ± 3.70	20.00 ± 3.00	29.50 ± 1.4	18.30 ± 6.5	3.30	3.40
5.0	16.00 ± 4.90	4.00 ± 0.90	9.30 ± 3.7	10.50 ± 4.4	3.60	5.40
L.S.D.(p 0.05)	12.70	13.30	18.80	13.00		

± S.E.

In contrast, using sucrose as an osmoticum, significant increase in shoot, hypocotyl, cotyledon and seedling dry weight was recorded at -0.1 bar of water potential (Fig. 3.4). However, radicle and shoot elongation as well as radicle dry weight decreased slightly. Growth of root and shoot were completely inhibited at higher water potentials induced with PEG or sucrose.

(iii) Imbibition

P. kesiya : The seeds imbibed at 5°C showed better germination percentage and rate as compared to control and seeds imbibed at higher temperature (Table 3.4). The imbibition of seeds at 5°C also resulted in maximum length of seedling parts and their biomass (Fig. 3.5). Imbibition for various periods at 5°C also indicated that 24 hr duration was optimum for germination percentage and rate as well as earliest days of peak value (Table 3.5). However, the growth of seedling parts was almost equal in seeds imbibed for 12 and 24 hr at 5°C except radicle length (Fig. 3.6).

S. khasiana : Seeds soaked in distilled water at various temperatures did not show any significant increase in seed germination and rate (Table 3.4). On the other hand, soaking of seeds at 35°C was inhibitory. The earlier and higher germination of about 48% ($p \leq 0.05$) was recorded in seeds imbibed for 6 hr at 15°C (Table 3.5). An imbibition temperature of 15°C

Fig. 3.4 : Effect of water stress (Sucrose, ... ; PEG, ——) on length of hypocotyl (O) and radicle (△), cotyledon expansion (□) and biomass of hypocotyl (x), radicle (▲), cotyledon (■), shoot (●) and seedling (●) of *Schima khasiana*. Vertical bars (L.S.D. $p=0.05$).

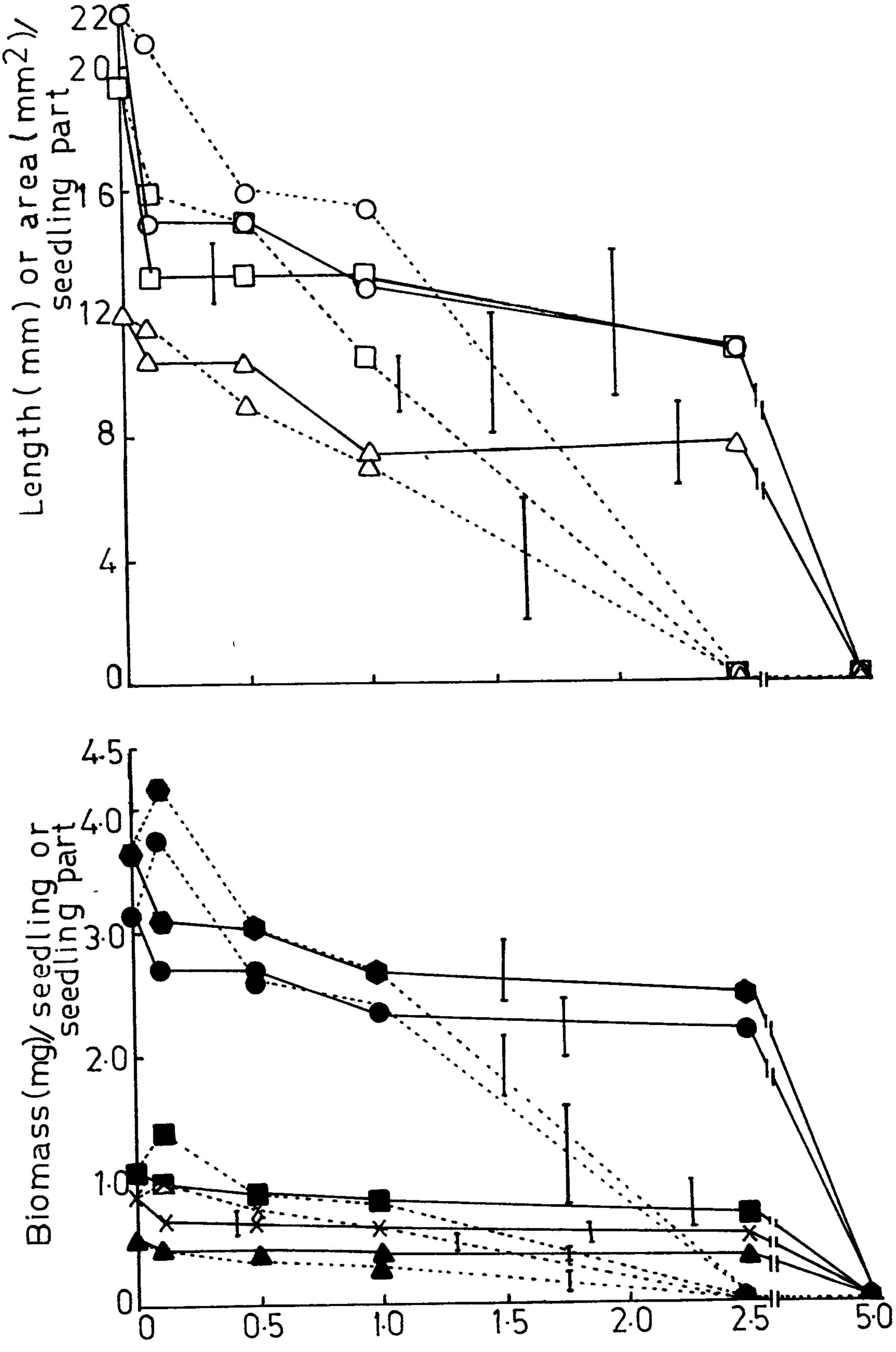

Length (mm) or area (mm2)/ seedling part
22
20
16
12
8
4
0
Biomass (mg)/ seedling or seedling part
4.5
4.0
3.0
2.0
1.0
0
0
0.5
1.0
1.5
2.0
2.5
5.0
Water potential (-bars)

Fig. 3.4

Table 3.4 : Effect of imbibition temperatures on seed germination in *Pinus kesiya* and *Schima khasiana*

Temperature (°C)	Germination (%)	Germination rate (% seed day^{-1})	Days to peak value
Pinus kesiya			
Control	57.00	21.70 ± 5.80	3.30
5	92.70	54.10 ± 4.35	1.75
15	85.15	43.00 ± 9.35	1.80
25	75.90	33.20 ± 1.50	3.00
35	71.00	21.70 ± 5.80	3.30
L.S.D. (p=0.05)	9.75		
Schima khasiana			
Control	38.50	34.70 ± 6.30	2.35
5	40.40	33.10 ±10.15	2.10
15	42.30	35.60 ± 8.50	2.00
25	42.30	32.80 ± 8.80	2.15
35	21.15	18.30 ± 4.10	4.15
L.S.D. (p=0.05)	13.40		

± S.E.

Fig. 3.5 : Effect of imbibition of *Pinus kesiya* seeds at different temperatures on length of hypocotyl (O), radicle (△), cotyledon (□) and biomass of hypocotyl (●), radicle (▲), cotyledon (■), shoot (x) and seedling (◆), C = control, verticale bars (L.S.D. $p=0.05$).

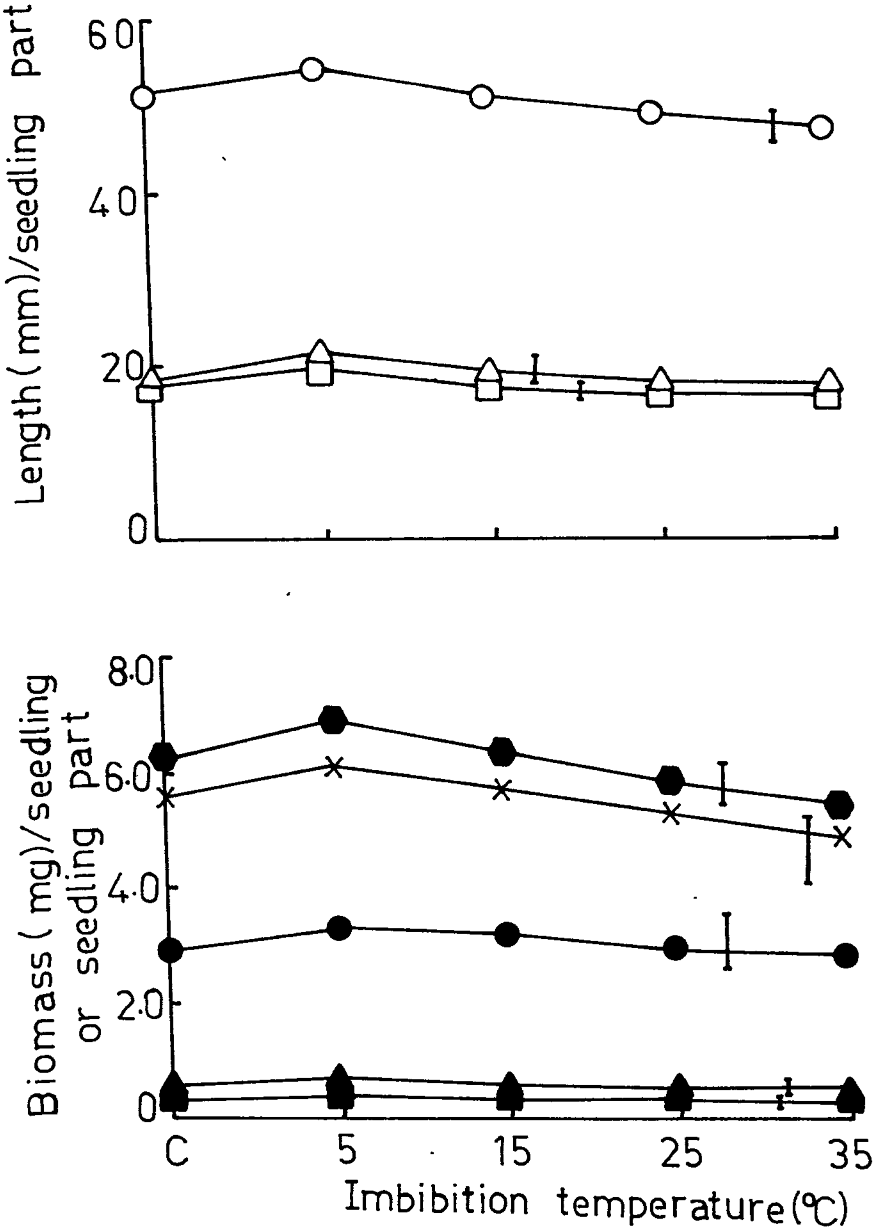

Length (mm)/seedling part
60
40
20
0
Biomass (mg)/seedling or seedling part
8.0
6.0
4.0
2.0
0
C
5
15
25
35
Imbibition temperature (°C)

Fig. 3.5

Table 3.5 : Effect of imbibition period on seed germination in *Pinus kesiya* and *Schima khasiana*

Imbibition Period (hr)	Germination (%)	Germination rate (% seed day^{-1})	Days to peak value
Pinus kesiya			
0 (Control)	57.00	21.70 ± 5.80	3.30
6	89.10	43.30 ± 6.00	2.00
12	91.70	46.00 ± 6.60	1.90
24 ✓	92.70	54.10 ± 4.35	1.75
48	87.10	29.50 ± 1.35	3.30
96	60.25	20.00 ± 2.70	4.40
L.S.D. ($p=0.05$)	11.80		
Schima khasiana			
0 (Control)	38.50	34.70 ± 6.30	2.35
3	38.50	34.70 ± 8.80	2.15
6	48.70	42.10 ±11.75	1.70
12	44.40	39.60 ±10.50	1.80
24	42.30	35.60 ± 8.50	2.10
L.S.D. ($p=0.05$)	6.30		

± S.E.

Fig. 3.6 : Effect of imbibition of *Pinus kesiya* at 5°C for different periods on length of hypocotyl (O), radicle (△), cotyledon (□) and biomass of hypocotyl (●), radicle (▲), cotyledon (■), shoot (x) and seedling (●). Vertical bars (L.S.D. $p=0.05$).

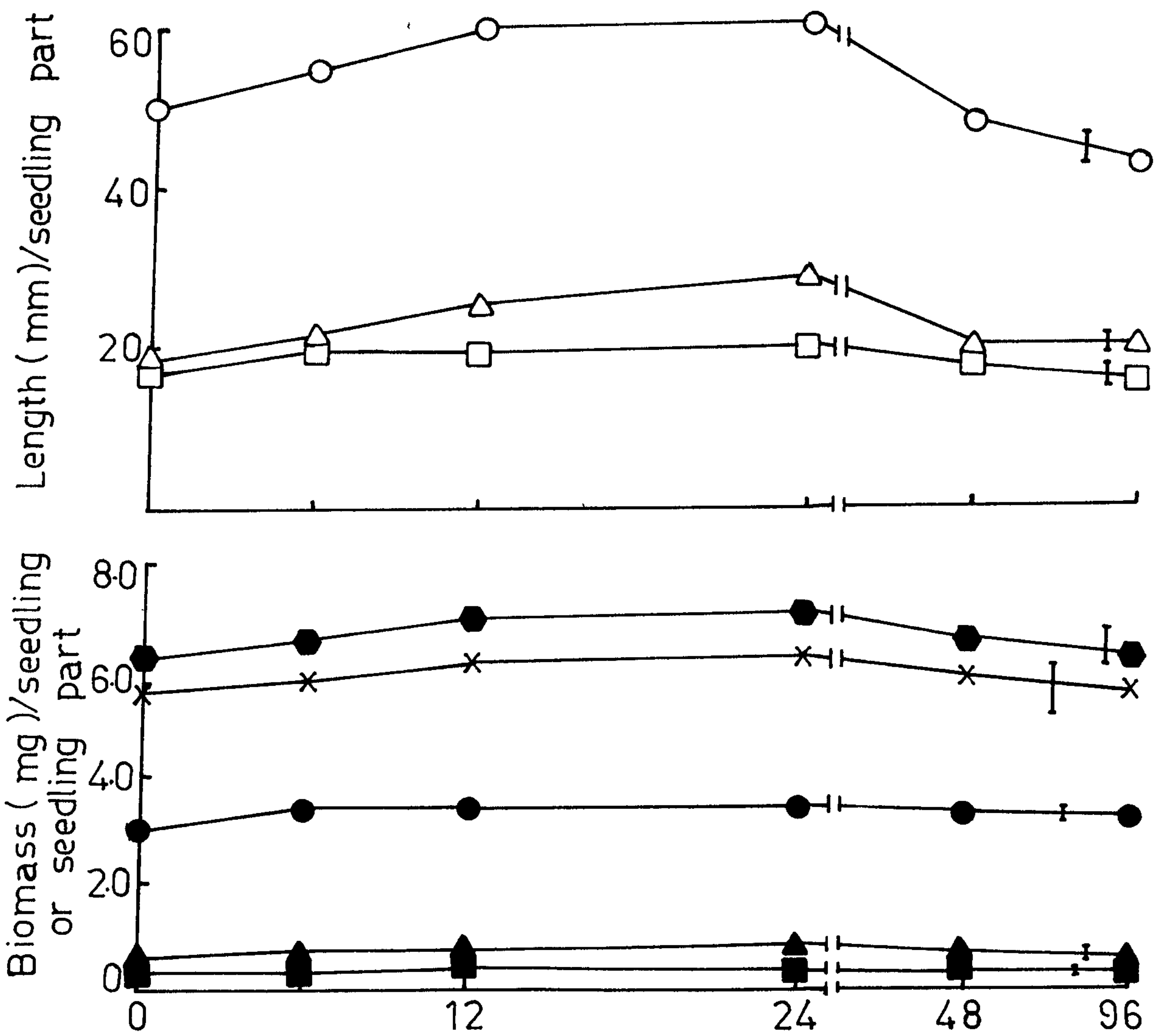

Length (mm)/seedling part
60
40
20
Biomass (mg)/seedling or seedling part
8.0
6.0
4.0
2.0
0
0
12
24
48
96
Imbibition period (hr)

Fig. 3.6

(Fig. 3.7) for 6 hr (Fig. 3.8) was found optimum for seedling growth.

(iv) Light

Photoperiod

P. kesiya : Amongst different photoperiods, 16 hr duration was found optimum for germination (Table 3.6); length and biomass of seedling parts (Fig. 3.9).

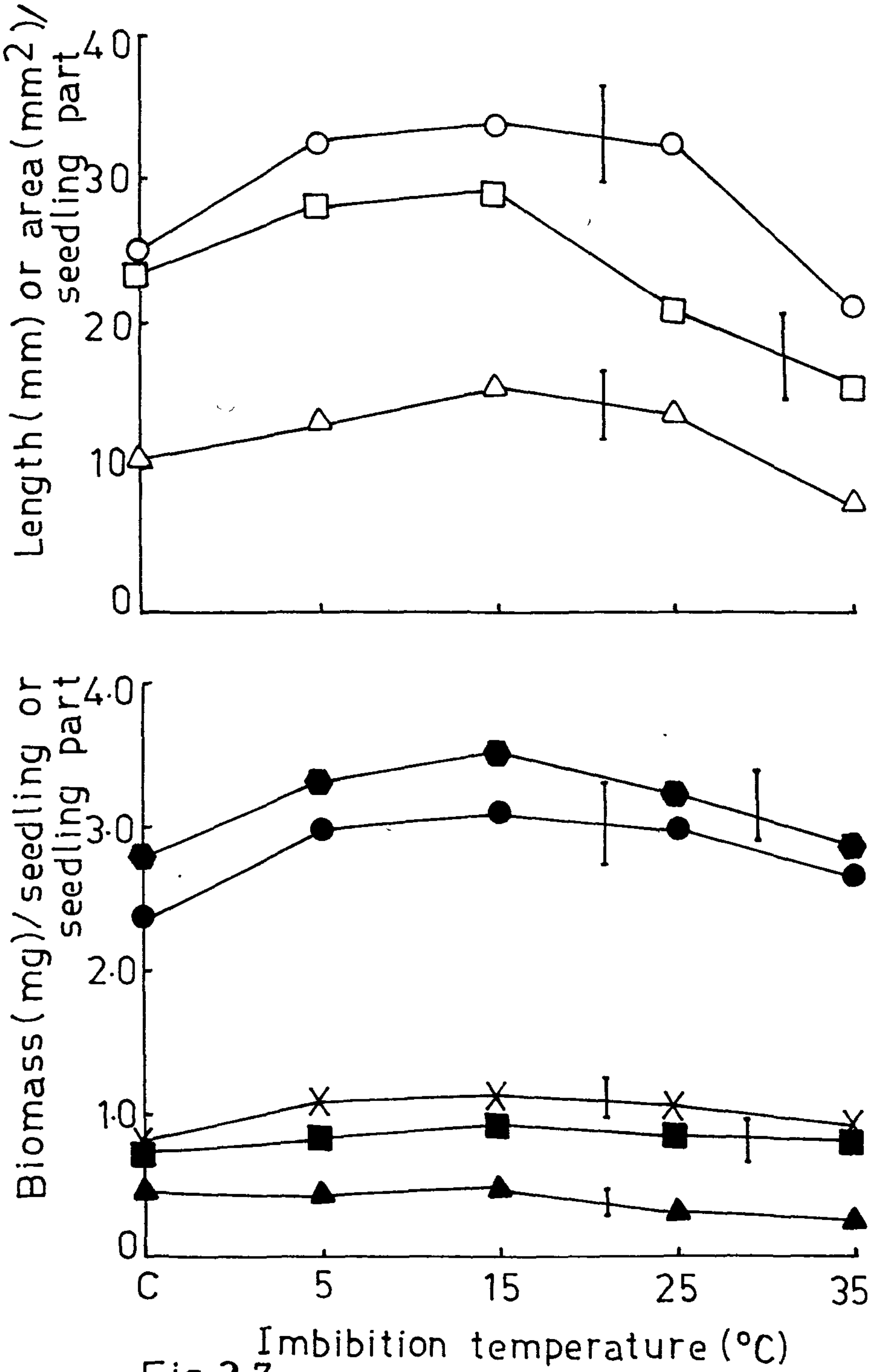
S. khasiana : The germination increased significantly ($p<0.05$) with 8-10 hr photoperiods (Table 3.6) as compared to seeds germinated in dark. However, with higher photoperiods the germination decreased. The cotyledon expansion and dry weight of seedling parts were higher at 8-10 hr photoperiods, while shoot elongation was found maximum in dark (Fig. 3.10).

Light intensity

P. kesiya : The light intensity from 1-10 lux resulted in better germination as compared to dark. A 3.0 lux light requirement was found optimum for fast germination (Table 3.7). The length and biomass of seedling were also maximum at this light intensity (Fig. 3.11).

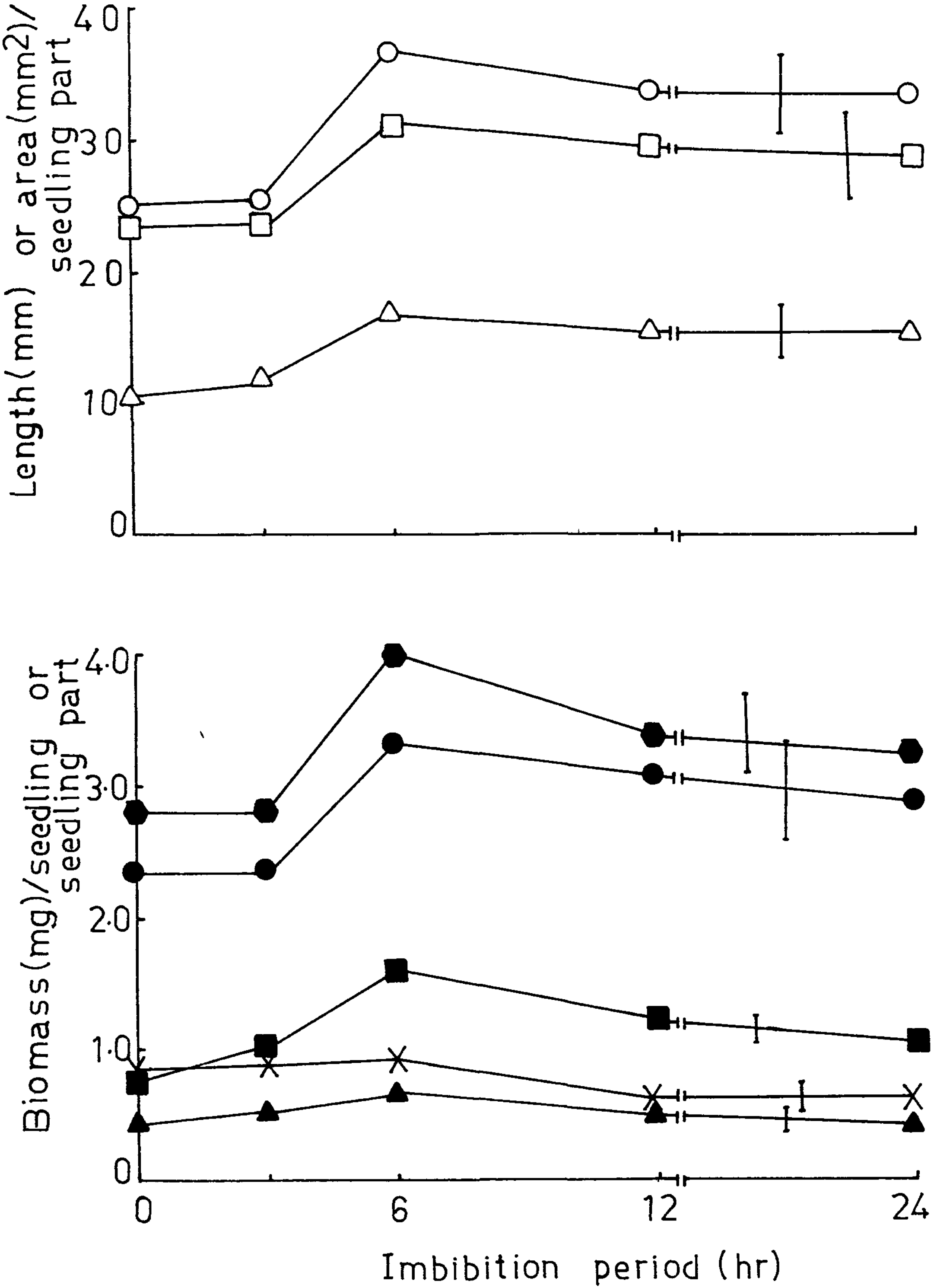
S. khasiana : A light of 25 lux resulted in maximum germination percentage and rate and also reduced the days to peak value (Table 3.7) which decreased subsequently at higher

Fig. 3.7 : Effect of imbibition of Schima khasiana seeds at different temperatures on length of hypocotyl (O) and radicle (△), cotyledon expansion (□) and biomass of hypocotyl (x), radicle (▲), cotyledon (■), shoot (●) and seedling (●), C = control, vertical bars (L.S.D. p=0.05).


Length (mm) or area (mm2)/ seedling part
40
30
20
10
0
Biomass (mg)/seedling or seedling part
4·0
3·0
2·0
1·0
0
C
5
15
25
35
Imbibition temperature (°C)

Fig·3·7

Fig. 3.8 : Effect of imbibition of Schima khasiana at 15°C for different periods on length of hypocotyl (O) and radicle (△), cotyledon expansion (□) and biomass of hypocotyl (x), radicle (▲), cotyledon (■), shoot (●) and seedling (●). Verticale bars (L.S.D. $p=0.05$).



Length(mm) or area(mm2)/seedling part
0
10
20
30
40
Biomass(mg)/seedling or seedling part
0
1.0
2.0
3.0
4.0
0
6
12
24
Imbibition period (hr)

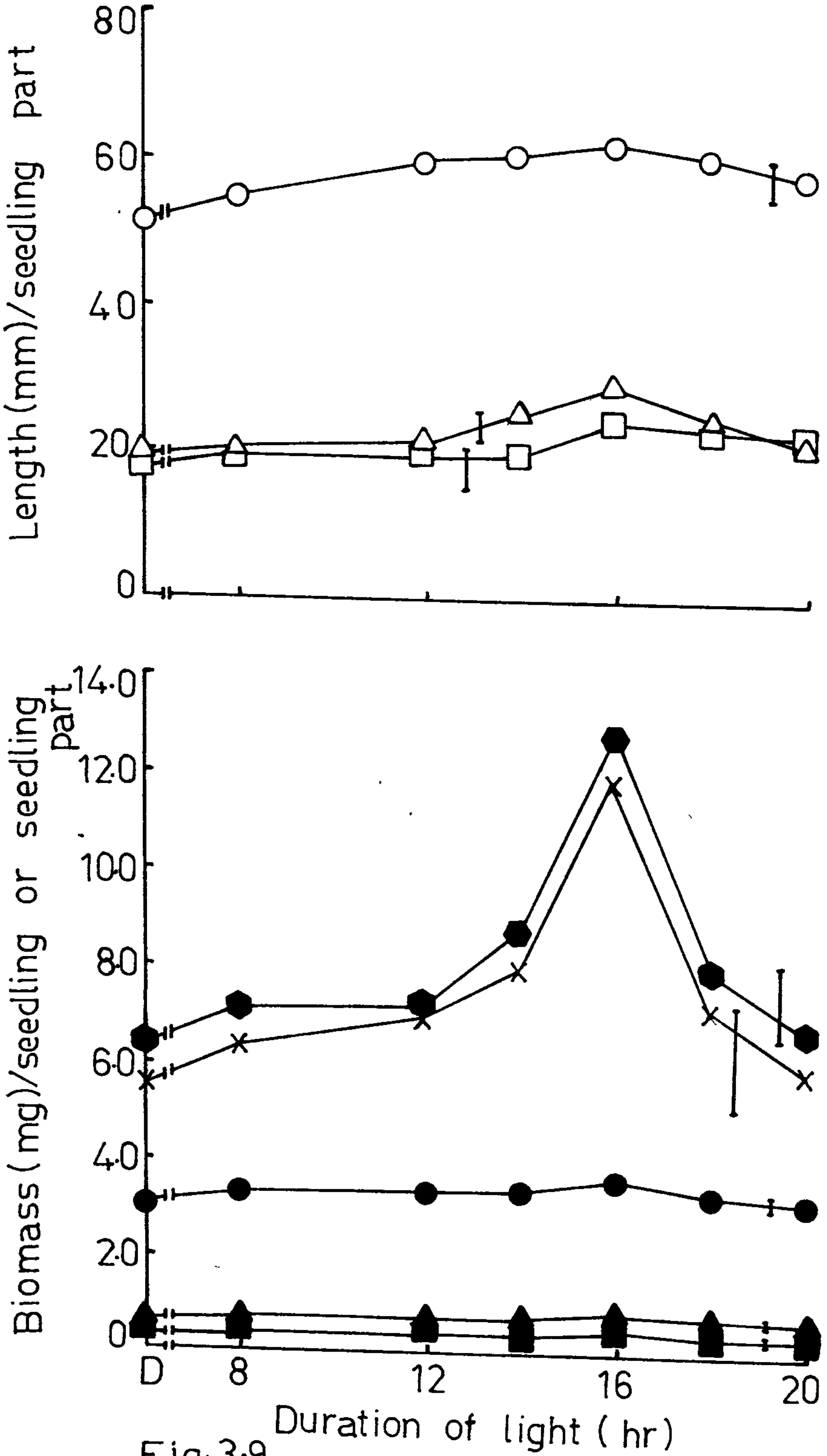
Fig. 3.8

Table 3.6 : Effect of photoperiod on seed germination in *Pinus kesiya* and *Schima khasiana*

Duration of light (hr)	Germination (%)	Germination rate (% seed day^{-1})	Days to peak value
Pinus kesiya			
Dark	62.75	23.40 ± 3.60	3.70
8	68.75	31.40 ± 4.05	2.90
12	69.10	46.00 ± 7.85	1.80
14	71.10	53.10 ± 6.05	1.70
16	91.70	57.90 ±12.80	1.35
18	72.70	53.10 ±13.85	1.35
20	68.75	47.40 ± 5.15	1.90
L.S.D. (p=0.05)	10.65		
Schima khasiana			
Dark	47.50	31.30 ± 3.10	3.00
6	57.50	42.00 ± 6.40	2.10
8	67.50	56.50 ±12.30	1.40
10	65.00	56.10 ±11.60	1.40
12	55.00	33.80 ± 4.25	2.60
16	52.50	33.30 ± 3.90	2.70
24	55.00	31.70 ± 3.50	2.90
L.S.D. (p=0.05)	11.40		

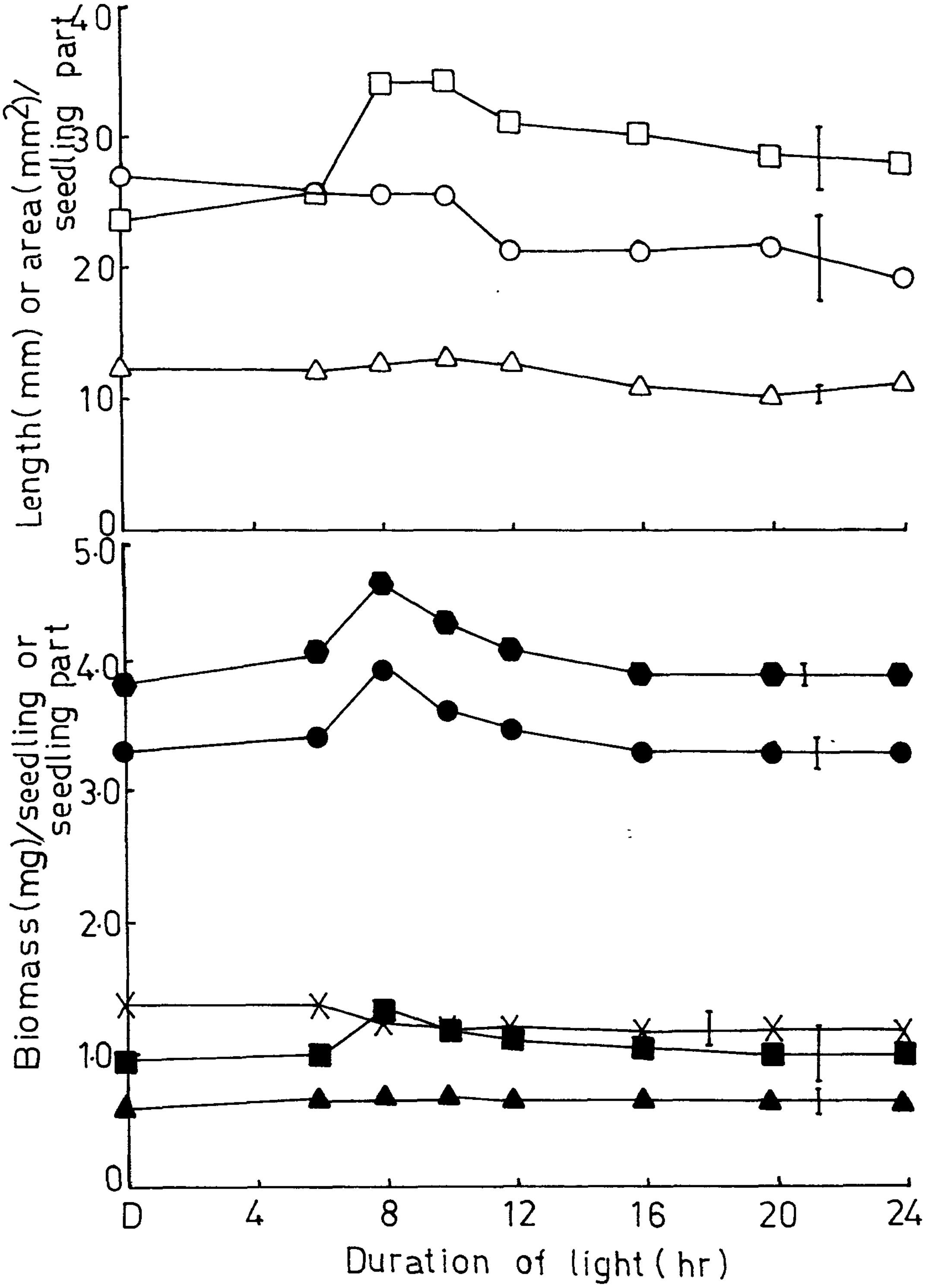
± S.E.

Fig. 3.9 : Photoperiodic effect on length of hypocotyl (O), radicle (△), cotyledon (□) and biomass of hypocotyl (●), radicle (▲), cotyledon (■), shoot (x) and seedling (●) of *Pinus kesiya*, D = dark. Vertical bars (L.S.D. p=0.05)


80
60
40
20
0
Length (mm)/seedling part
14·0
12·0
10·0
8·0
6·0
4·0
2·0
0
Biomass (mg)/seedling or seedling part
D
8
12
16
20
Duration of light (hr)

Fig·3·9

Fig. 3.10 : Photoperiodic effect on length of hypocotyl (O) and radicle (△), cotyledon expansion (◻) and biomass of hypocotyl (x), radicle (▲), cotyledon (■), shoot (⬮) and seedling (●) of *Schima khasiana*, D = dark. Vertical bars (L.S.D. p=0.05).



Length (mm) or area (mm^2)/ seedling part
40
30
20
10
0
Biomass (mg)/seedling or seedling part
5·0
4·0
3·0
2·0
1·0
0
D
4
8
12
16
20
24
Duration of light (hr)

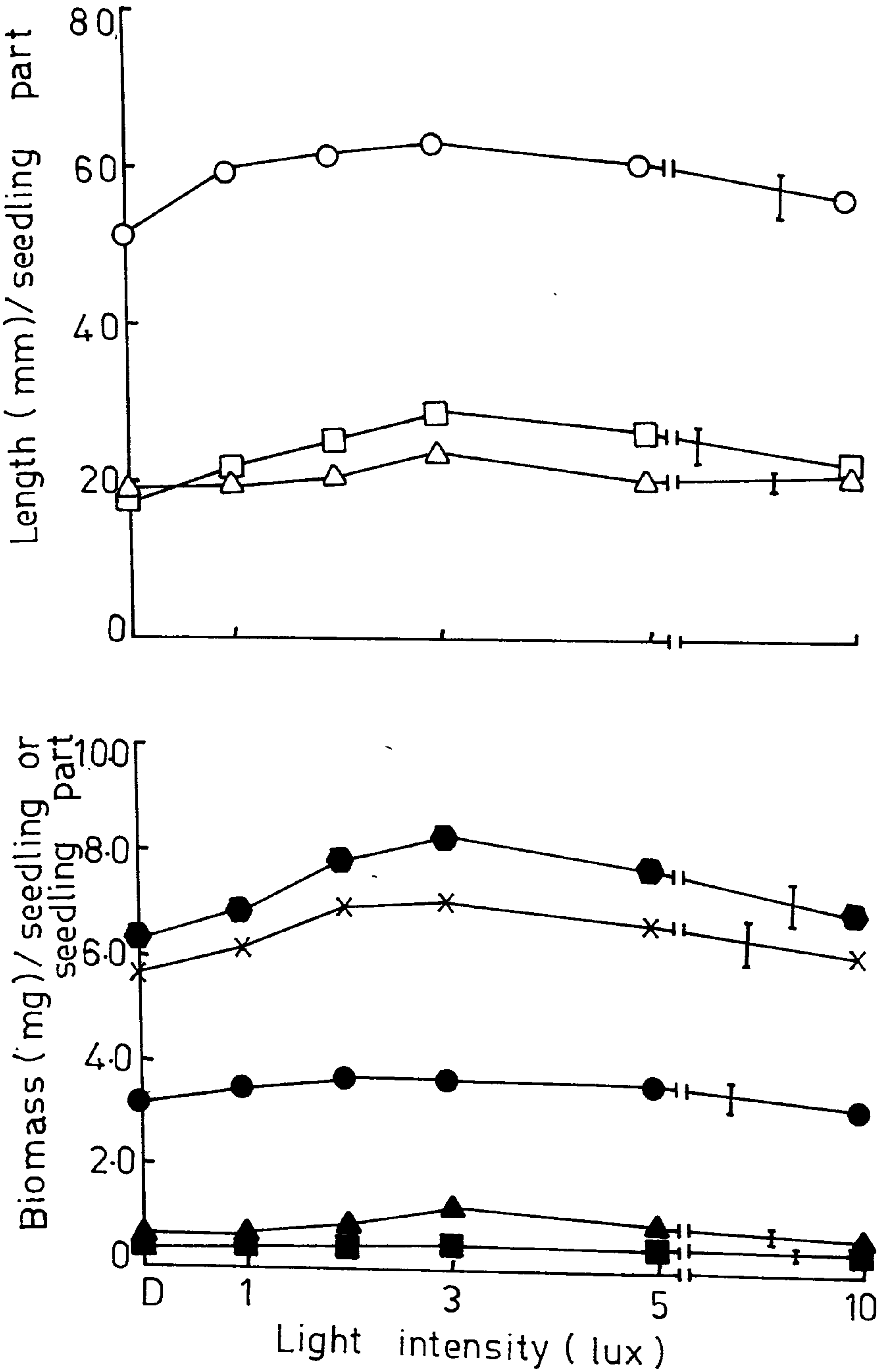
Fig. 3·10

Table 3.7 : Influence of light intensity on seed germination in *Pinus kesiya* and *Schima khasiana*

Light intensity (lux)	Germination (%)	Germination rate (% seed day^{-1})	Days to peak value
Pinus kesiya			
Dark	62.75	23.40 ± 3.60	3.70
1	91.70	31.30 ± 3.10	3.00
2	91.70	36.50 ± 2.60	2.65
3 ✓	91.70	53.20 ± 5.10	1.75
5	91.70	48.00 ± 5.60	1.90
10	91.40	42.50 ± 0.60	2.35
L.S.D. (p=0.05)	8.75		
Schima khasiana			
Dark	47.50	31.30 ± 3.10	3.00
1	47.50	31.40 ± 4.10	2.90
5	50.00	36.50 ± 2.60	2.60
10	50.00	54.10 ± 4.40	1.80
25	65.00	57.90 ±12.80	1.30
50	60.00	53.10 ± 6.10	1.70
100	50.00	43.30 ± 6.00	2.00
L.S.D. (p=0.05)	6.70		

± S.E.

Fig. 3.11 : Effect of light intensity on length of hypocotyl (O), radicle (△), cotyledon (□) and biomass of hypocotyl (●), radicle (▲), cotyledon (■), shoot (x) and seedling (●) of *Pinus kesiya*, D = dark. Vertical bars (L.S.D. $p=0.05$).


80
60
40
20
0
Length (mm)/ seedling part
10·0
8·0
6·0
4·0
2·0
0
Biomass (mg)/ seedling or seedling part
D
1
3
5
10
Light intensity (lux)

Fig·3·11

light intensities. There was a concomitant increase in cotyledon expansion and seedling dry weight with increase in light intensities. Conversely shoot elongation was found to decline upto 25 lux and subsequently no change was noticeable (Fig. 3.12).

Wavelength

P. kesiya : The red light irradiation of seeds resulted in higher germination, rate and seedling growth and reduced the days to peak value as compared to control (Table 3.8 and Fig. 3.13). The far-red irradiation of seeds reversed the stimulatory effect of red light.

(v) Temperature

P. kesiya : The effect of constant temperature showed that germination and seedling growth increased upto 25°C which subsequently decreased by increasing the temperature (Table 3.9). On the other hand, the diurnal temperature of 20/15°C resulted in maximum germination. The diurnal temmoperiodicity of 35/20°C was optimum for length and biomass of seedling parts (Fig. 3.14). A slight increase in growth was also noticed at 25/15°C temperature.

S. khasiana : The optimum and earliest germination were recorded at 20/10°C amongst the various constant and

Fig. 3.12 : Effect of light intensity on length of hypocotyl (○) and radicle (△), cotyledon expansion (□) and biomass of hypocotyl (x), radicle (▲), cotyledon (■), shoot (●) and seedling (⬢) of *Schima khasiana*, D = dark. Vertical bars (L.S.D. p=0.05).

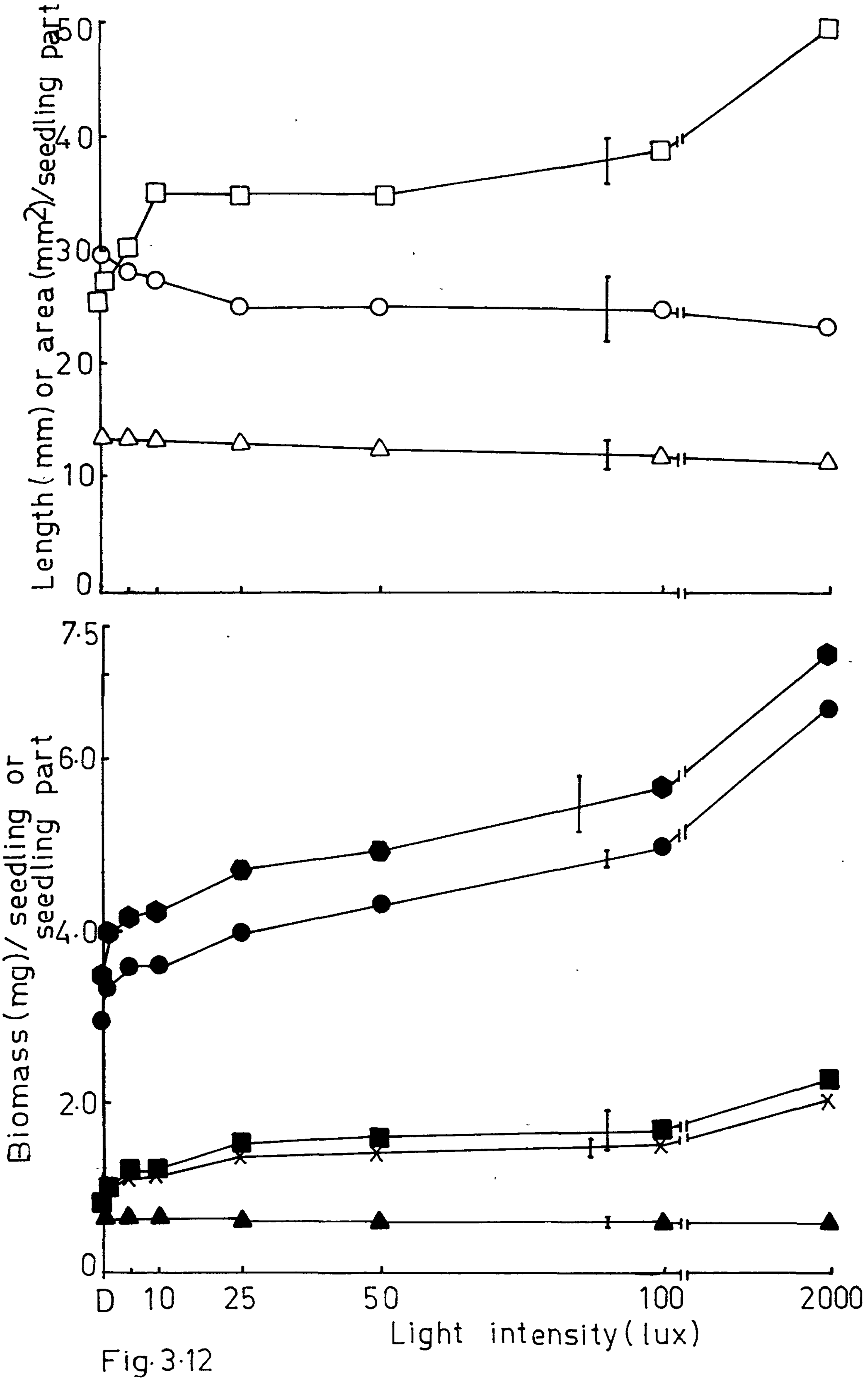

Length (mm) or area (mm^2)/seedling part
0
10
20
30
40
50
Biomass (mg)/ seedling or seedling part
0
2.0
4.0
6.0
7.5
D
10
25
50
100
2000
Light intensity (lux)

Fig. 3.12

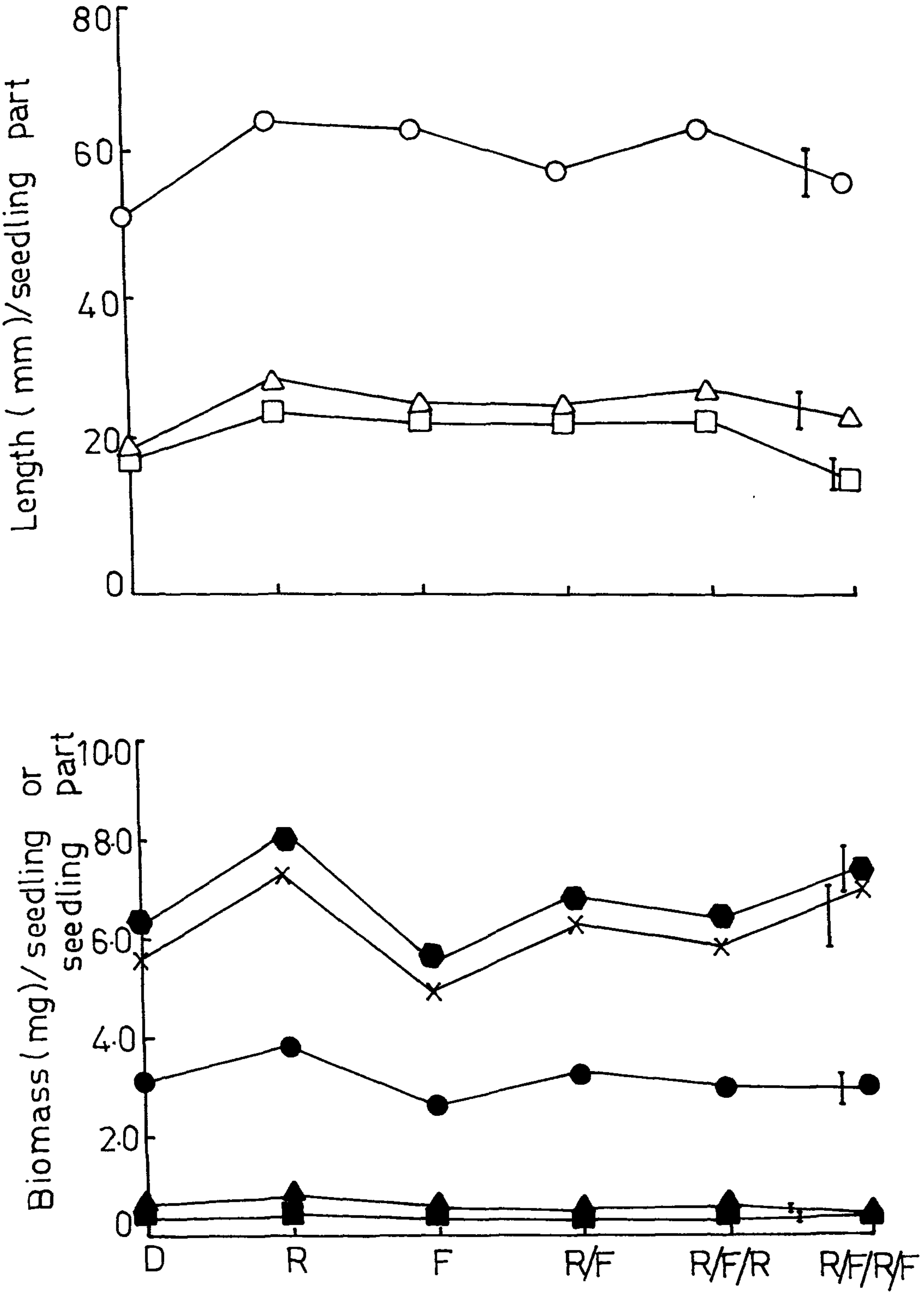
Table 3.8 : Effect of red and far-red radiation on seed germination in *Pinus kesiya*

Treatment	Germination (%)	Germination rate (% seed day^{-1})	Days to peak value
Dark	62.75	23.40 ± 3.60	3.70
R	86.15	43.00 ± 9.35	1.80
F	68.80	28.10 ± 3.30	3.25
R/F	68.80	31.40 ± 4.10	2.85
R/F/R	86.15	43.00 ± 9.35	1.80
R/F/R/F	69.10	31.40 ± 4.10	2.85
L.S.D. (p=0.05)	12.15		

R = Red; F = Far-red

± S.E.

Fig. 3.13 : Response of *Pinus kesiya* seeds to red and far-red radiations on length of hypocotyl (O), radicle (△), cotyledon (□) and biomass of hypocotyl (●), radicle (▲), cotyledon (■), shoot (x) and seedling (●). D = dark. Vertical bars (L.S.D. $p=0.05$).



80
60
40
20
0
Length (mm)/seedling part
10·0
8·0
6·0
4·0
2·0
0
Biomass (mg)/seedling or seedling part
D
R
F
R/F
R/F/R
R/F/R/F
Light irradiation

Fig·3·13

Table 3.9 : Effect of temperature on seed germination in *Pinus kesiya*

Temperature (°C)	Germination (%)	Germination rate (% seed day^{-1})	Days to peak value
15	76.85	14.20 ± 1.00	6.90
20	86.15	20.80 ± 1.60	4.50
25	90.00	30.30 ± 3.85	3.00
30	72.60	20.90 ± 4.15	3.80
35	62.75	11.80 ± 2.65	6.50
20/15	88.90	25.70 ± 3.80	3.40
25/15	73.90	20.50 ± 2.60	4.40
30/15	69.00	27.20 ± 4.35	3.15
35/15	85.80	25.50 ± 3.20	3.50
30/20	73.35	20.40 ± 0.70	4.85
35/20	83.15	25.50 ± 4.00	3.70
35/25	78.75	26.70 ± 5.10	3.00
L.S.D. (p=0.05)	10.80		

± S.E.

Fig. 3.14 : Effect of temperature on length of hypocotyl (O), radicle (△), cotyledon (□) and biomass of hypocotyl (●), radicle (▲), cotyledon (■), shoot (x) and seedling (●) of *Pinus kesiya*. Vertical bars (L.S.D. $p=0.05$).

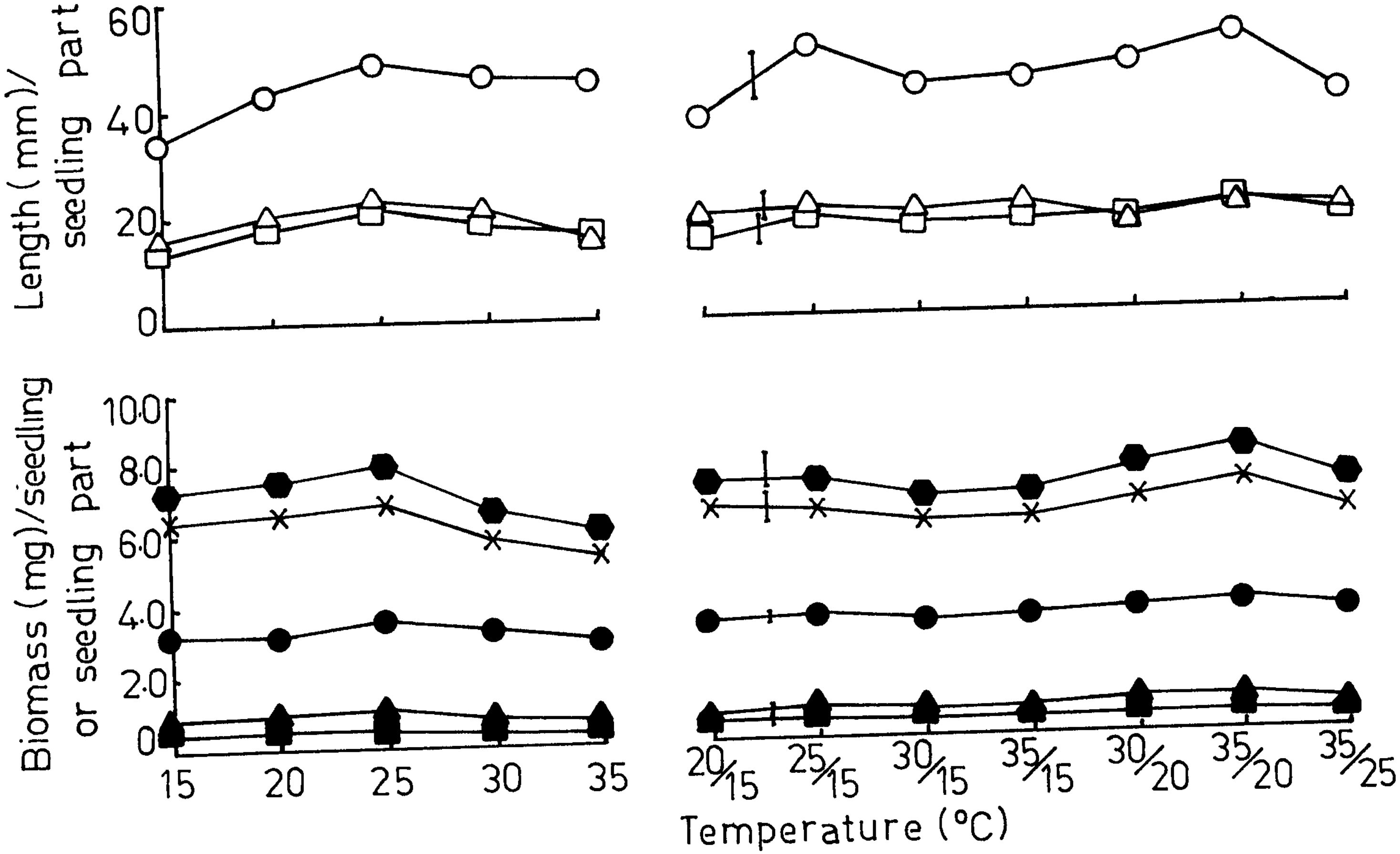

Length (mm)/
seedling part
60
40
20
0
Biomass (mg)/seedling
or seedling part
10.0
8.0
6.0
4.0
2.0
0
15
20
25
30
35
20/15
25/15
30/15
35/15
30/20
35/20
35/25
Temperature (°C)

Fig. 3·14

diurnal temperature treatments (Table 3.10). However, the maximum growth was recorded at 25/15°C (Fig. 3.15) which was higher than optimum temperature for seed germination.

(vi) Growth Regulators

P. kesiya : All the concentrations of growth regulators used, promoted germination of P. kesiya seeds. Seeds treated with IAA (25 mg/l), GA (150-200 mg/l), FAP (1 mg/l), AA (25-50 mg/l) showed better germination percentage, rate and also lower days to peak value as compared to control. On the other hand, 2,4-D treatment resulted in better germination percentage and rate at 100 mg/l and 25 mg/l concentrations, respectively (Table 3.11). There was a decrease in germination with growth regulator treatments beyond the aforesaid concentrations. Amongst the different growth regulators used, GA showed maximum germination percentage, rate and least days to peak value. Comparative study of various growth regulators on seedling growth, revealed that GA and AA showed higher radicle and shoot elongations whereas higher cotyledon growth was noted in FAP and AA treatments (Fig. 3.16). With 2,4-D treatment, abnormal seedlings were produced having reduced radicle. The dry weight of seedling parts was maximum in GA treated seeds.

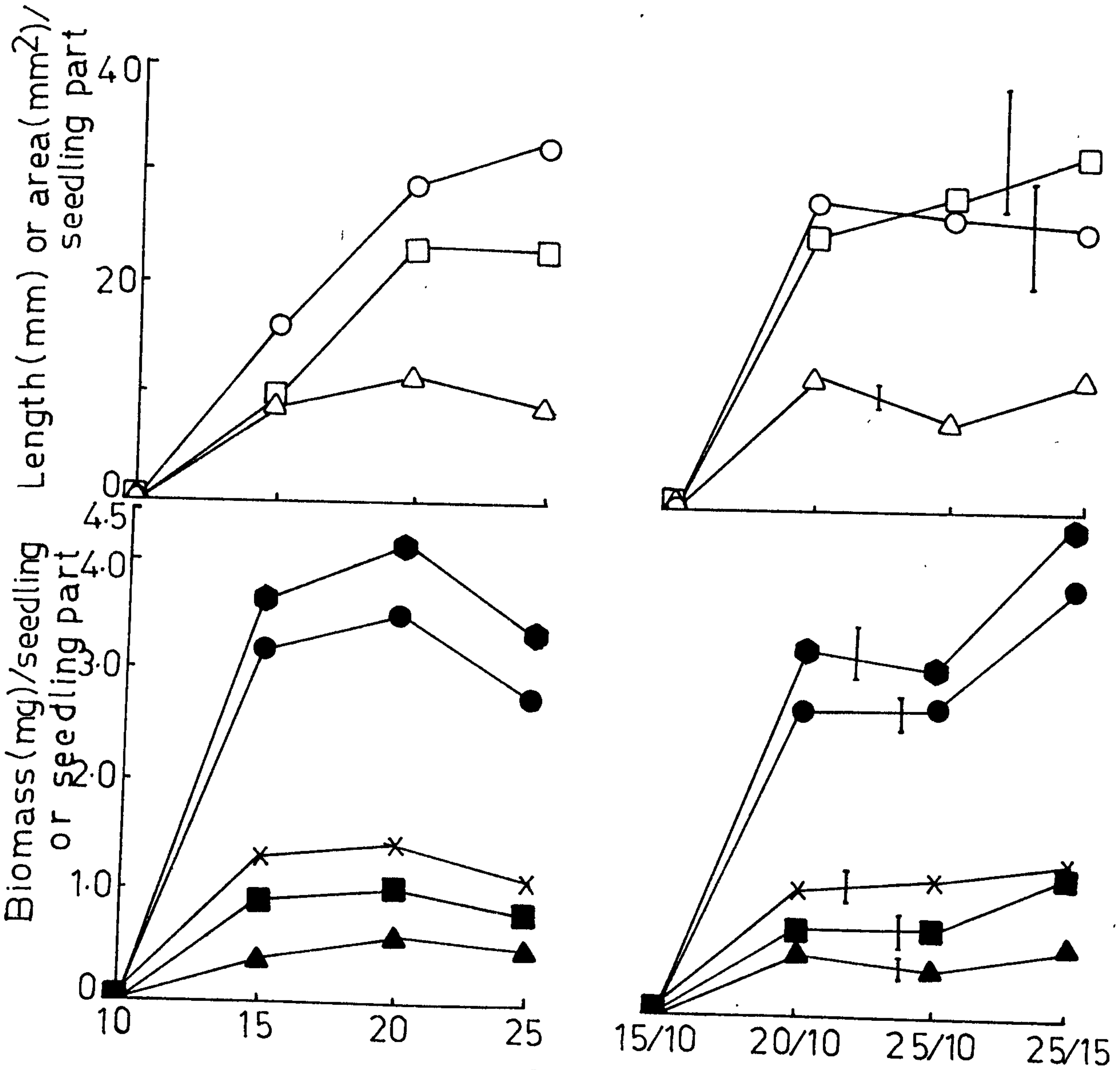
S. khasiana : Different concentrations of growth regulator treatments stimulated germination in this species.

Table 3.10 : Effect of temperature on seed germination in *Schima khasiana*

Temperature (°C)	Germination (%)	Germination rate (% seed day⁻¹)	Days to peak value
10	0	0	0
15	50.00	29.90 ± 4.50	3.70
20	47.50	34.10 ± 5.60	2.50
25	37.50	33.40 ± 3.90	2.70
15/10	52.50	25.80 ± 1.40	3.80
20/10	55.00	38.00 ± 8.90	2.00
25/10	42.50	33.80 ± 4.75	2.70
25/15	35.00	34.70 ± 6.30	2.40
L.S.D. ($p=0.05$)	6.70		

± S.E.

Fig. 3.15 : Effect of temperature on length of hypocotyl (O) and radicle (△), cotyledon expansion (□) and biomass of hypocotyl (x), radicle (▲), cotyledon (■), shoot (⬮) and seedling (●) of *Schima khasiana*. Vertical bars (L.S.D. $p=0.05$).


Length (mm) or area (mm²)/ seedling part
40
20
0
Biomass (mg)/seedling or seedling part
4·5
4·0
3·0
2·0
1·0
0
10
15
20
25
15/10
20/10
25/10
25/15
Temperature (°C)

Fig·3·15

Table 3,11 : Effect of IAA, 2,4-D, GA, FAP and AA on seed germination in *Pinus kesiya*

Treatment (concentration mg/l)	Germination (%)	Germination rate (% seed day^{-1})	Days to peak value
Control	75,00 ± 4,80	21,30 ± 2,20	4,35
IAA			
1	75.00 ± 4.60	30,80 ± 1,60	4,10
10	75.00 ± 6.70	32,10 ± 4,00	3,80
25	88.50 ± 5.20*	33,90 ± 4,20*	3,75
50	86.25 ± 6.00	27,10 ± 1,80	5,50
100	86.25 ± 5.20	26,60 ± 0,60	5.80
2,4-D			
10	75.00 ± 5.00	28,30 ± 6,50	5,40
25	75.00 ± 2.25	43.20 ± 8,30*	3,20
50	83.50 ± 3.25	37.70 ± 3,50*	3,90
100	92.50 ± 3.40*	33.50 ± 3.20	4,60
200	88.80 ± 5.90	35.10 ± 1.50	5,00
GA			
10	81.50 ± 5.00	35.10 ± 1.50	4.00
25	81.50 ± 4.20	35.30 ± 4.60	2.50
50	81.50 ± 4.20	40.00 ± 5.60*	2.25
100	82.50 ± 8,75*	44,50 ± 2,25*	2,20
150	95.25 ± 2.70*	54.50 ± 2,50*	1,80
200	97.50 ± 2.50*	53,50 ± 3,20*	1,80
250	94.00 ± 5.20*	48.50 ± 5.00*	2,00
FAP			
0.5	87.50 ± 2.75	34.50 ± 1.25	4.00
1.0	93.50 ± 2.75*	43.90 ± 4.20*	1.90
5.0	73.50 ± 6.50	43.90 ± 5.20*	2.00
10.0	71.00 ± 2.25	37.10 ± 4.25	2.50
AA			
1	75.00 ± 3.40	34.50 ± 1.50	4.00
10	81.50 ± 4.00	41.80 ± 2.75*	3.00
25	95.00 ± 4.30*	48.00 ± 9.00*	2.00
50	90.00 ± 3.00*	47.90 ± 6.00*	2.25
100	81.50 ± 5.00	35.80 ± 1.40	3.80

± S.E.

* Significant at $p \leqslant 0.05$ level.

Fig. 3.16 : Effect of seed treatment with growth regulators on seedling growth of *Pinus kesiya* and *Schima khasiana*, C = control, (1) IAA, (2) 2,4-D, (3) GA, (4) FAP and (5) AA.

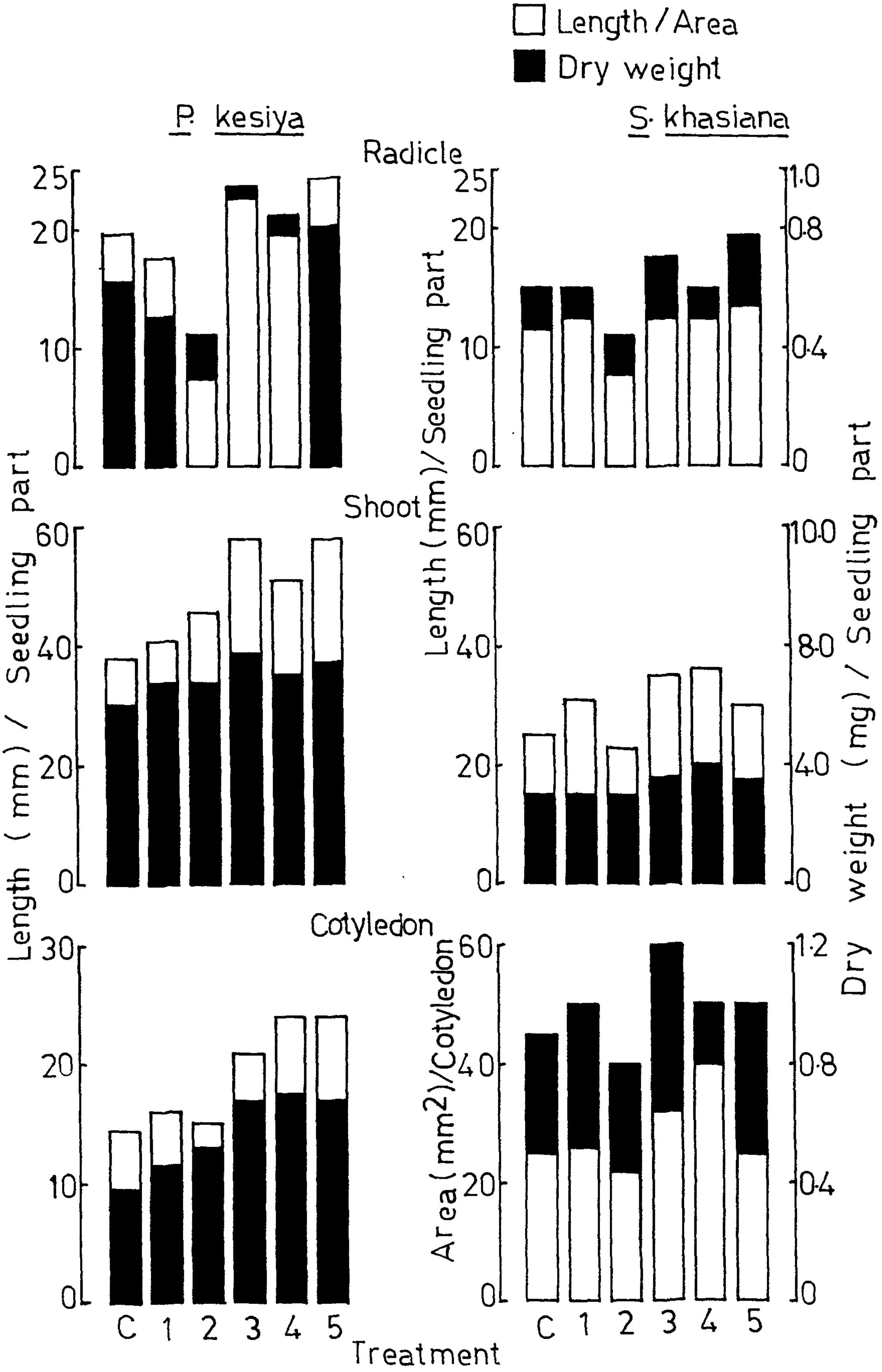

Length / Area
Dry weight
P. kesiya
S. khasiana
Radicle
Shoot
Cotyledon
Length (mm) / Seedling part
Length (mm)/Seedling part
Area (mm^2)/Cotyledon
Dry weight (mg) / Seedling part
0
10
20
25
0
20
40
60
0
10
20
30
0
0.4
0.8
1.0
0
4.0
8.0
10.0
0
0.4
0.8
1.2
C
1
2
3
4
5
Treatment

Fig. 3.16

Table 3.12 : Effect of IAA, 2,4-D, GA, FAP and AA on seed germination in *Schima khasiana*

Treatment (concentration mg/l)	Germination (%)	Germination rate (% seed day^{-1})	Days to peak value
Control	25.00 ± 4.70	16.80 ± 2.10	5.35
IAA			
1	25.00 ± 4.40	16.80 ± 2.25	5.30
10	36.20 ± 2.00*	18.90 ± 2.25*	4.80
25	29.10 ± 3.25	15.30 ± 1.85	5.90
50	25.00 ± 3.25	13.00 ± 3.25	7.70
100	25.00 ± 3.80	13.00 ± 3.25	7.70
2,4-D			
10	25.00 ± 3.90	18.20 ± 4.60	4.00
25	30.00 ± 5.40*	21.60 ± 3.90*	3.95
50	24.50 ± 5.20	14.30 ± 3.25	5.30
100	22.00 ± 5.20	13.50 ± 1.00	7.10
GA			
1	25.00 ± 3.25	19.60 ± 2.25	4.55
10	25.00 ± 3.25	23.20 ± 3.10*	3.80
25	33.10 ± 3.80*	26.20 ± 2.25*	3.60
50	24.50 ± 3.80	19.60 ± 2.10	4.70
100	24.50 ± 3.80	16.70 ± 1.50	5.65
FAP			
0.05	36.50 ± 2.20*	30.60 ± 3.00*	3.10
0.10	45.00 ± 2.40*	37.60 ± 4.10*	2.45
0.50	38.00 ± 3.25*	29.50 ± 3.10*	3.10
1.00	36.50 ± 1.50*	22.00 ± 2.00	4.25
AA			
1	30.00 ± 3.80*	20.70 ± 2.20	4.45
10	33.50 ± 5.00*	29.20 ± 3.10	3.15
25	25.00 ± 3.25	21.60 ± 3.40	3.95
50	25.00 ± 2.00	18.30 ± 1.70	5.10

± S.E.

* Significant at $p \leq 0.05$ level.

Treatment of seeds with IAA (10 mg/l), 2,4-D (25 mg/l), GA (25 mg/l), FAP (0.1 mg/l) and AA (10 mg/l) resulted in higher germination percentage, rate and lower days to peak value as compared to control (Table 3.12). A decrease was noticed in germination beyond the aforesaid concentrations. Amongst different growth regulators, FAP treatment exhibited maximum germination and rate and least days to peak value. FAP and AA treatments resulted in maximum cotyledon expansion and radicle elongation, respectively. However, higher shoot elongation was observed in GA and FAP treatments (Fig. 3.16). A reduction in the growth of seedling was found in 2,4-D treated seeds. The total dry weight of seedling was maximum in FAP treatment.

Discussion

Germinating seeds are very sensitive to H^+ ion concentration of the medium. A slight change in pH level may lead to a significant disruption in metabolism (Ovcharov, 1969). The substrate acidity (pH 2.0) was reported to inhibit seed germination and early seedling growth in Douglas-fir (McColl and Johnson, 1983). Ogner and Teigen (1980) reported that irrigation of spruce plants with higher acidic solutions like pH 2.5 and 3.0 resulted in greater accumulation of Al^{3+} ions and reduction in Ca^{2+}, Fe^{3+} and Mn^{2+} ions. But these changes did not affect the plant growth immediately. Among coniferous species, acidic substrates either had no effect as observed in hemlock or promoted seed germination as in white pine (Baldwin, 1934; Lee and Weber, 1980). Raynal et al. (1982) reported that hemlock seeds germinated well at pH 4.0. During the present study, P. kesiya seeds showed maximum germination percentage at pH 4.8 whereas a better rate of germination resulted at pH 6.0 and 6.4 amongst the various pH treatments. There were no significant relationship between pH and seed germination of P. rigida (Maull, 1963) and P. banksiana (Vaartaja, 1957). While mildly acidic to neutral pH exhibited higher germination and growth of various parts in S. khasiana, the alkaline and highly acidic pH were toxic in this species. The mildly acidic to neutral pH was also reported stimulatory for germination and growth of Achillea millefolium (Stjepanovič and Corovic, 1963). Abougendia and Redman (1979) noted varying effects of acidity on germination in conifers depending upon the ionic characteristics of the substrate solution which resulted in leaching of the various substances from the seed itself. Hence, in these experiments it was essential to change the treatment solution frequently to prevent the changes in pH level caused by seed leachates.

Tree seeds are very sensitive to availability of water (Baker, 1950) which determines the germination and early seedling survival. It was recorded that the germination energy of Populus deltoides (Farmer and Bonner, 1967), *P. ciliata* (Singh and Singh, 1983), *Acacia rigidula*, *A. berlandieri* and *Forliera angustifolia* (Everitt, 1983) decreased as moisture stress increased. During the present study on *P. kesiya* and *S. khasiana* seeds, water potential beyond -2.5 and -0.5 bars, respectively, inhibited the germination and seedling growth. The water potential created with sucrose was stimulatory for seed germination and seeling growth at -2.5 bar in *P. kesiya*, and for early germination as well as maximum dry weight at -0.1 bar in *S. khasiana*. Under stress condition, the deterioration in seedling growth of *P. deltoides* was also reported. Inhibition in germination by increasing water stress may possibly be related to the moisture deficit in the seeds below the threshold (Larson and Schubert, 1969; Everitt, 1983). The moisture deficit may lead to the degradation and inactivation of biochemical processes. The prevention of seed germination by an osmoticum has been reported on account of reduced water supply and appreciable respiration during seed germination (Hegarthy, 1977). The increase in shoot growth in *S. khasiana* at -0.1 bar and early germination and higher seedling growth in *P. kesiya* at -2.5 bars were probably due to nutrient availability from the substrate.

Seed germination and seedling growth of *P. kesiya* were greatly improved by imbibing the seeds in water at 5°C. To improve seed germination, the soaking of seeds has been tried by many workers. Toole *et al.* (1961) reported that imbibition of

P. virginiana seeds at 5°C and subsequent incubation at 25°C resulted in better germination. Similar treatment also stimulated the seed germination in southern pines (Barnett, 1971). The induction of seed germination in loblolly pine occurred due to leaching of inhibitors and an increase in endogenous levels of growth stimulators during the process of stratification (Paul et al., 1973). Bevington and Hoyle (1981) noted that chilling treatment at 3°C or red light initiated the phytochrome system in Betula resulting in higher germination. Marreno (1962) mentioned that P. carribea seeds soaked in water for a day or two showed better germination. In case of P. patula, soaking for 18 hr was found suitable (Ghosh et al., 1974). On the other hand, P. elliottii required 10 days of soaking for hastening the germination (Foot, 1967). In the present study, imbibition of P. kesiya seeds for 12 and 24 hr resulted in higher germination and growth whereas radicle growth was much higher in seeds imbibed for 24 hr. Casas (1964) noted in case of cacao tree that 25% moisture content of the seeds resulted in 100% germination. Soaking of S. khasiana seeds at 15°C for 6 hr might have resulted in activation of the physiological process leading to fast germination because non-dormant seeds require a certain amount of water for resumption of physiological process (Kramer and Kozlowski, 1979). An increase in germination of alder and parsley seeds after 24 hr incubation was observed (Khudyakov and Zinov'ev, 1964). Imbibition of seeds at high temperatures and durations resulted in delayed germination and slow growth of P. kesiya and S. khasiana probably due to higher moisture contents of seeds. It has been reported in southern pines that higher moisture content of seeds inhibited the total germination and delayed the peak value (Campbell, 1982). The soaking of maize seeds for higher periods resulted in decrease in cytokinin content during swelling (Julin-Tegelman, 1979). The soaking requirement of both the species was differed due to their seed coat constraints.

It has been reported that early successional and pioneer species regenerate in gaps (Denslow, 1980; Vázquez-Yanes, 1980). P. kesiya and S. khasiana are early successional species. The germination and seedling growth except shoot elongation in S. khasiana were enhanced when seeds were germinated in light conditions. The stimulatory effects of light in seed germination have been reported in many species (Sarvas, 1950; Jones, 1961; Shukla and Ramakrishnan, 1981; Campbell, 1982). Generally, 8-12 hr day length is necessary for maximum germination and growth in most forest trees (Kozlowski, 1971). During the present study, optimum germination of P. kesiya and S. khasiana seeds resulted with 3 lux for 16 hr and with 25 lux for 8 hr, respectively. A higher requirement of photoperiods in case of Pseudotsuga menziesii, Betula (Jones, 1961) and Duabanga sonneratioides (Shukla and

Ramakrishnan, 1981) have been reported. Jones (1961) reported the stimulation of seed germination in many trees species by low light intensities upto 100 lux. It is clear from the results that P. kesiya is a low light requiring species under long photoperiods exhibiting its wide distribution at high altitude. The present findings in S. khasiana are closely related to the conditions of light and temperature prevalent at high altitude (1800m) of Meghalaya during early rainfall of late April and early May when the germination in Schima occurs under natural conditions. The marked differences in seed germination under dark and low light intensities and photoperiods, is in contrast to the findings of Boojh and Ramakrishnan (1982) on the same species where non-significant changes were reported under dark and continuous light (500-600 lux) conditions. Besides, these authors have reported the maximum germination at 0-2 cm depth of soil under natural conditions where a light intensity of 500-600 lux could not be possible for 24 hr (Wooley and Stoller, 1978). The increase in cotyledon expansion and yield of seedling parts were observed at higher light intensities in S. khasiana which was probably due to increased light mediated process like photosynthesis, as reported in Robinia pseudoaccacia (Marshall and Kozlowski, 1974). The inhibition in shoot elongation at higher light intensities is possibly related to maturation of seedling axes after emergence.

An irradiation of far-red (20 min) almost reversed the stimulatory effect of red light (10 min) on seed germination in P. kesiya during the present investigation. It was found that red irradiation alone or red/far-red/red irradiations showed better germination, while irradiations of far-red, red/far-red and red/far-red/red/far-red inhibited seed germination in P. kesiya suggesting the involvement of phytochrome system. This is consistent with findings of Toole et al. (1961) in case of P. virginiana and Nyman (1961) in P. sylvestris where a phytochrome mediated process was reported. An increase in polysome formation mediated by phytochrome action has been reported in P. thunbergii germinating seeds (Yamamoto et al., 1975). Sasaki and Asakawa (1974) reported in P. thunbergii seeds isolated from cones in the dark, a promotory effect of red light on germination which was reversed by an increased quantity of far-red light. Light has been reported to cause increased protein synthesis (Yamamoto and Sasaki, 1977) and also in RNA containing poly A^+ (Yamamoto, 1982) in P. thunbergii seeds. The reversal effects of red/far-red/red and red/far-red/red/far-red showed an increase and decrease in length of various seedling parts, respectively. However, a reverse pattern was obtained for biomass of seedling parts which could be due to thinning and thickening of hypocotyl.

Temperature also causes a significant change in seed germination of many species. During the present study, a constant temperature of 25°C was found optimum for seed germination in P. kesiya. However, diurnal thermoperiodicity of 20/15°C and 35/20°C showed earlier germination and better seedling growth, respectively. A peak germination of southern pines has been reported to occur at 24°C (Barnett, 1979). In P. contorta the germination was equal from 20-30° (Critchfield, 1957). An almost similar trend was also reported during seedling emergence of Engleman spruse and lodgepole pine at 16-25°C (Kaufmann and Eckard, 1977), but reduction in germination was recorded at 35°C. On the other hand, there are reports in literature regarding diurnal thermoperiodicity accelerating seed germination in P. densiflora, P. thunbergii (Asakawa, 1959), P. elliottii, P. taeda (Dunlap and Barnett, (1982), Heliocarpus (Vázquez-Yanes and Orozco-Segovia, 1982). Lang (1965) mentioned that the optimum conditions of different species varies depending upon their requirement which may be either physiological or genetical. The existence of thermoperiodic behaviour of P. kesiya under long photoperiod is related to its wide distribution at high elevation of North-East India. It has been well-established that development of daily fluctuation of temperature under natural conditions act as an environmental indicator of appropriate conditions for seed germination and seedling establishment in many species

(Ellern and Tadnor, 1967; Vázques-Yanes and Orozco-Segovia, 1982). *S. khasiana* showed maximum seed germination and seedling growth at 20/10°C and 25/15°C, respectively. However, these findings are in contrast to the constant temperature optimum of 15°C reported in the same species by Boojh and Ramakrishnan (1982). Moreover, the constant temperature could not exist under natural conditions of high altitude of Meghalaya at the time of germination in this species. The occurrance of diurnal thermoperiodicity in early successional species has been reported (Rao and Singh, 1985). The higher temperature requirement for seedling emergence compared to that for germination was reported in a number of species (Stepanov, 1946). The thermoperiodic behaviour for seedling emergence in *P. kesiya* and *S. khasiana* may be a possible factor for conferring adaptability under natural condition of high altitude of Meghalaya.

In general, storage of seeds results in partial loss of viability due to disturbances in metabolism and deficiencies of certain chemicals (Ovcharov, 1969; Khan, 1977; Bewley and Black, 1982). GA, kinins, ABA, auxins, ethylene, vitamins and other regulatory substances are vitally important and their application to seeds intensify the metabolism and improve germination and plant productivity (Bonner, 1976; Lewak, 1984; Mehanna *et al.*, 1985; Raghava, 1985; Singh, 1985).

During the present study, one year-stored seeds of P. kesiya and S. khasiana showed better germination when treated with various growth regulators, viz., IAA, 2,4-D, GA, FAP and AA. The stimulation of seed germination by growth regulator has been reported in a number of woody plants (Biswas et al., 1972; Shafiq, 1979, 1980; Shibakusa, 1980; Tinus, 1982; Kumari and Kohli, 1984; Mehanna et al., 1985; Singh, 1985). It is noted that germination response of seeds to growth regulators is highly variable which generally depends upon both the external and internal factors. The growth regulators may easily penetrate the seed at their optimum concentration and also be available at the site of action (Bonner, 1976; Tinus, 1982). The higher germination in P. kesiya and S. khasiana were obtained with GA (150 and 200 mg/l) and FAP (0.1 mg/l) treatments, respectively. The seeds of both species may lack these concentrations during the beginning of germination in one-year-stored seeds. It is reported that GA (250-1,000 mg/l) application to under developed embryos of sub-tropical species stimulate the seed germination (Grushvitskii and Limar, 1965). The increase in cytokinin content of seeds at the beginning of germination has been reported in many cases (ref. Khan, 1977). Mehanna et al. (1985) reported the enhancement of germination of Nemaguard peach seeds by benzylaminopurine. The soaking of tung seeds in solutions of auxins and AA have been reported to improve the seed germination and seedling growth (Chatterjee, 1960). The high level of AA content and its utilization in embryonic axes during seed germination was observed (Chinoy *et al*., 1973).

Plant growth and development are greatly influenced by phytohormones. During the study on seedling growth, GA, FAP and AA were found highly effective in increasing the growth of seedling parts in *P. kesiya* and *S. khasiana*. 2,4-D treatment resulted in abnormal seedlings in both the species which could be due to its phytotoxic effects on seedling development (Kozlowski and Sasaki, 1968; Sasaki *et al*., 1968; Wu *et al*., 1971). Simakin (1966) mentioned that radicle of apple seeds first reacted to IAA, other embryonic parts to 2,4-D and perisperm responded to succinic acid. During the present investigation, *P. kesiya* seeds showed maximum radicle and shoot elongation in GA and AA treatments while cotyledon growth was higher in FAP and AA treated seeds. In *S. khasiana* seeds, radicle length and cotyledon expansion were higher in AA and FAP treatments, respectively. Shoot elongation was higher in GA and FAP treatments. A differential response of various seedling parts to different growth regulators was observed in both *P. kesiya* and *S. khasiana*.

Chapter IV

BIOCHEMICAL CHANGES DURING SEEDLING DEVELOPMENT

Introduction

The seed germination is an unique developmental phenomenon in the life of plants and its complexity is shown up by the intricate interactions between axes and storage tissue or organ, i.e., endosperm of cotyledons (Mayer and Marbach, 1981). Hence, the regulation of enzyme activity is under the influence of embryonic axes. The young regions of plants are characterized by reduced state which switches over to an oxidized state as the tissue becomes mature and differentiated. The enzyme system responsible for synthesis of membrane components like fatty acids, nucleosides and nucleotides, purines and pyrimidines all become active early in germination. The oxidases have been implicated in many metabolic changes (Mayer and Shain, 1974; Mayer and Poljakoff-Mayber, 1982). Auxin inactivating system has an important physiological role in the regulation of IAA (Fielding and Hall, 1978; Palmieri et al., 1978). Phenolic compounds are co-factors of certain enzymes and thus regulate the growth and development of plants (Sarapuu, 1965; Runkova et al., 1972; Butt, 1979; Duke and Vaughn, 1982) through changes in the activities of phenol oxidase, IAA-oxidase, and peroxidase (Podstolski et al., 1974; Butt, 1979; Barendse, 1983). The role of enzymes in relation to germination has been focussed by several workers (Scandalios, 1974; Gaspar et al., 1977; Dendsay and Sachar, 1982; Barendse, 1983; Pitel et al., 1984; Miller, 1985). The value of isoenzymes as biochemical and genetic markers of forest trees has been demonstrated in various studies like characterization of populations, species differentiation, pollination studies and evolutionary aspects (Durzan, 1966; Feret and Bergman, 1976; Kohli and Tandon, 1982; Cheliak and Pitel, 1984). Although there are numerous studies on isoenzyme expression during germination of agricultural crops (Scandalios, 1974; Gaspar et al., 1977) but only a few are concerned with the tree species (ref. Pitel et al., 1984).

In conifers the higher content of lipid exists in mega-gametophytic tissue and it is converted or utilized through sugars via glyoxylate cycle during germination in the presence of glyoxylate enzymes (Firenzuoli et al., 1968a; Ching, 1970). Further, Firenzuoli et al.,(1968b) have reported the transfer of sugars to embryos for their growth promotion. Iso-citrate lyase is a prominent enzyme of glyoxysome cycle in conifers and its control mechanism by growth regulators has been studied (Bilderback, 1974; Vu and Biggs, 1979).

In many cases, the exogenous application of known growth regulators could substitute for the seedling axes or embryo. The control of seed germination by external application of hormones reflects the natural control mechanism (Khan, 1971; Mayer and Shain, 1974). The changes in property of membranes, in response to growth regulators, is known to play a prominent role in controlling seed germination by affecting the rate of hydration, enzyme release, ion transport and concentration, pH and inhibitor contents (Tao and Khan, 1977; Penel et al., 1984). On the other hand, growth inhibitors decisively interfere with physiological process of seed germination (Dendsay and Sachar, 1978, 1982; Barendse, 1983; Lewak, 1984).

Enzyme activity is greatly influenced by environmental factors through change in hormones synthesis (Smith et al., 1977). The regulatory role of growth regulators on enzyme activity is very complex due to the action of both environmental factors and growth regulators on specific enzymes (Barendse, 1983). Effects of growth regulators on seed germination, seedling growth and enzyme metabolism have been widely studied (Wareing and Saunders, 1971; Mehta et al., 1974a, b; Balasimha and Tewari, 1979; Dendsay and Sachar, 1982; Barendse, 1983; Sankhla et al., 1985). The number of different enzymes affected by growth regulators is large and they cover a wide range of metabolic activities (Letham et al., 1978; Dendsay and Sachar, 1982; Barendse, 1983; Penel et al., 1984; Miller, 1985; Sankhla et al., 1985; Sarkar and Choudhuri, 1985). But the mechanism by which seed germination and early seedling growth affected by growth regulators and modified with changes in enzymes, phenols and nucleic acid contents is poorly understood in forest trees.

Materials and Methods

Pinus kesiya seeds were soaked in IAA (25 mg/l), 2,4-D (100 mg/l), GA (150 mg/l), FAP (1 mg/l) and AA (25 mg/l) for 24 hours at 5°C while *Schima khasiana* in IAA (10 mg/l) 2,4-D (25 mg/l), GA (25 mg/l), FAP (0.1 mg/l) and AA (1 mg/l) for 6 hr at 15°C and germinated as described in chapter III, In case of *P. kesiya,* embryo and endosperm were separated after seed treatment, radicle and cotyledon emergence stages, whereas in *S. khasiana,* into seedling axes and cotyledons at radicle, plumule and cotyledon emergence.

Fresh weight and moisture content determinations

Fresh weight of different parts of both the species as mentioned above were recorded during different stages of germination. The dry weight was determined by oven drying the materials at 60 ± 1°C for 48 hr for estimation of moisture content. It is expressed on percentage fresh weight basis.

Enzyme extraction

Acetone powders of different parts were homogenized in chilled mortar and pestle with 20 volumes of its weight in 0.2M phosphate buffer (pH 6.8). In case of *P. kesiya,* 0.3% sodium dodecyle sulphate (W/W) was used in the extracting buffer as phenolic scavenger. The homogenates were filtered through two layers of muslin cloth and centrifuged at 10,000 rpm for 30 min at 0-4°C and the supernatants were used for enzyme assay.

Assay of enzymes

Catalase : The activity of catalase (E.C. 1.11.1.6) was measured by the method of Mahadevan and Sridhar (1982). In the assay mixture, 2.7 ml phosphate buffer (0.1M, pH 6.0) and 0.2 ml extract were taken. After adjusting the absorbance to zero, 0.1 ml H_2O_2 (1% V/V) was added. Absorbance was recorded at 230 nm for 75 sec at 15 sec interval. The activity is expressed as ΔA min^{-1} mg^{-1} protein. The controls were maintained using boiled extracts.

Peroxidase : The peroxidase (EC 1.11.1.7) activity was measured following Mahadevan and Sridhar (1982). In a 6 ml reaction mixture, 2.5 ml pyrogallol (0.1 M), approrpriate amounts of phosphate buffer (0.1 M, pH 6.8) and enzyme were taken and the absorption was calibrated to zero. To this 0.5 ml of H_2O_2 (1% V/V) was added and the tube inverted immediately to mix the contents. The change in absorbance was recorded at 20 sec interval for 3 min at 420 nm. The activity is expressed as Δ A min^{-1} mg^{-1} protein.

IAA-oxidase : The enzyme activity was measured by the method of Tandon and Arya (1982). In the reaction mixture, first 2,4-dichlorophenol (DCP) was added to 0.2 M phosphate buffer (pH 6.8) then manganese chloride ($MnCl_2$) and enzyme, and at last IAA. The total volume of the reaction mixture was 5 ml and the final concentrations of DCP, $MnCl_2$ and IAA was 0.2 mM. The reaction mixture was incubated in dark at 37°C in a shaking water both. After 1 hr, 2 ml of Salkowski reagent was added to terminate the reaction. Following 1 hr wait, the absorbance of the mixture was recorded at 530 nm. The amount of IAA destroyed was calculated from a standard curve of IAA. The enzyme activity is expressed as mg IAA oxidized hr^{-1} mg^{-1} protein.

Polyphenol oxidase : The polyphenol oxidase (EC 1.10.3.1) activity was measured using the method of Mahadevan and Sridhar (1982). In a 6 ml reaction mixture, 2 ml enzyme extract and 3 ml phosphate buffer (0.1M, pH 6.8) were taken. After calibration of absorbance to zero at 495 nm, 1 ml of 0.1 M substrate (pyrogallol for *P. kesiya* and catechol for *S. khasiana*) was added. The change in absorbance was recorded at 20 sec interval for 3 min. The activity is expressed as ΔA min^{-1} mg^{-1} protein.

Protein :

Protein was estimated following the method of Lowry *et al.* (1951). Protein was precipitated by adding 10% trichloroacetic acid (TCA) to tissue extract in a ratio of 1:1 (V/V) and centrifuged at 6,000 rpm for 10 min. The supernatant was discarded and precipitate was washed twice with distilled water. It was dissolved in 1 ml of 0.5 N NaOH and diluted to desired volume. The volume of diluted extract was made to 1.5 ml with distilled water. To this, 5 ml freshly prepared alkaline solution (1 ml of 1% Copper sulphate and 1 ml of 2.7% sodium potassium tartarate mixed with 100 ml of 2% sodium carbonate solution) was added and allowed to stand for 10 min at room temperature. After incubation 0.5 ml Folin-ciocaltaeu reagent (equally diluted with water) was added with immediate shaking and held at room temperature for 20 min and colour was read at 750 nm. Protein content is expressed as μg seed^{-1} part or seedling^{-1} part using standard curve prepared by bovine serum albumin.

Extraction of phenols

The tissue was cut into pieces and plunged into boiling ethyl alcohol (96%) and boiled for 5-10 min. After cooling, it was crushed in a mortar and pestle. The homogenate was passed through two layers of muslin cloth and debris was re-extracted with 80% ethyl alcohol for 3 min. Crude extracts were mixed together and centrifuged at 6,000 rpm for 10 min. The supernatant was used for estimations of phenols using the procedure of Mahadevan and Sridhar (1982) with slight modification. The phenol contents are expressed as mg g^{-1} fresh weight.

Total phenol : The reaction mixture contained 1 ml extract, 1 ml Folin-ciocaltaeu reagent (1:10 V/V) and 2 ml 20% Na_2CO_3. After thorough mixing it was boiled for exactly 1 min and cooled under running tap water. The volume of reaction mixture was made to 25 ml with distilled water and the intensity of colour was read at 650 nm. A blank cotaining all the reagents minus extract was used to adjust the absorbance to zero. The total phenol content was calculated using a standard curve prepared with catechol.

O-dihydroxyphenol : To 1 ml of alcohol extract, were added 2 ml HCl (0.05N), 1 ml Arnow's reagent (10 g sodium nitrate and 10 g sodium molybdate in 100 ml distilled water) and 2 ml NaOH (1N). Soon after the addition of the alkali, pink colour appeared. The colour was diluted by raising the volume to 10 ml with distilled water and the absorbance was read at 515 nm. Amount of O-dihydroxyphenol was calculated using a standard curve prepared with caffeic acid.

Extraction and estimation of nucleic acids

The procedure described by Fusimura *et al.* (1980) was used for the extraction and estimation of nucleic acids.

500 mg of tissues from different stages of seedling emergence were cut and plunged into 5 ml of boiling ethanol (95%) and macerated in a mortar and pestle using acid washed sand. The homogenate was centrifuged for 15 min at 10,000 rpm.

The pellet was extracted twice with 80% ethanol and centrifuged. The residue was re-extracted twice in chilled distilled water and centrifuged. The pellet was suspended in 10 ml of 5% perchloric acid (PCA) and allowed to stand for 30 min in ice bath. After centrifugation, the residue was extracted in 10 ml mixture of diethyl ether:alcohol (2:1) and centrifuged as mentioned above. The pellet contained nucleic acids and proteins.

The pellet was suspended in 5 ml of chilled 1N PCA and incubated at 4°C for 42 hr. After incubation, the extract was centrifuged at 4°C for 15 min. The supernatant was used for ribonucleic acid (RNA) estimation. The residue was suspended in 10 ml of 1N PCA and incubated for 30 min at 90°C in water bath. After incubation, the extract was cooled and centrifuged. The supernatant was collected for estimation of dioxyribonucleic acid (DNA).

The absorbance of DNA and RNA solutions were read at 260 nm in a UV spectrophotometer and their quantities were calculated using standard curve prepared with calf thymus DNA and yeast extract RNA. The nucleic acids are expressed as mg g^{-1} fresh weight of tissues.

Electrophoresis

For electrophoretic studies, fresh materials were crushed with 0.2 M phosphate buffer pH (6.8) in a chilled mortar and pestle. The homogenates after filtering through two layers of muslin cloth were centrifuged for 30 min at 10,000 rpm. The supernatants were used for qualitative analyses of proteins and peroxidase.

The polyacrylamide gels were prepared following Davis (1964). Each batch of gels were prepared by mixing one part of stock solution A (containing 24 ml 1N HCl, 18.3 g Tris, 0.115 ml NNN'N'-tetramethylethylenediamine and volume was made upto 50 ml in distilled water), two parts of stock solution B (28g acrylamide, 0.735 N N'-methylene-bisacrylamide, dissolved in distilled water to make the volume to 100 ml), one part of distilled water and four parts of 1.4 g/l ammonium per sulphate solution freshly prepared. Tris glycine (pH 8.4) buffer was used as an electrolyte.

Protein samples were mixed with freshly prepared 40% sucrose solution and the mixture containing 0.2-0.4 mg protein (Pitel et al. 1984) was applied to the gel column in cathodic chamber. A drop of 1% bromophenol blue in distilled water was added in the mixture as tracking dye. The gels were electrophoresised at 4°C with 1mA per tube for first 10 min and subsequently 3mA per tube to allow the tracking dye to reach the bottom of each gel.

Protein bands were localized following the methods of Durzan (1966). The gels were fixed in 10% TCA for 30 min followed by staining with 0.1% amido block in 7% acetic acid for 30 min. After destaining by washing with 7% acetic acid the gels were stored in distilled water. Peroxidases were stained by placing the gels in a solution containing 15 ml of 1% H_2O_2 (V/V) in 2% benzidine in 20% acetic acid (W/V) in distilled water as described by Pitel *et al.* (1984).

The bands were categorized as dense, medium and light depending on the intensity of the colour and the gel patterns were compared visually. Zymograms were made based on the Rf values of protein and peroxidase bands.

Results

Fresh weight and moisture content

P. kesiya : The fresh weight of embryo increased during all the stages of seedling emergence in control as well as with growth regulator treatments. The endosperm showed an increase in growth upto the radicle protrusion stage and thereafter it declined during the cotyledon emergence except for 2,4-D treatment (Table 4.1). The growth regulator treatments did not show any significant change in the growth of embryo in the treated seeds while higher growth was recorded with IAA, GA, FAP and AA treatments at radicle and cotyledon emergence stages. The growth regulator treatments resulted in higher growth of

Table 4.1 : Effect of growth regulators on fresh weight (mg) of embryo and endosperm of *Pinus kesiya* in treated seeds and during seedling emergence

Treatment	Seed part	Treated seeds	Radicle emergence stage	Cotyledon emergence stage
Control	Embryo	2.25 ± 0.06	3.80 ± 0.04	46.60 ± 0.85
	Endosperm	12.80 ± 0.13	14.00 ± 0.05	11.10 ± 0.25
IAA	Embryo	2.15 ± 0.02	11.00 ± 0.25	56.60 ± 0.25
	Endosperm	12.80 ± 0.17	16.35 ± 0.30	9.45 ± 0.05
2,4-D	Embryo	2.30 ± 0.08	3.00 ± 0.06	41.20 ± 2.00
	Endosperm	15.60 ± 0.13	12.85 ± 0.32	8.95 ± 0.15
GA	Embryo	2.40 ± 0.05	11.80 ± 1.00	52.40 ± 0.45
	Endosperm	12.50 ± 0.50	18.80 ± 0.13	7.10 ± 0.05
FAP	Embryo	2.15 ± 0.01	6.85 ± 0.73	50.90 ± 0.30
	Endosperm	10.30 ± 0.00	17.20 ± 0.62	12.65 ± 0.15
AA	Embryo	2.35 ± 0.02	7.60 ± 0.65	53.50 ± 1.05
	Endosperm	10.35 ± 0.15	17.65 ± 0.55	10.20 ± 0.10

± S.E.

endosperm as compared to the control at radicle emergence stage. Conversely, an inhibition in growth was obtained at the cotyledon emergence stage except FAP treatment.

The moisture content of both embryo and endosperm increased in control as well as in all the growth regulator treatments during different stages of seedling emergence. After seed treatment, the moisture content was highest in the endosperm of 2,4-D treated seeds. The higher moisture content of both embryo and endosperm was also recorded in all the growth regulator treatments except for 2,4-D as compared to control at the radicle and cotyledon emergence stages (Table 4.2).

S. khasiana : The increase in fresh weight of seedling axes and cotyledons was recorded during different stages of seedling emergence in control as well as growth regulator treatments except in cotyledons of 2,4-D treatment during the cotyledon emergence phase. As compared to control, the seed treatment with growth regulators showed higher fresh weight of cotyledons at radicle emergence except for FAP treatment while IAA treatment increased the growth of both the seedling parts. At the time of plumule emergence, the growth of seedling axes and cotyledons were higher in all treatments except 2,4-D as compared to control. The growth of seedling axes and cotyledons were higher in IAA, GA, FAP and AA treatments at cotyledon

Table 4.2 : Effect of growth regulators on percentage moisture content of embryo and endosperm of *Pinus kesiya* in treated seeds and during seedling emergence

Treatment	Seed part	Treated seeds	Radicle emergence stage	Cotyledon emegence stage
Control	Embryo	66.70	73.70	86.60
	Endosperm	42.20	53.00	76.10
IAA	Embryo	51.20	86.35	87.40
	Endosperm	44.60	59.65	83.10
2,4-D	Embryo	61.20	70.00	83.60
	Endosperm	51.00	52.10	75.50
GA	Embryo	64.60	87.30	86.65
	Endosperm	24.10	65.40	78.75
FAP	Embryo	53.60	83.25	86.50
	Endosperm	34.00	57.60	77.50
AA	Embryo	48.60	83.60	87.10
	Endosperm	34.40	62.00	75.00

emergence phase, excepting GA in cotyledons. 2,4-D inhibited the growth of both the seedling parts at various stages of seedling emergence (Table 4.3).

The moisture content of both the seedling parts increased during plumule emergence. During cotyledon emergence it was almost stationary in seedling axes except 2,4-D treatment, where an increase was recorded. In case of cotyledons, the moisture content increased during cotyledon emergence in all the treatments except 2,4-D. At the radicle emergence phase, the moisture content was higher in cotyledons with growth regulator treatments as compared to control, and at plumule emergence with IAA, FAP and AA treatments. A lower moisture content of seedling axes at radicle emergence in 2,4-D treatment as compared to control and in both the seedling parts at plumule and cotyledon emergence phases were recorded (Table 4.4).

Catalase

P. kesiya : The enzyme activity decreased in embryo during radicle emergence in control as well as in growth regulator treatments except FAP. However, the activity increased in control as well as in all treatments between radicle and cotyledon emergence stages except FAP treatment. During radicle emergence stage, the enzyme activity of endosperm

Table 4.4 : Effect of growth regulators on percentage moisture content of seedling axes and cotyledons of <u>Schima khasiana</u> during seedling emergence

Treatment	Seedling part	Stages of seedling **development**		
		Radicle emergence	Plumule emergence	Cotyledon emergence
Control	Seedling axes	84.30	94.50	94.00
	Cotyledons	50.70	75.00	90.00
IAA	Seedling axes	88.10	93.30	94.10
	Cotyledons	61.50	77.60	91.75
2,4-D	Seedling axes	54.30	58.80	79.40
	Cotyledons	52.00	66.00	66.25
GA	Seedling axes	80.00	93.50	94.50
	Cotyledons	60.00	74.25	94.15
FAP	Seedling axes	82.70	95.30	95.00
	Cotyledons	66.80	81.50	91.00
AA	Seedling axes	83.30	95.00	94.00
	Cotyledons	62.50	82.30	91.25

increased with 2,4-D and FAP treatments while in control as well as in other growth regulator treatments a decrease was recorded. An inhibition of catalase activity also resulted during cotyledon emergence phase except 2,4-D and FAP treatments. As compared to control, all growth regulator treatments except AA resulted in lower enzyme activity in embryo after seed treatment. With all the growth regulator treatments, the activity was also less as compared to control during radicle and cotyledon emergence stages. However, GA and AA treatments resulted in higher activity at the latter stage. As compared to control, the enzyme activity was higher in endosperm of GA treated seeds, with all the growth regulators at radicle and cotyledon emergence stages (Table 4.5).

S. khasiana : The enzyme activity increased in both seedling axes and cotyledons during seedling emergence in control as well as in growth regulator treatments except in seedling axes of 2,4-D treated seeds. At radicle emergence, the growth regulator treatments resulted in higher enzyme activity in both the seedling parts as compared to control. But an inhibition was recorded with IAA, 2,4-D and AA treatments at plumule emergence and with IAA, 2,4-D and GA at cotyledon emergence phase. While FAP enhanced the activity in both the seedling parts, AA resulted in higher activity only in seedling axes at cotyledon emergence stage (Table 4.6).

Table 4.5 : Effect of growth regulators on catalase activity ($\Delta A\ min^{-1}\ mg^{-1}$ protein) of embryo and endosperm of *Pinus kesiya* in treated seeds and during seedling emergence

Treatment	Seed part	Treated seeds	Radicle emergence stage	Cotyledon emergence stage
Control	Embryo	29.20±0.15	5.20±0.00	5.85±0.10
	Endosperm	23.90±0.60	3.40±0.15	1.25±0.00
IAA	Embryo	6.40±0.05	2.80±0.05	3.60±0.65
	Endosperm	5.00±0.05	4.40±0.00	3.90±0.10
2.4-D	Embryo	7.40±0.05	2.50±0.05	3.50±1.15
	Endosperm	3.70±0.00	4.00±0.02	5.25±0.30
GA	Embryo	21.80±0.15	4.90±0.05	8.60±0.20
	Endosperm	56.20±0.90	3.40±0.10	2.60±0.20
FAP	Embryo	2.25±0.01	3.85±0.05	3.50±0.25
	Endosperm	3.00±0.05	4.10±0.02	6.40±0.15
AA	Embryo	29.70±0.20	3.00±0.10	9.70±0.25
	Endosperm	14.25±0.00	5.30±0.05	3.00±0.20

± S.E.

Table 4.6 : Effect of growth regulators on catalase activity (ΔA min^{-1} mg^{-1} protein) of seedling axes and cotyledons of *Schima khasiana* during seedling emergence

Treatment	Seedling parts	Stages of seedling emergence		
		Radicle emergence	Plumule emergence	Cotyledon emergence
Control	Seedling axes	0.03±0.01	0.60±0.04	3.80±0.25
	Cotyledons	0.04±0.01	0.50±0.03	4.15±0.40
IAA	Seedling axes	0.04±0.01	0.05±0.00	1.95±0.10
	Cotyledons	0.07±0.02	0.25±0.01	1.20±0.20
2,4-D	Seedling axes	0.90±0.01	0.60±0.05	0.85±0.20
	Cotyledons	0.06±0.01	0.15±0.00	0.25±0.05
GA	Seedling axes	0.05±0.01	0.80±0.01	1.75±0.05
	Cotyledons	0.12±0.01	0.65±0.04	1.20±0.10
FAP	Seedling axes	0.15±0.04	1.10±0.05	4.30±0.10
	Cotyledons	0.06±0.02	2.70±0.10	6.00±0.10
AA	Seedling axes	0.15±0.05	0.50±0.06	7.25±0.10
	Cotyledons	0.09±0.01	0.40±0.04	0.80±0.05

± S.E.

Peroxidase

P. kesiya : The enzyme activity increased in embryo of control and treated seeds during different stages of seedling emergence except for 2,4-D treatment where it decreased at cotyledon emergence stage. While in endosperm, enzyme activity increased during radicle emergence and later during cotyledon emergence stage, it showed a decreasing trend. After seed treatment with growth regulators, except AA, a higher enzyme activity was observed in both embryo and endosperm as compared to control. The inhibition in activity was recorded with all the growth regulator treatments at radicle emergence except GA treatment, where it was higher. At cotyledon emergence phase, 2,4-D and GA inhibited the peroxidase activity in embryo whereas IAA, FAP and AA inhibited it in endosperm (Table 4.7).

S. khasiana : In seedling axes, peroxidase activity decreased in control during plumule emergence as compared to radicle emergence phase, while with IAA, GA, FAP and AA treatments it increased. Conversely, during cotyledons emergence the activity decreased in control as well as with all growth regulator treatments. On the other hand, the enzyme activity increased in cotyledons of control as well as growth regulator treatments except 2,4-D during seedling development. At radicle emergence stage, enzyme activity was less in seedling

Table 4.7 : Effect of growth regulators on peroxidase activity (ΔA min^{-1} mg^{-1} protein) of embryo and endosperm of *Pinus kesiya* in treated seeds and during seedling emergence

Treatment	Seed part	Treated seeds	Radicle emergence stage	Cotyledon emergence stage
Control	Embryo	0.04±0.01	1.20±0.04	2.20±0.06
	Endosperm	0.04±0.00	0.65±0.02	0.50±0.01
IAA	Embryo	0.17±0.01	0.25±0.00	5.50±0.02
	Endosperm	0.15±0.01	0.50±0.01	0.45±0.01
2,4-D	Embryo	0.24±0.00	0.25±0.01	0.15±0.03
	Endosperm	0.16±0.01	0.60±0.01	0.55±0.01
GA	Embryo	0.06±0.01	1.75±0.03	1.80±0.04
	Endosperm	0.10±0.01	1.35±0.03	0.90±0.01
FAP	Embryo	0.13±0.01	0.65±0.02	4.85±0.02
	Endosperm	0.14±0.01	0.60±0.01	0.35±0.06
AA	Embryo	0.04±0.01	0.40±0.00	2.65±0.02
	Endosperm	0.03±0.00	0.65±0.01	0.40±0.01

± S.E.

axes of all the growth regulator treatments as compared to control. At plumule and cotyledon emergence phase, the enzyme activity was higher with IAA, FAP and AA treatments excepting the last treatment in case of cotyledon during cotyledon emergence. The enzyme activity increased with growth regulator treatments in cotyledons of all the stages of seedling emergence except 2,4-D at cotyledon emergence phase where it decreased (Table 4.8).

IAA-oxidase

P. kesiya : The enzyme activity increased in embryo of control as well as with growth regulators except IAA and 2,4-D treatments at radicle emergence phase. In case of endosperm, the enzyme activity decreased during radicle emergence in control, IAA and 2,4-D treatments whereas GA, FAP and AA treatments resulted in increase in the activity. During cotyledon emergence, in endosperm, the activity was less in control and GA treatments. The enzyme activity could not be detected in embryo of control and GA treatment as well as in both embryo and endosperm of AA treated seeds. But at radicle and cotyledon emergence stages the activity was lower in both the seed parts in all the treatments except embryo in GA and AA treatments as compared to control. In endosperm, enzyme activity was higher with IAA, 2,4-D and GA treatments after seed treatment stage and with GA and AA at radicle and cotyledon

Table 4.8 : Effect of growth regulators on peroxidase activity ($\Delta A\ \mathrm{min}^{-1}\ \mathrm{mg}^{-1}$ protein) of seedling axes and cotyledons of *Schima khasiana* during seedling emergence

Treatment	Seedling part	Stages of seedling development		
		Radicle emergence	Plumule emergence	Cotyledon emergence
Control	Seedling axes	2.30±0.08	1.65±0.03	0.65±0.04
	Cotyledons	0.45±0.03	0.45±0.03	0.80±0.05
IAA	Seedling axes	1.70±0.04	4.00±0.01	2.85±0.01
	Cotyledons	0.70±0.01	1.20±0.01	2.95±0.11
2,4-D	Seedling axes	0.50±0.02	0.50±0.03	0.40±0.03
	Cotyledons	0.80±0.01	0.80±0.01	0.30±0.01
GA	Seedling axes	0.65±0.02	0.85±0.01	0.30±0.04
	Cotyledons	0.50±0.01	0.70±0.02	1.60±0.04
FAP	Seedling axes	1.50±0.13	1.80±0.05	1.35±0.05
	Cotyledons	1.05±0.01	1.15±0.04	1.50±0.01
AA	Seedling axes	1.65±0.05	2.50±0.14	0.60±0.03
	Cotyledons	0.70±0.02	1.15±0.05	3.10±0.09

± S.E.

emergence phases (Table 4.9).

S. khasiana : The enzyme activity was not observed during the course of seedling development in both seedling axes and cotyledons of control. The activity was recorded during plumule emergence in cotyledons of IAA, 2,4-D and GA treated seeds and seedling axes in IAA treatment. At cotyledon emergence, higher activity in seedling axes was noted in IAA, FAP and AA treatments whereas in cotyledons the maximum activity was recorded in FAP treatment (Table 4.10).

Polyphenol oxidase

P. khasiana : In general the increase in enzyme activity was observed in embryo of control as well as of growth regulator treated seeds **during seedling emergence** except 2,4-D. In case of endosperm, the activity was higher during radicle emergence phase in all the treatments. Conversely, it declined during cotyledon emergence except for AA treatment. The enzyme activity was higher in both embryo and endosperm of growth regulator treated seeds as compared to control at all the stages of seedling development (Table 4.11).

S. khasiana : In the seedling axes of control, the enzyme activity was **similar** during **radicle and plumule emergence stages.**

Table 4.9 : Effect of growth regulators on IAA-oxidase activity (mg IAA oxidized hr^{-1} mg^{-1} protein) of embryo and endosperm of *Pinus kesiya* in treated seeds and during seedling emergence

Treatment	Seed part	Treated seeds	Radicle emergence stage	Cotyledon emergence stage
Control	Embryo	-	0.70±0.00	1.20±0.03
	Endosperm	0.03±0.01	0.25±0.01	0.10±0.01
IAA	Embryo	0.20±0.01	0.05±0.00	0.50±0.07
	Endosperm	0.19±0.01	0.05±0.01	0.05±0.01
2,4-D	Embryo	0.23±0.01	0.10±0.02	0.65±0.01
	Endosperm	0.18±0.01	0.05±0.01	0.05±0.02
GA	Embryo	-	0.30±0.03	1.35±0.22
	Endosperm	0.08±0.00	0.65±0.05	0.20±0.01
FAP	Embryo	0.02±0.01	0.20±0.02	0.60±0.07
	Endosperm	0.03±0.01	0.05±0.01	0.05±0.01
AA	Embryo	-	0.25±0.01	1.50±0.08
	Endosperm	-	0.45±0.01	0.40±0.01

± S.E.

\- Activity not seen.

Table 4.10 : Effect of growth regulators on IAA-oxidase activity *(mg IAA oxidized hr^{-1} mg^{-1} protein) of seedling axes* and cotyledons of *Schima khasiana* during seedling emergence

Treatment	Seedling part	Stages of seedling development		
		Radicle emergence	Plumule emergence	Cotyledon emergence
Control	Seedling axes	-	-	-
	Cotyledons	-	-	-
IAA	Seedling axes	-	1.05±0.01	1.25±0.01
	Cotyledons	-	0.05±0.01	0.15±0.04
2,4-D	Seedling axes	-	-	0.25±0.04
	Cotyledons	-	0.15±0.01	0.10±0.02
GA	Seedling axes	-	-	0.05±0.01
	Cotyledons	-	0.21±0.01	0.25±0.02
FAP	Seedling axes	-	-	1.20±0.25
	Cotyledons	-	-	1.00±0.01
AA	Seedling axes	-	-	1.30±0.04
	Cotyledons	-	-	0.85±0.00

± S.E.

\- Activity not seen.

Table 4.11 : Effect of growth regulators on polyphenol oxidase activity (ΔA min^{-1} mg^{-1} protein) of embryo and endosperm of *Pinus kesiya* in treated seeds and during seedling emergence

Treatment	Seed part	Treated seeds	Radicle emergence stage	Cotyledon emergence stage
Control	Embryo	0.01±0.00	0.05±0.01	0.10±0.01
	Endosperm	0.01±0.00	0.05±0.01	0.04±0.01
IAA	Embryo	0.09±0.01	0.23±0.01	2.49±0.06
	Endosperm	0.07±0.01	0.32±0.01	0.21±0.01
2,4-D	Embryo	0.13±0.02	0.11±0.00	0.10±0.04
	Endosperm	0.10±0.01	0.46±0.03	0.25±0.01
GA	Embryo	0.01±0.00	0.10±0.01	0,07±0.01
	Endosperm	0.04±0.00	0.05±0.00	0.03±0.01
FAP	Embryo	0.05±0.00	0.54±0.01	2.10±0.01
	Endosperm	0.05±0.00	0.46±0.01	0.22±0.01
AA	Embryo	0.01±0.00	0.04±0.01	0.12±0.01
	Endosperm	0.02±0.00	0.06±0.01	0.08±0.01

± S.E.

However, it increased in IAA treatment. The other growth regulators inhibited the activity in seedling axes at this stage. While at cotyledon emergence stage, activity declined in seedling axes of control and growth regulator treatments except in case of FAP treatment. In cotyledons, the activity increased during different stages of seedling emergence except for FAP and AA treatments during plumule and cotyledon emergence phases, respectively. The enzyme activity was higher in seedling axes with all growth regulator treatments at radicle emergence; with IAA, GA and AA treatments at plumule emergence, and with IAA, FAP and AA treatments at cotyledon emergence phase as compared to control. On the other hand, the acitivity was lower in cotyledons with all the growth regulator treatments except FAP at radicle emergence phase. However, at plumule emergence stage it was higher with GA treatment (Table 4.12). The enzyme activity was higher in cotyledons with IAA and GA treatments at cotyledon emergence stage as compared to control.

Protein

P. kesiya : In embryo, protein content increased during seedling emergence in control and growth regulator treatments, while it declined in endosperm. As compared to control, the protein content in embryo was higher in all the growth regulator treated seeds except 2,4-D. At radicle emergence stage the

Table 4.12 : Effect of growth regulators on polyphenol oxidase activity (ΔA min^{-1} mg^{-1} protein) of seedling axes and cotyledons of *Schima khasiana* during seedling emergence

Treatment	Seedling part	Stages of seedling development		
		Radicle emergence	Plumule emergence	Cotyledon emergence
Control	Seedling axes	0.15±0.03	0.15±0.01	0.10±0.02
	Cotyledons	0.15±0.03	0.15±0.04	0.20±0.01
IAA	Seedling axes	0.40±0.02	0.55±0.01	0.35±0.01
	Cotyledons	0.10±0.01	0.15±0.00	0.35±0.01
2,4-D	Seedling axes	0.35±0.00	0.10±0.01	0.05±0.01
	Cotyledons	0.10±0.01	0.15±0.01	0.20±0.04
GA	Seedling axes	0.25±0.01	0.20±0.01	0.10±0.01
	Cotyledons	0.05±0.01	0.25±0.01	0.70±0.00
FAP	Seedling axes	0.50±0.03	0.15±0.01	0.15±0.02
	Cotyledons	0.20±0.03	0.10±0.01	0.10±0.01
AA	Seedling axes	0.50±0.04	0.20±0.00	0.15±0.01
	Cotyledons	0.10±0.02	0.15±0.01	0.10±0.01

± S.E.

protein content in embryo was higher with AA treatment whereas in all other treatments it was lower than control. Lower protein content was observed in endosperm of treated seeds and at radicle and cotyledon emergence phases as compared to control. However, it enhanced with IAA, FAP and AA treatment at cotyledon emergence phase (Table 4.13).

S. khasiana : In seedling axes, protein content increased during seedling emergence in control and growth regulator treatments. In cotyledons, the protein content increased during plumule emergence in all the treatments. However, it declined during cotyledon emergence. In IAA, GA, FAP and AA treated seeds the protein content was higher in seedling axes as compared to control at radicle and plumule emergence stages. At cotyledon emergence stage only with IAA and AA treatments higher protein content in seedling axes were observed. In case of cotyledons, all growth regulator treatments resulted in higher protein content at radicle, plumule and cotyledon emergence stages excepting FAP and AA treatments. The inhibition in protein content was observed with 2,4-D treatment in both seedling axes and cotyledons during seedling growth (Table 4.14).

Total Phenol

P. kesiya : The total phenol contents increased in embryo during seedling development in control and growth [illegible]

Table 4.13 : Effect of growth regulators on protein content (μg seed^{-1} part) of embryo and endosperm of *Pinus kesiya* in treated seeds and during seedling emergence

Treatment	Seed part	Treated seeds	Radicle emergence stage	Cotyledon emergence stage
Control	Embryo	13.00±0.40	28.30±0.70	64.80±2.00
	Endosperm	61.00±0.20	45.00±0.05	21.60±0.25
IAA	Embryo	19.50±0.00	22.60±0.75	68.50±7.25
	Endosperm	49.30±0.40	31.90±0.45	22.30±0.20
2,4-D	Embryo	11.90±0.95	21.10±0.60	33.35±4.95
	Endosperm	43.50±0.20	35.15±0.45	18.25±0.45
GA	Embryo	21.35±0.65	28.15±1.05	90.10±1.30
	Endosperm	28.90±0.45	19.10±0.30	10.10±0.35
FAP	Embryo	22.20±0.25	24.90±3.00	25.35±0.85
	Endosperm	47.20±0.20	36.15±0.30	31.60±0.90
AA	Embryo	23.00±0.00	31.25±0.55	78.30±3.40
	Endosperm	39.10±0.20	34.10±0.00	39.10±0.20

± S.E.

Table 4.14 : Effect of growth regulators on protein content (Ug seedling^{-1} part) of seedling axes and cotyledons of *Schima khasiana* during seedling emergence

Treatment	Seedling part	Stages of seedling development		
		Radicle emergence	Plumule emergence	Cotyledon emergence
Control	Seedling axes	10.10±0.15	10.90±0.80	34.65±1.15
	Cotyledons	220.70±3.40	370.30±0.00	195.20±9.20
IAA	Seedling axes	31.00±0.40	39.60±0.50	52.50±3.15
	Cotyledons	230.20±1.60	370.40±7.75	240.10±9.10
2,4-D	Seedling axes	10.10±0.06	10.50±0.75	23.00±1.25
	Cotyledons	195.20±2.65	300.00±5.60	205.00±3.50
GA	Seedling axes	15.00±0.40	21.70±2.05	22.40±1.95
	Cotyledons	400.50±5.30	430.00±2.50	330.00±4.00
FAP	Seedling axes	16.40±0.20	29.50±3.50	34.40±0.50
	Cotyledons	270.50±0.10	400.50±2.35	180.25±6.65
AA	Seedling axes	14.30±2.15	24.00±0.10	40.50±0.60
	Cotyledons	245.70±1.50	460.00±2.35	125.60±9.80

± S.E.

regulator treatments. It decreased in endosperm and seed coat during radicle emergence except for an increase in endosperm of GA and AA treated seeds (Table 4.15). The growth regulator treatments resulted in higher phenol content in embryo and lower in endosperm and seed coat as compared to control during seedling development excepting GA and AA treatments in case of endosperm.

S. khasiana : The total phenol contents decreased in seedling axes during plumule and cotyledon emergence stages in control and growth regulator treated seeds whereas it increased during plumule emergence in IAA and 2,4-D treatments. In case of cotyledons, phenol content increased during seedling development except during cotyledon emergence in GA treatment. An inhibition was recorded in phenol contents of seed coat during seedling development. 2,4-D and GA treatments of the seeds resulted in an increase in phenol contents of seedling axes and cotyledons as compared to control at different stages of seedling development whereas IAA treatment exhibited higher phenol contents at plumule and cotyledon emergence stages (Table 4.16).

O-dihydroxyphenol

P. kesiya : The increase in O-dihydroxyphenol content was recorded in both embryo and endosperm during seedling development in control and growth regulator treatments except IAA and

Table 4.15 : Effect of growth regulators on total phenol content (mg g^{-1} fresh weight) of embryo, endosperm and seed coat of Pinus kesiya in treated seeds and during seedling emergence

Treatment	Seed part	Treated seeds	Radicle emergence stage	Cotyledon emergence stage
Control	Embryo	0.30±0.00	1.25±0.01	2.30±0.04
	Endosperm	0.55±0.03	0.55±0.00	0.75±0.02
	Seed coat	0.45±0.00	0.30±0.02	0.30±0.00
IAA	Embryo	0.35±0.02	1.10±0.01	2.30±0.02
	Endosperm	0.45±0.04	0.35±0.01	0.55±0.01
	Seed coat	0.40±0.03	0.25±0.03	0.25±0.01
2,4-D	Embryo	0.75±0.06	1.25±0.03	2.45±0.04
	Endosperm	0.45±0.03	0.40±0.01	0.40±0.01
	Seed coat	0.35±0.03	0.20±0.01	0.25±0.01
GA	Embryo	0.35±0.03	1.40±0.02	2.45±0.04
	Endosperm	0.30±0.00	0.45±0.02	0.90±0.02
	Seed coat	0.30±0.06	0.20±0.02	0.35±0.05
FAP	Embryo	0.65±0.04	1.30±0.02	2.15±0.04
	Endosperm	0.50±0.04	0.45±0.01	0.55±0.01
	Seed coat	0.40±0.02	0.20±0.01	0.20±0.01
AA	Embryo	0.70±0.04	1.25±0.01	2.40±0.00
	Endosperm	0.45±0.00	0.50±0.01	0.85±0.02
	Seed coat	0.45±0.04	0.20±0.02	0.30±0.00

± S.E.

Table 4.16 : Effect of growth regulators on total phenol content ($mg\ g^{-1}$ fresh weight) of seedling axes, cotyledons and seed coat of <u>Schima</u> <u>khasiana</u> during seedling emergence

Treatment	Seedling part	Stages of seedling development		
		Radicle emergence	Plumule emergence	Cotyledon emergence
Control	Seedling axes	1.00±0.00	0.50±0.01	0.40±0.01
	Cotyledons	0.80±0.03	1.20±0.03	1.30±0.02
	Seed coat	0.30±0.01	0.25±0.00	0.15±0.01
IAA	Seedling axes	0.80±0.03	1.60±0.05	0.70±0.02
	Cotyledons	0.70±0.03	2.25±0.00	2.40±0.06
	Seed coat	0.25±0.01	0.25±0.01	0.20±0.01
2,4-D	Seedling axes	3.80±0.00	5.25±0.18	3.50±0.13
	Cotyledons	1.05±0.03	0.10±0.09	2.35±0.06
	Seed coat	0.25±0.02	0.20±0.01	0.25±0.01
GA	Seedling axes	3.20±0.00	1.55±0.08	1.25±0.00
	Cotyledons	1.20±0.07	2.35±0.07	1.90±0.06
	Seed coat	0.20±0.01	0.20±0.01	0.20±0.01
FAP	Seedling axes	1.00±0.00	0.45±0.01	0.40±0.01
	Cotyledons	0.55±0.03	1.10±0.03	1.25±0.01
	Seed coat	0.25±0.01	0.25±0.01	0.10±0.01
AA	Seedling axes	0.80±0.03	0.50±0.01	0.50±0.00
	Cotyledons	0.55±0.05	0.80±0.02	1.75±0.03
	Seed coat	0.20±0.01	0.20±0.01	0.10±0.00

± S.E.

AA treatments. As compared to control, IAA treatment resulted in higher O-dihydroxyphenol content in embryo and endosperm after seed treatment, in embryo with all the treatments at radicle emergence, and with IAA, 2,4-D and FAP at cotyledon emergence stage. The higher content of O-dihydroxyphenol in endosperm at both radicle and cotyledon emergence stages were recorded with 2,4-D treatment and with FAP at cotyledon emergence stage only (Table 4.17).

S. khasiana : The O-dihydroxyphenol content decreased in seedling axes during seedling development except for control, FAP and AA treatments. In cotyledons, it decreased with IAA and 2,4-D treatments during seedling development. It increased with GA during plumule emergence and with FAP and AA treatments during cotyledon emergence stage. The O-dihydroxyphenol content decreased in IAA and GA treatments during cotyledon emergence stage (Table 4.18).

Nucleic acids

P. kesiya : The endosperm contained more DNA and RNA as compared to embryo during all stages of seedling development. During radicle emergence, both DNA and RNA contents in embryo and endosperm increased as compared to imbibed seeds. However, these declined during cotyledon emergence stage (Table 4.19).

S. khasiana : The DNA contents in both seedling axes and cotyledons decreased during seedling development except for an

Table 4.17 : Effect of growth regulators on O-dihydroxyphenol content (mg g^{-1} fresh weight) of embryo, endosperm and seed coat of *Pinus kesiya* in treated seeds and during seedling emergence

Treatment	Seed part	Treated seeds	Radicle emergence stage	Cotyledon emergence stage
Control	Embryo	0.01±0.001	0.08±0.001	0.14±0.001
	Endosperm	0.02±0.001	0.03±0.000	0.04±0.002
	Seed coat	0.03±0.003	0.03±0.001	0.02±0.001
IAA	Embryo	0.03±0.001	0.12±0.005	0.26±0.002
	Endosperm	0.08±0.002	0.02±0.001	0.02±0.002
	Seed coat	0.03±0.001	0.04±0.003	0.03±0.001
2,4-D	Embryo	0.01±0.001	0.11±0.002	0.20±0.001
	Endosperm	0.03±0,001	0.06±0.002	0.13±0.002
	Seed coat	0.02±0.001	0.02±0.001	0.02±0.001
GA	Embryo	0.01±0.002	0.11±0.002	0.13±0.001
	Endosperm	0.02±0.001	0.03±0.001	0.03±0.002
	Seed coat	0.04±0.001	0.05±0.002	0.02±0.001
FAP	Embryo	0.01±0.001	0.14±0.002	0.21±0.004
	Endosperm	0.02±0,002	0.03±0.001	0.10±0.001
	Seed coat	0.03±0.001	0.02±0.002	0,02±0.001
AA	Embryo	0.01±0.003	0.11±0.002	0.13±0.001
	Endosperm	0.03±0.001	0.03±0.001	0.01±0.001
	Seed coat	0.03±0.001	0.03±0.001	0.01±0.003

± S.E.

Table 4.18 : Effect of growth regulators on O-dihydroxyphenol content (mg g^{-1} fresh weight) of seedling axes, cotyledons and seed coat of *Schima khasiana* during seedling emergence

Treatment	Seedling part	Stages of seedling development		
		Radicle emergence	Plumule emergence	Cotyledon emergence
Control	Seedling axes	0.03±0.005	0.01±0.002	0.02±0.003
	Cotyledons	0.03±0.003	0.03±0.001	0.09±0.002
	Seed coat	0.01±0.001	0.01±0.002	0.01±0.001
IAA	Seedling axes	0.09±0.004	0.06±0.002	0.02±0.002
	Cotyledons	0.38±0.008	0.24±0.005	0.16±0.015
	Seed coat	0.01±0.001	0.03±0.002	0.01±0.001
2,4-D	Seedling axes	0.50±0.040	0.45±0.001	0.30±0.040
	Cotyledons	0.15±0.002	0.09±0.005	0.10±0.005
	Seed coat	0.02±0.001	0.03±0.004	0.03±0.004
GA	Seedling axes	0.20±0.002	0.08±0.006	0.04±0.001
	Cotyledons	0.08±0.010	0.18±0.010	0.10±0.001
	Seed coat	0.02±0.002	0.02±0.002	0.01±0.001
FAP	Seedling axes	0.03±0.001	0.02±0.002	0.03±0.003
	Cotyledons	0.03±0.002	0.03±0.003	0.12±0.002
	Seed coat	0.01±0.001	0.02±0.001	0.02±0.002
AA	Seedling axes	0.02±0.004	0.01±0.001	0.02±0.001
	Cotyledons	0.01±0.004	0.01±0.003	0.15±0.025
	Seed coat	0.01±0.002	0.01±0.001	0.02±0.001

± S.E.

Table 4.19 : Changes in nucleic acid contents (mg g^{-1} fresh weight) during imbibition and seedling emergence in *Pinus kesiya*

Nucleic acid	Seed part	Imbibed seeds	Radicle emergence stage	Cotyledon emergence stage
DNA	Embryo	1.70±0.05	1.70±0.11	1.50±0.20
	Endosperm	1.65±0.07	1.80±0.16	1.65±0.25
RNA	Embryo	10.20±1.25	14.30±1.50	13.20±1.75
	Endosperm	16.80±1.40	18.40±1.80	16.20±2.25

± S.E.

increase in the former at plumule emergence stage. In both seedling axes and cotyledons, the RNA content decreased at plumule emergence stage which subsequently increased during cotyledons emergence (Table 4.20).

Electrophoresis

P. kesiya

Peroxidases : In embryo, 6, 9 and 10 bands appeared after imbibition, radicle and cotyledon emergence, respectively (Fig. 4.1A). The increase in number of bands with Rfs 0.05, 0.10, 0.16, 0.26, 0.29, 0.47 and 0.60 during radicle emergence and Rfs 0.02, 0.23 and 0.65 during cotyledon emergence were observed. This is also accompanied with increase in enzyme activity of embryo during seedling emergence (Table 4.7). In endosperm, 3, 15 and 10 bands were observed on gels after imbibition, radicle and cotyledon emergence phases, respectively (Fig. 4.1A). A large number of new bands having Rfs 0.04, 0.06, 0.11, 0.43, 0.49, 0.55, 0.59, 0.65, 0.82, 0.87, 0.90 and 0.94 were recorded during radicle emergence while bands of Rfs 0.04, 0.65, 0.83, 0.87, 0.90 and 0.94 were not detected in endosperm during emergence of cotyledons. This is also correlated with peroxidase activity recorded during seedling and cotyledon emergence stages (Table 4.7).

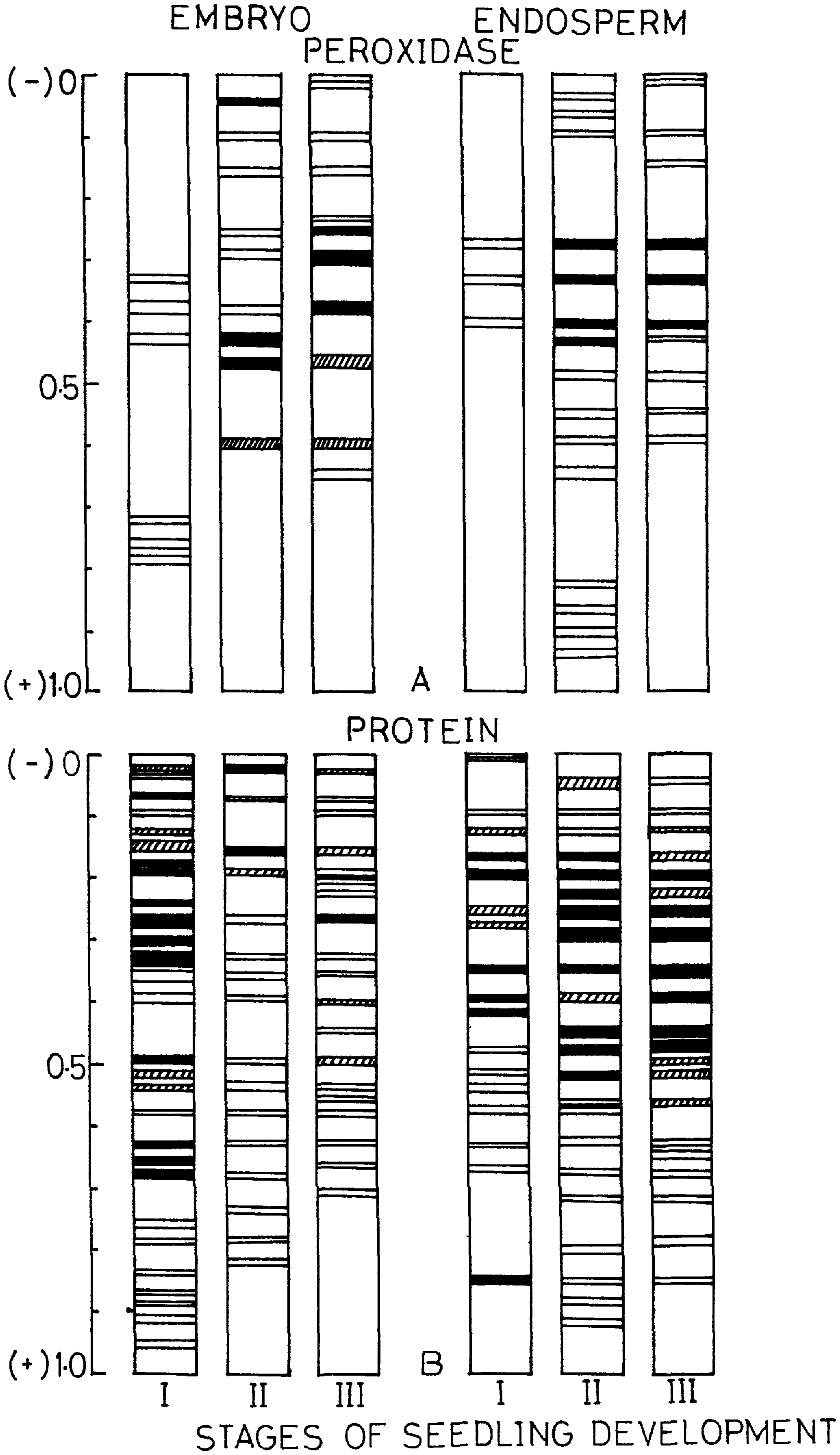
Proteins : In embryo 28, 16, 19 bands were recorded after imbibition, radicle and cotyledon emergence, respectively

Table 4.20 : Changes in nucleic acid contents (mg g^{-1} fresh weight) during seedling emergence in *Schima khasiana*

Nucleic acid	Seedling part	Stages of seedling development		
		Radicle emergence	Plumule emergence	Cotyledon emergence
DNA	Seedling axes	1.40±0.12	2.00±0.15	1.40±0.10
	Cotyledons	1.90±0.25	1.70±0.05	1.60±0.15
RNA	Seedling axes	19.50±1.25	12.90±2.10	18.90±2.30
	Cotyledons	24.60±2.20	21.00±1.50	21.60±2.40

± S.E.

Fig. 4.1 : Electrophoretic changes in peroxidases (A) and proteins (B) in embryo and endosperm during (I) imbibing seeds, (II) radicle emerged and (III) cotyledon emergence phases of *Pinus kesiya*. Increasing band intensity (▭ ▨ ▬).



EMBRYO
ENDOSPERM
PEROXIDASE
(-)0
0·5
(+)1·0
A
PROTEIN
(-)0
0·5
(+)1·0
B
I
II
III
I
II
III
STAGES OF SEEDLING DEVELOPMENT

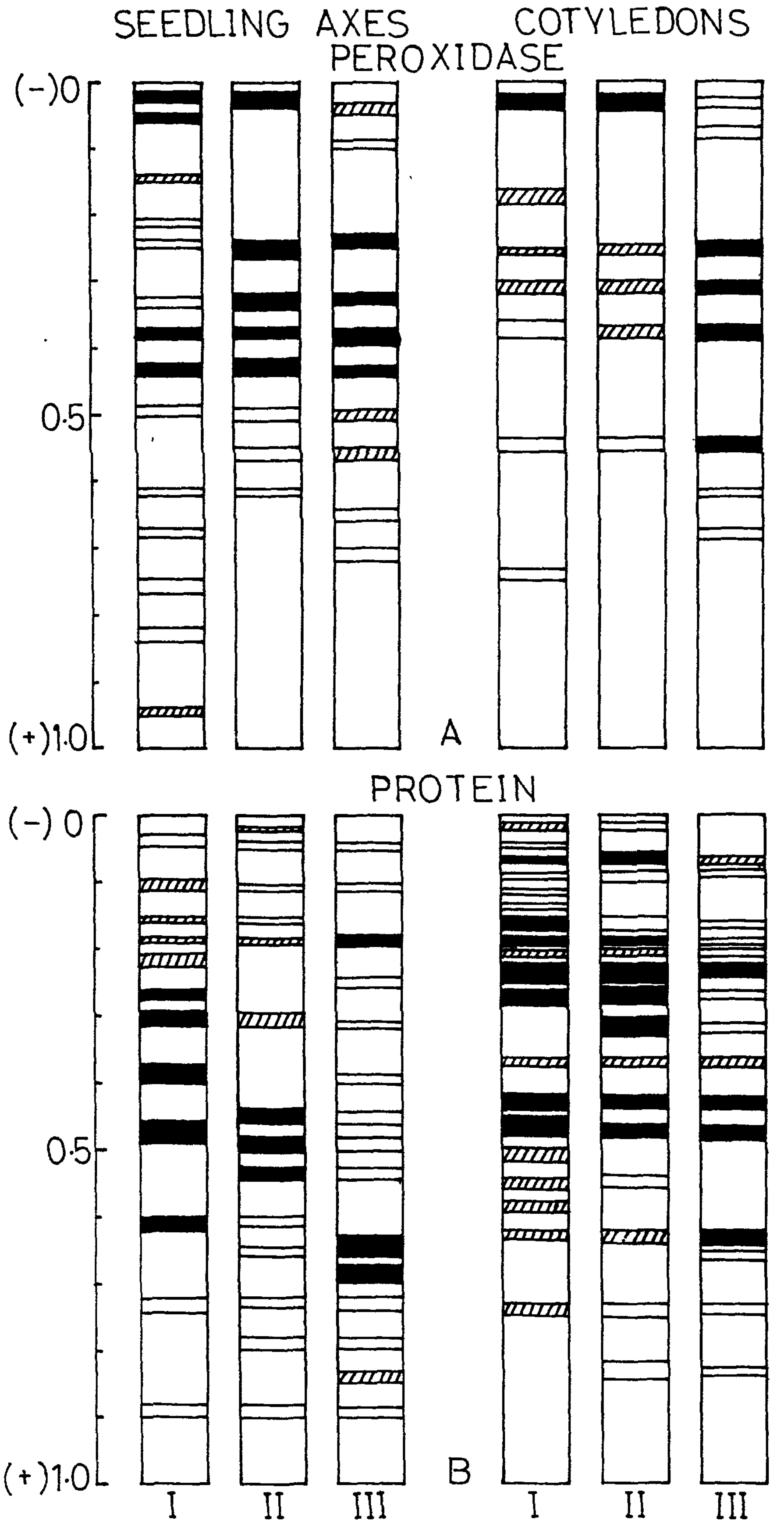
Fig· 4·1

(Fig. 4.1B). While two new bands of Rfs 0.74 and 0.82 appeared during radicle emergence, 5 new bands of Rfs 0.21, 0.23, 0.45, 0.56 and 0.71 appeared during the cotyledon emergence phase. The appearance of new bands during seedling emergence is also in accordance with increase in protein content of embryo (Table 4.13). In endosperm 17, 22 and 21 bands appeared on gels using extracts of imbibed seeds, radicle and cotyledon emegence stage, respectively (Fig. 4.1B). During emergence of radicle, bands of Rfs 0.02, 0.28, 0.42 and 0.54 were not detected and also during cotyledon emergence bands with Rfs 0.58, 0.88 and 0.92 were absent. This was consistent with decrease in protein content of endosperm during seedling emergence (Table 4.13).

S. khasiana

Peroxidases : In seedling axes 14, 8 and 10 bands were noticed during stages of radicle, plumule and cotyledon emergence, respectively (Fig. 4.2A). Correspondingly, a decrease in enzyme activity was also recorded during seedling emergence (Table 4.8). During plumule emergence, bands of Rfs 0.06, 0.15, 0.22, 0.68, 0.75, 0.83 and 0.94 were absent. However, 1 new band of Rf 0.56 appeared at this stage. On the other hand, during cotyledon emergence, band of Rf 0.62 was not detected but 3 new bands with Rfs 0.09, 0.65 and 0.71 were recorded. In cotyledons, 7, 5 and 8 bands appeared during radicle, plumule

Fig. 4.2 : Electrophoretic changes in peroxidases (A) and proteins (B) in seedling axes and cotyledons during (I) radicle emergence phase, (II) plumule emergence phase and (III) cotyledon emergence phase in *Schima khasiana*. Increasing band intensity (▭ ▨ ■).


SEEDLING AXES
COTYLEDONS
PEROXIDASE
(–)0
0·5
(+)1·0
A
PROTEIN
(–) 0
0·5
(+)1·0
B
I
II
III
I
II
III
STAGES OF SEEDLING DEVELOPMENT

Fig· 4·2

and cotyledon emergence, respectively. During plumule emergence, bands of Rfs 0.18 and 0.74 were not detected. The intensity of band of Rf 0.38 was more. During cotyledon emergence, 3 new bands of Rfs 0.08, 0.62 and 0.68 were observed. The increase in enzyme activity was also recorded in cotyledons at cotyledon emergence stage (Table 4.8).

Proteins : In seedling axes, 12, 14, 15 bands were observed at stages of radicle, plumule and cotyledon emergence, respectively. During plumule emergence, 4 new bands of Rfs 0.02, 0.54, 0.65 and 0.79 were recorded. One band of Rf 0.47 of radicle emergence stage perhaps got split up into two bands of Rfs 0.45 and 0.49, observed at plumule emergence stage. But during emergence of cotyledons, a band of Rf 0.39 present in radicle emergence phase was re-recorded (Fig. 4.2B). The increase in protein content of seedling axes was also observed during seedling emergence (Table 4.14). In cotyledons, 19, 16 and 15 bands were detected during radicle, plumule and cotyledon emergence phases, respectively (Fig. 4.2B). During plumule emergence, 2 new bands of Rfs 0.42 and 0.33 were developed whereas two bands of Rfs 0.02 and 0.53 were not detected during emergence of cotyledons. The increase in protein content during plumule emergence phase and subsequently decrease during cotyledon emergence were recorded (Table 4.14).

Discussion

The soaking of seeds in growth regulators like IAA, GA, FAP and AA solutions exhibited an increase in the seedling growth and moisture content in both P. kesiya and S. khasiana except 2,4-D treatment. The increase in growth is due to cell enlargement and differentiation of active embryo (Khan, 1977; Chinoy and Saxena, 1978) while suppression in growth by 2,4-D is due to its toxic nature (Prasad, 1984).

Many oxidative enzymes are present in seeds and changes in them lead to onset of germination (Mayer and Poljakoff-Mayber, 1982). A number of terminal oxidases are associated with membranes and the change in properties of membranes bear a prominent role in controlling seed germination by affecting the rate of hydration, respiration, enzyme release and hormone content etc. (Tao and Khan, 1977; Rodríguez and Sanchez-Tamís, 1986). Auxins, GA, FAP and AA regulate the endogenous IAA content during germination and development, by change in enzyme activities or synthesis of auxin (Galston and Davis, 1969; Gaspar et al., 1977, Mehta and Chinoy, 1978; Lee, 1980; Saleh and Hamberg, 1980; Dendsay and Sachar, 1982; Trewavas, 1982; Zajączlowski et al., 1983).

The increase in catalase activity was recorded in endosperm of P. kesiya with growth regulator treatments as compared to control at radicle and cotyledon emergence stages and also in GA treated seeds. In conifers, the actual site of catalase involvement in seed for mobilization of triglyceride may be the endosperm. The presence of high levels of lipids in conifers also suggests the occurrence of enzymes of glyoxysome cycle (isocitrate lyase, matate synthetase and catalase) in germinating seeds (Firenzuoli *et al.*, 1968a; Ching, 1970). An inhibition in catalase activity in embryo resulted at both radicle and cotyledon emergence stages except for GA and AA treatments at the latter stage. In case of *S. khasiana* the catalase activity was higher in seedling axes and cotyledons in all growth regulator treatments as compared to control except 2,4-D. However, IAA and 2,4-D treatments were slightly inhibitory. An increase in the micro-bodies during development suggests an increase in catalase activity and protein bodies of sweet potato root tissues (Esaka and Asahi, 1979; Esaka *et al.*, 1983). The catalase activity regulates the level of H_2O_2 which may be the function of plant growth regulators as reported during development of sunflower plants (Sarkar and Choudhury, 1985). The increase in catalase activity of *Cicer arietinum* with GA and AA (Mehta, *et al.*, 1974a) and of *Cajanas cajan* (Mehta *et al.*, 1974b) with AA treatments have been reported. Seed potatoes soaked in GA resulted in decrease in catalase and increase in peroxidase activities during early germination (Puzina, 1976). Henry and Jordan (1977) also showed a depression in the activities of catalase, peroxidase and polyphenol oxidase with GA and IAA treatments of the excised apical section of pea seedlings. But the increase in peroxidase and catalase with AA suggests an upsurge in oxidative processes through free radical monodehydroascorbic acid in the energy transfer during seedling development of Plantago (Mehta and Chinoy, 1978). Kinetin has been reported to activate the IAA-oxidizing enzyme in roots of Lens culminaris but not the catalase activity which led to a decrease in IAA content (Gaspar and Xhauffaire, 1967).

In control of P. kesiya, the peroxidase activity was higher in embryo as compared to endosperm suggesting the actual energetics involved with the embryo during germination. Peroxidase and IAA oxidase activities have been found associated with the active differentiation in cucumber seedlings (Halevy, 1964) and it can be regulated by the translocation waves of auxin (Sánchez-Bravo et al., 1986). The developmental fluctuation in the measured levels of peroxidase activity were also observed in pea (Gibson and Liu, 1978). They also reported that such fluctuation could be due to either non-physiologically identical isoenzymes of peroxidase which might be responsible for these developmental fluctuations. As compared to control, the growth regulators suppressed the peroxidase and IAA-oxidase activities, during radicle emergence except GA treatment in P. kesiya. The decrease in peroxidase activity in growth regulator treatments accompanied with overall increase in fresh weight of S. *khasiana* has been observed during the radicle emergence while IAA oxidase activity was not detected during this stage. Gaspar *et al.* (1977) emphasized the presence of a sufficiently low peroxidase activity and a minimum auxin level of the embryo which may be responsible for the onset of germination of dormant and non-dormant wheat seeds. The same may increase the production of α-amylase. The inverse correlation between peroxidase and radicle emergence with application to IAA, FAP and AA in P. *kesiya* and also in S. *khasiana* with all growth regulators except 2,4-D suggests the possible role of peroxidase and IAA oxidase in regulation of IAA level which could govern the redox potential and cell division (Kamiski, 1971; Lee, 1980). During germination the peroxidase activity was higher in GA treated seeds of P. *kesiya*. A similar observation has been recorded during the germination of sweet potato seeds treated with GA (Puzina, 1976). Besides, a number of growth hormones have been reported to inhibit the peroxidase and IAA-oxidase activities in many species (Elkinawy and Raa, 1973; Ram *et al.*, 1976; Balasimha, *et al.*, 1977; Saleh, 1981; Dendsay and Sachar, 1982). A direct correlation between growth and peroxidase activity has been found during cotyledon emergence in P. *kesiya* and S. *khasiana* in response to IAA, FAP, AA and GA treatments excepting the last treatment in the latter species. While all the growth regulators enhanced the IAA-oxidase activity in

P. kesiya, the treatments of IAA, 2,4-D and FAP increased the activity of IAA-oxidase in treated seeds. The maximum increase in the activities of both peroxidase and IAA-oxidase at cotyledon emergence stage in *S. khasiana* with FAP treatment suggests that cotyledons are the possible site of accumulation of synthesis of IAA.

Similar interpretation was given for cotyledons of *Lupinus albus* during seedling gdevelopment (Elkinawy, 1982). Saleh and Hamberg (1980) have mentioned an increase in the amount of endogenous IAA in seeds of *Phaseolus*, *Pinus*, *Zea* as well as in young bean plants with kinetin treatment. In number of studies an increase in IAA-oxidase and peroxidase has been reported during seedling development with application of various growth regulators (Dziewanowska and Lewak, 1975; Brunner, 1978; Dendsay and Sachar, 1978; 1982; Mehta and Chinoy, 1978; Balasimha and Tewari, 1979). 2,4-D inhibited the peroxidase activity and seedling growth in both the embryo and endosperm of radicle and cotyledon protruded seeds of *P. kesiya* and throughout seedling development in *S. khasiana*. The activity of peroxidase with application to 2,4-D has been shown to slightly improve in Jerusalem artichoke tissue (Morell and Démétriadés, 1955). Conversely, inhibition in the enzyme activity in relation to seedling growth in *Trigonella foenum-graeuum* (Balasimha *et al*., 1976) and in wheat (Fluckiger, 1977) were reported.

In many cases, a close correlation has been found between the enhanced activity of polyphenol oxidase and the concentration of phenolic substances (Stelgig *et al.*, 1972; Mayer and Harel, 1979; Vaughn and Duke, 1984). Polyphenol oxidase activity increased with seedling emergence in both embryo and endosperm of *P. kesiya* except in endosperm of cotyledon emergence phase. In general, this increase was accompanied with an increase in total phenol and O-dihydroxyphenol contents. It has been suggested that polyphenol oxidase has been involved in the biosynthesis of flavonoids (Gaspar, 1965; Runkova *et al.*, 1972, Sutfeld and Wiermann, 1976; Butt, 1979; Duke and Vaughn, 1982). The decrease in phenol contents of seed coats in both the species with growth regulator treatments reveal the leaching of phenolic inhibitors. Kumar *et al.* (1979) mentioned the presence of rutin, quercetin, α-resorcyclic acid, protocatechuic acid, p-hydrobenzoic and vanillic acid in leachates of *Cicer* seeds. The growth regulators enhanced the polyphenol oxidase activity in both embryo and endosperm of *P. kesiya* during seedling development except 2,4-D treatment. It has been observed that higher phenol content was directly proportional to the increase in fresh weight of seedling. The increase in growth could be due to increase in polyphenols which are found to be associated with an increase in protein and IAA contents (Galston and Davis, 1969; Schneider and Wightman, 1974; Barendse, 1983).

In response to IAA, 2,4-D, GA and AA treatments of S. khasiana, the polyphenol oxidase activity increased in seedling axes and decreased in cotyledons as compared to control, during radicle emergence stage. However, FAP treatment resulted in higher activity in both the seedling parts. During plumule emergence, 2,4-D and FAP inhibited the activity in seedling axes and cotyledons, respectively. During cotyledon emergence stage, it was observed that IAA enhanced the polyphenol oxidase activity in both seedling axes and cotyledons. The higher content of total phenol was observed during plumule and cotyledon emergence in IAA, 2,4-D and GA treatments and O-dihydroxyphenol during radicle, plumule and cotyledon emergence in both seedling axes and cotyledons as compared to respective controls. A direct relationship was seen between polyphenol oxidase and total phenol content. The possible involvement of phenol oxidase in oxidation of IAA has been reported (Potapov et al., 1973). The higher activity of IAA-oxidase, peroxidase and polyphenol oxidase in growth zone of Lupinus roots was also observed. A number of growth regulators were found to affect differently the level of polyphenol oxidase during growth (Henry and Jordan, 1977; Taneja and Sachar, 1977a,b; Balasimha and Tewari, 1979). The increase in polyphenol and O-dihydroxyphenol contents with growth promoters i.e., IAA, GA, FAP and AA indicated their involvement in metabolic pathways during seedling development. The increase in polyphenols resulted in a higher number of protein bands (Feucht and Schmid, 1980; Schmid and Feucht, 1981). The increase in protein content of embryo and seedling axes of P. kesiya and S. khasiana, respectively, may be correlated with the growth of the tissue. The decrease in protein content in endosperm of P. kesiya exhibited the mobilization of soluble material for seedling development. The marked increase in protein content of seedlings with IAA, GA and AA during cotyledon emergence could probably be related to photosynthetic activity of cotyledons (Huber and Sankhla, 1974a). AA and FAP treatments resulted in higher protein contents in endosperm as compared to control. This indicates less utilization of protein content during seedling development in these treatments. The protein content was found higher in cotyledons as compared to seedling axes in S. khasiana. The cotyledon is regarded as storage organ in many angiosperm. Under the influence of different growth regulators, protein could be mobilized differently to developing seedling axes (Kumari and Kohli, 1984). The changes in protein content may be related with the initial stages of differentiation (Patterson and Trewavas, 1967).

The RNA content was higher in extra-embrynol parts like endosperm in P. kesiya and cotyledons in S. khasiana during emergence of radicle. The increase in RNA contents in extra-embryonal part is suggestive of stimulation of the radicle protrusion from the seeds through hydrolization of protein content for developing embryos. This is also true for extra-embryonal parts of many seeds like *Cassia occidentalis* (Kumari and Kohli, 1984), Douglas-fir (Ching, 1966) and Castor bean (Martin and Northecote, 1981). In general, both RNA and DNA contents increased during radicle emergence. The higher amount of DNA during radicle emergence except embryo of *P. kesiya* is indicative of higher rate of cell division as mentioned by several workers (ref. Mayer and Poljakoff-Mayber, 1982).

The present study on electrophoresis of extracts of *P. kesiya* and *S. khasiana* during seedling emergence indicated that during germination and emergence of seedlings, many changes occur in the level of isomaric nature and number of bands of peroxidases and proteins. The 7 and 12 new bands of peroxidase and 2 and 8 new bands of proteins in embryo and endosperm tissues of *P. kesiya*, respectively, were observed during emergence of radicle. The appearance of new bands of peroxidase and proteins in embryo and change in intensity of bands exhibit an important function of peroxidase and synthesis of proteins for the onset of radicle protrusion from the seed. The increase in number of bands of peroxidase and proteins in endosperm is possibly due to synthesis of metabolites and growth factors responsible for active growth and differentiation of embryo during initiation of germination. The detection of new bands and changes in activities during germination is in accordance with results obtained in other species of Pinus (Conkle, 1971; Ramaiah et al., 1971; Pitel et al., 1984). In general, metabolic activity of seed begins with imbibition and increases during subsequent germination. The increase in activity is related to the physiological, anatomical or morphological events occuring during germination (Mayer, 1977). After emergence of plumule in S. khasiana, the decrease in the number of bands of peroxidase in both seedling axes and cotyledons was recorded along with decrease in its cactivity. Such changes could be due to a conformational change of the isoenzymes of peroxidase or due to interaction between enzyme sub-units. The increase and decrease in protein bands of seedling axes and cotyledons to developing seedling for its growth during emergence of plumule and cotyledon in S. khasiana with further growth and differentiation, the number or intensity of peroxidase iso-enzymes changed in embryo and endosperm of P. kesiya and seedling axes and cotyledons of S. khasiana during emergence of cotyledons. Similar results were reported in other tree species in which activity of peroxidase has been found to be low in dry and imbibed seeds, but the number and intensity of peroxidase bands increased with further development (Thévénot, 1977; Pitel et al., 1984). It has been seen that certain peroxidase and protein bands are specific and they may be possibly involved in tissue differentiation or morphogenetic changes in developing seedlings of both the species. The occurrence of specific iso-enzymes have been reported in *Quercus alba* (Mayberry and Foret, 1977) and *Pinus* (Conkle, 1971; Pitel *et al.*, 1984). Many of the consistently occuring bands represent storage proteins, whereas others of lower concentration may be enzymes necessary for development of embryo during germination process (Durzan, 1966). However, the physiological role attributable to certain enzymes is complex and for that they are known to change their structure and specificity in the presence of small molecules which induce conformational change in the protein.

Chapter V

SUMMARY

The regeneration of pioneer and important trees is important because the forest resources are fast dwindling due to indiscriminate felling of forest trees. Pinus kesiya Royle ex Gord. and Schima khasiana Dyer are two economically important trees of high altitude in North-East India. The seed is crucial intermediary step in the life of trees. The germination in relation to biochemical approach has received little attention in forest trees. During the present study, requirements of seed storage, germination, seedling growth as well as regulation of certain biochemical aspects during seedling emergence have been studied.

Longevity of seed is extremely important for the regeneration of plants and raising the nursery through direct seeding. The seeds of pioneer and early successional plants loose their viability very soon under unfavourable conditions. During the present study, both P. kesiya and S. khasiana seeds stored at 3-5° C in sealed bags showed optimum viability and germination. While storage of S. khasiana seeds at laboratory temperature (15-22° C) in open and over CaO showed complete loss of germination capacity within 4 months, it was retained in the seeds stored in sealed bags. One-year-stored seeds under various conditions exhibited a decrease in yield in both the species.

The early emergence and higher growth were recorded after a year in S. khasiana as compared to P. kesiya under field conditions. The seasonal variations showed that the conditions prevalent at the study site during August and September for *P. kesiya* and *S. khasiana*, respectively, might be appropriate periods to raise the seedlings under controlled conditions.

A number of environmental factors and growth regulators affect the resumption of embryonic growth. The early germination was recorded in *P. kesiya* at pH 6.0 and 6.4, however, total germination was higher at pH 4.8. The slightly acidic to neutral pH solutions enhanced the seed germination and seedling growth in *S. khasiana*. The water stress of -2.5 bars stimulated the seed germination and seedling growth in *P. kesiya* and beyond this limit a decrease was noted. Osmotic potentials above -1.0 bar created by both PEG and sucrose significantly inhibited the seed germination ($P \leq 0.05$) and decreased the seedling growth in *S. khasiana*. However, sucrose at -0.10 bar as osmoticum enhanced the seedling growth. Imbibition of seeds at varying temperature and duration showed better seed germination and seedling growth at 5° C in *P. kesiya* for 24 hr. In *S. khasiana*, the soaking of seeds at 15° C for 6 hr resulted in maximum germination and early seedling growth.

The responses of light and temperature on seedling emergence in both *P. kesiya* and *S. khasiana* seeds were studied.

The maximum seed germination and seedling growth resulted under a 3.0 lux light of 16 hr photoperiod in P. kesiya while in S. khasiana, illuminance of 25 lux for 8-10 hr photoperiod resulted in rapid and maximum germination. On the other hand cotyledon expansion and seedling yield in S. khasiana were better at higher light intensities. The red radiation enhanced seed germination and seedling growth in P. kesiya, whereas far-red reversed the stimulatory effect of red light. Amongst the different temperature treatments, diurnal temperatures of 20/15°C and 20/10°C resulted in higher seed germination in P. kesiya and S. khasiana, respectively. However, higher seedling growth in P. kesiya and S. khasiana, were recorded at diurnal temperature of 35/20 and 25/15° C, respectively. The effect of growth regulators on seed germination of one-year-stored seeds of P. kesiya and S. khasiana were studied. The growth regulators viz., IAA (25 mg/l), 2,4-D (25 mg/l and 100 mg/l), GA (150-200 mg/l), FAP (1 mg/l) and AA (25-50 mg/l) stimulated seed germination in P. kesiya. In S. khasiana soaking of seed in growth regulators like IAA (10 mg/l), 2,4-D (25 mg/l), GA_3 (25 mg/l), FAP (0.1 mg/l) and AA (10 mg/l) enhanced the germination percentage. 2,4-D produced abnormal seedlings in both the species. Studies of different growth regulators on seedling growth revealed that GA and FAP were highly permissive in P. kesiya and S. khasiana, respectively, for stimulation of seed germination and seedling growth.

Application of growth regulators affect the enzyme levels during seed germination. The catalase activity was higher in endosperm of GA treated seeds and also with other growth regulators during radicle emergence in *P. kesiya*. In *S. khasiana*, catalase activity was significantly higher in seedling axes of 2,4-D, FAP and AA during radicle emergence. 2,4-D resulted in an inhibition in the enzyme activity during cotyledon emergence in both the seedling parts. The peroxidase activity in *P. kesiya* was inhibited in embryo and endosperm of growth regulator treatments except GA at radicle emergence stage. The cotyledons of *S. khasiana* showed an increase in peroxidase activity with seedling development. Growth regulators inhibited the enzyme activity in seedling axes but increased in the cotyledons during radicle emergence. 2,4-D resulted in an inhibition in peroxidase activity in both the species. The decrease in IAA-oxidase activity was recorded in the embryo of growth regulators treatment during radicle emergence as compared to control in *P. kesiya*. The inhibition was also noted in endosperm with IAA, 2,4-D and FAP treatments. The higher IAA-oxidase activity was observed in seedling axes as compared to cotyledon during cotyledon emergence in *S. khasiana*. In *P. kesiya* all the growth regulators enhanced the polyphenol oxidase activity in embryo and endosperm. In general an increase in polyphenol oxidase activity was observed in seedling axes of *S. khasiana* in growth regulator treatments whereas a decrease in cotyledons was recorded.

. Total phenol and O-dihydroxyphenol contents increased in the embryo during its growth and a decrease in total phenol of endosperm and seed coat was observed in P. kesiya. In S. khasiana, total phenol content declined in seedling axes and seed coat, whereas an increase was observed in cotyledons. During cotyledon emergence, 2,4-D enhanced the O-dihydroxyphenol content in seedling axes whereas other growth regulators in cotyledons.

An increase in protein content of embryo and seedling axes of P. kesiya and S. khasiana, respectively, was observed in developing seedlings. In P. kesiya, a marked increase in protein content of embryo with IAA, GA and AA during cotyledon emergence was observed. However, a decrease was noted in endosperm except FAP and AA treatments. The higher protein content was observed in cotyledons as compared to seedling axes in S. khasiana. The protein content increased with the growth in this species except in cotyledons during cotyledon emergence stage. An inhibition in protein contents with 2,4-D during radicle and plumule emergence in both the seedling parts was observed as compared to respective controls. Initially, no change in DNA content of embryo in P. kesiya was recorded while endosperm showed an increase in its content during radicle emergence. RNA content was higher in both the seed parts during radicle emergence which subsequently declined during cotyledon emergence. In *S. khasiana*, seedling axes showed an increase in DNA content during emergence of plumule which declined later. RNA content in both seedling parts decreased at plumule emergence stage and subsequently increased during cotyledon emergence in seedling axes.

Isoperoxidases and protein bands were localised on polyacrylamide gels during imbibition and germination in *P. kesiya* and also during various stages of seedling emergence in *S. khasiana*. The emergence of radicle and cotyledons resulted in dramatic increase in peroxidase isoenzymes in embryo of *P. kesiya* and also during radicle emergence in endosperm tissues. In seedling axes of *S. khasiana*, a higher number of bands appeared while further growth, i.e. plumule emergence phase, resulted in decrease in number of higher intense bands. The dramatic increase in protein bands of *P. kesiya* embryo were noticed while in endosperm these decreased. In *S. khasiana* an increase in number of bands in seedling axes was observed at different stages of seedling emergence. However, a reverse picture was obtained for cotyledons. The change in bands pattern may be on account of tissue differentiation during seedling emergence.

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APPENDIX

LIST OF PUBLICATION OF THE CANDIDATE

1. Verma, A.N. and P. Tandon, 1983. Seed germination and seedling growth in *Pinus kesiya* with reference to environmental factors. Proc. Natn. Symp. Adv. Front. Pl. Sci. (Eds. H.C. Arya, N. Sankhla, M.N. Tewari, N.S. Sekhawat, S.D. Purohit), pp. 288-289, Jodhpur University Press, Jodhpur.

2. Verma, A.N. and P. Tandon, 1984. Seed germination and seedling growth in *Pinus kesiya* Royle ex Gord. I. Influence of imbibition, substrate pH and moisture level. Proc. Indian natn. Sci. Acad. B50 (3): 326-331.

3. Verma, A.N. and P. Tandon, 1984. Seed germination and seedling growth in *Pinus kesiya* Royle ex Gord. II. Light and temperature requirements. Proc. Indian natn. Sci. Acad. B50 (5): 512-518.

4. Verma, A.N. and P. Tandon, 1987. Effect of storage on germination in *Schima khasiana* and *Pinus kesiya* seeds. In Production and Conservation Forestry (Eds. P.K. Khosla and D.K. Khurana) (In press).