Biochemical Adaptations in *Pinus kesiya* Royle ex Gord Population Growing at Different Altitudes

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Biochemical adaptability of *Pinus kesiya* populations growing abundently in and around Shillong (92° 4′ E Longitude and 25° 2′ N Latitude) at the mean altitudes of 1000, 1400 and 1800 m from sea level were studied with respect to changes at molecular level in the seed lot and during initial development of the seedling. Quantitative and qualitative variations in structural proteins and changes in the activities and isoenzyme band patterns of peroxidase and esterase has been correlated to the altitudinal variations.

INTRODUCTION

The survival capacity of any plant depends on its inbuilt potentialities to germinate, grow, differentiate and reproduce under the existing environmental factors. During their adaptations plants undergo certain morphological, anatomical or/and physiological modifications which are achieved ultimately by altering some mechanisms at hormonal or purely biochemical levels. For the last few years a little attention has been given to the genetic basis of adaptations in relation to forest trees. Dhillon *et al.* (1978) reported the absence of any significant variation in the amount of DNA w. r. t. germinating seeds from five different populations of *Pinus rigida* growing in contrasting ecological situations (35° 53′ N to 45° 06′ N Latitude). It was felt interesting to study the changes, if any, in the products of gene function that are brought about by different environmental conditions. Preliminary attempts were made to study altitudinal effects (stored in the seeds) in *Pinus kesiya* Royle. populations growing abundently in and around Shillong (92° 4′ E Longitude ; 25° 2′ Latitude).

Sampling

The mosaic of natural geo-climatic conditions of North-Eastern India ranges from plain land nearing sea level to an altitude of over 2200 m ; from moderate to worlds' heaviest annual rainfall (Cheerapunji area); tropical to temperate climatic conditions and various soil types. The seeds were collected in the month of March from the plants of *P. kesiya* growing at three different mean altitudinal ranges—namely, Sirwat Forest, Mawpat (1000 m), Riat Khawan Reserve Forest, Jowai (1400 m) and upper shillong forest (1800 m).

Cultivation

A calculated number of seeds of the three collections were washed and soaked separately in distilled water (Agpaoa and Pulmano, 1978). One third from each lot were removed after 24 h of soaking for cell free extraction and the rest were sown over wet cotton bed in petri dish. One day after sowing, half of the sprouted seeds from each lot were processed for preparing the cell free plant extract, whereas, the second half was allowed to germinate further under normal existing conditions of the room. After ten days of sowing per cent germination was calculated and the seedlings were processed for plant extraction.

Preparation of cell free plant extract

The samples were homogenized in a glass homogenizer at 4°C using acid washed sand and 0·2 M phosphate buffer pH 7·0 containing 1 mM polyethylene glycole to prevent enzyme inactivation by phenolic compounds. The homogenate was centrifuged at 26,000 x g for 30 min at 0°C and the supernatant collected. The concentration of different extracts was equalised w.r.t. their soluble protein content (Lowry *et al.*, 1951).

Protein content

The samples were crushed in acetone and freed from pigments and phenolic compounds by repeated washing with acetone and finally with ether and air dried. The dried white powder thus obtained was used for estimation of protein content expressed as mg/g dry wt. (Lowry *et al.*, 1951).

Enzyme activity

The specific enzyme activities of peroxidase and esterase (expressed as μ kat/g protein) were measured essentially following the methods of Mitra *et al.* (1970) and Kohli (1978), respectively.

Acrylamide gel electrophoresis

The separation of biomolecules was done on 8·0 per cent polyacrylamide disc gel electrophoresis—running buffer: Lithium hydroxide pH 8·1 (for proteins) and Tris-glycine pH 8·3 (for enzymes). The method was essentially similar to the one described by Brewer (1970). Soluble proteins were detected using mixture of Amido-black and Coomassie blue (Kohli and Sawhney, 1980); simultaneous coupling Azo-dye method as described by Kohli (1978) was followed for marking isoenzymes of esterase. Peroxidase isoenzymes were detected using Benzidine-H_2O_2 mixture (Kohli, 1978).

RESULTS

Germination

Whereas, almost all the seeds from Jowai and Upper Shillong collection germinated, the lowest germination percentage was recorded in the seeds from Mawpat area (Table I).

TABLE I: Per cent germination of *Pinus kesiya* seed collected from different altitudes

S.No.	Collection	Mean altitude (m)	per cent seeds germinated
1.	Mawpat Forest	1000	54·50
2.	Jowai Forest	1400	95·00
3.	Upper Shillong Forest	1800	97·25

Total protein content

The total protein content was almost the same in all the three collections, initially. General increase in the content was observed with the seedling development in all the cases and the values almost coincides in the three collections at any particular developmental stage.

Electrophoretic pattern of soluble protein

A comparison of the electrophoretic profiles of water soluble proteins from three different stages as presented in figure 1, reveals that initially 20 protein bands were available in each of the three collections. Of these as many as 18 were identical owing to their similar Rm values. Two new protein bands, of which one was identical, appeared in all the collections after one day of germination. In Upper Shillong collection, one new protein band made its appearence after 10 days of germination, whereas in others there was no new synthesis.

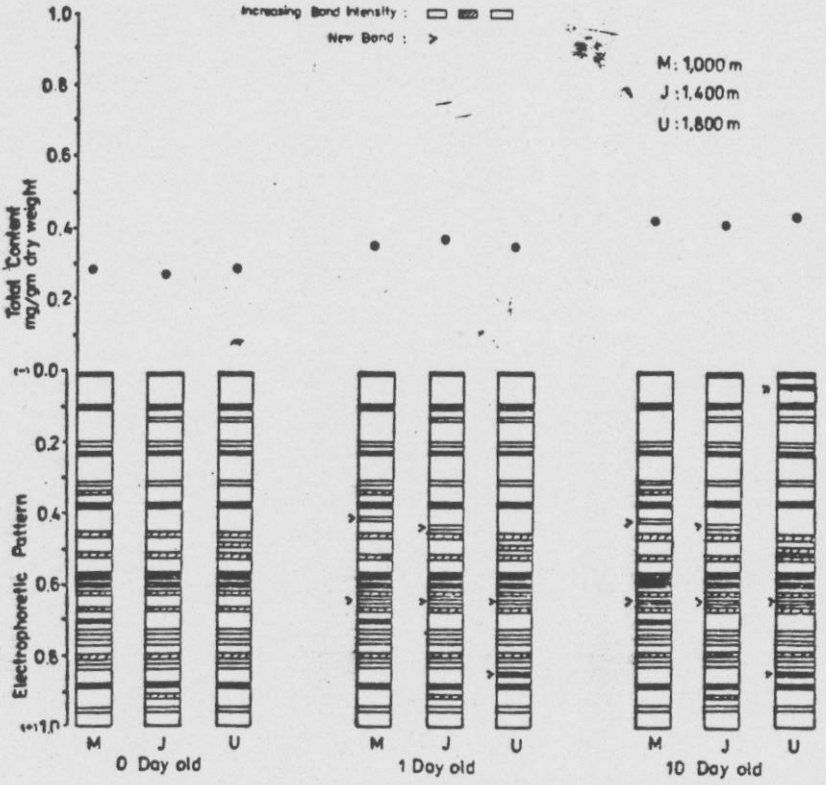

Fig. 1 : Total protein content and electrophoretic pattern.

Total enzyme activity

The specific activities of each of the two enzyme systems peroxidase and esterase studied were almost same in all the three collections. However, these activities increased initially with active growth, almost equally in all the three cases.

Isoenzyme pattern

Peroxidase (E. C. 1·11·1·7) : Initially 4 isoenzyme bands, but all at different Rm values were noticed in all the three collections. The pattern of new isoenzymes synthesized *de novo* was, however, different. Thus, whereas, as many as 5 isoenzymes appeared new in Mawpat and Jowai collections, only three new bands were noticed in Upper Shillong collection in response to 1 day germination. Of the newly synthesized bands only one (Rm 0·90) was identical. In 10 day old seedlings 2 new bands were noticed which were identical in the collections from Jowai and Upper Shillong (Fig. 2).

Esterase (E.C. 3·1·1·2) : Initially (zero day stage) two identical isoenzymens of esterase represented by 2 bands were observed. In 1 day germinated seeds 2 new bands—one (Rm 0·30) with low and the

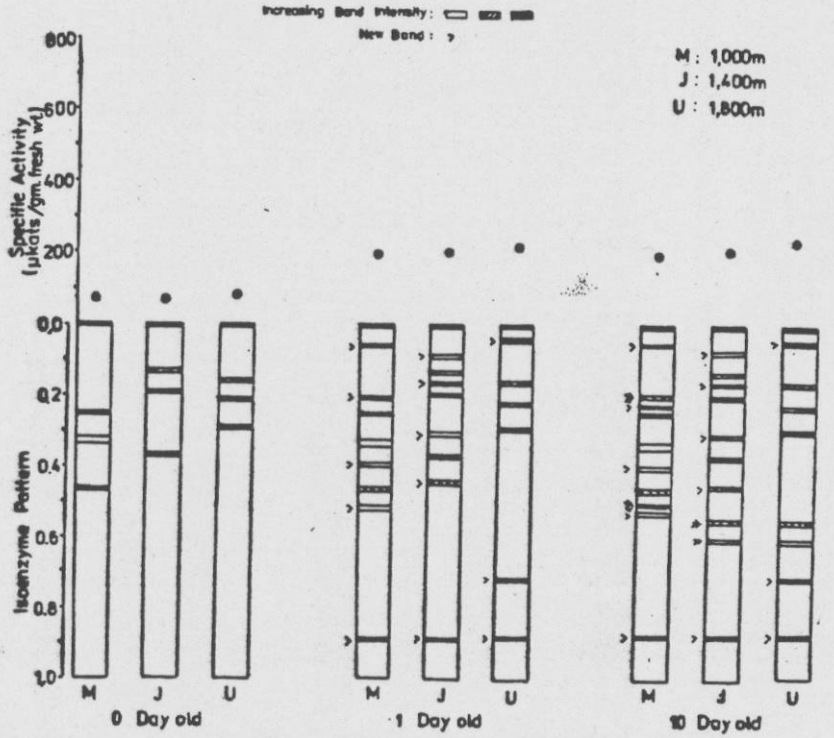

Fig. 2 : Specific activity of peroxidase and isoenzyme pattern.

second (Rm 0·74) with high electrophoretic mobility appeared new in all the three collections (Fig. 3). However, in addition to these two, whereas, 2 more were noticed new in the Jowai collection only one more appeared in the samples from Upper Shillong. One of these two additional bands in Jowai collection was similar to that from Upper Shillong. At the 10 day old seedling stage 1, 1 and 2 bands appeared new in Mawpat, Jowai and Upper Shillong collections, respectively. Here also one new band (Rm 0·80) was identical in Jowai and Upper Shillong collections.

DISCUSSION

In the current investigation, in order to understand and correlate various biomolecular adaptations related with the altitudinal effects, the effective adaptive changes stored in the genomes (in the seeds) were exploited. The genome got activated with the activation of seed germination and the adaptive changes got expressed during the stages of initial seedling development.

It is seen that total protein content in activated seeds was almost same in all the collections and the new protein synthesized is equally added in all the three cases during the development, so that, the increase is also almost identical. Initially the soluble structural proteins are also

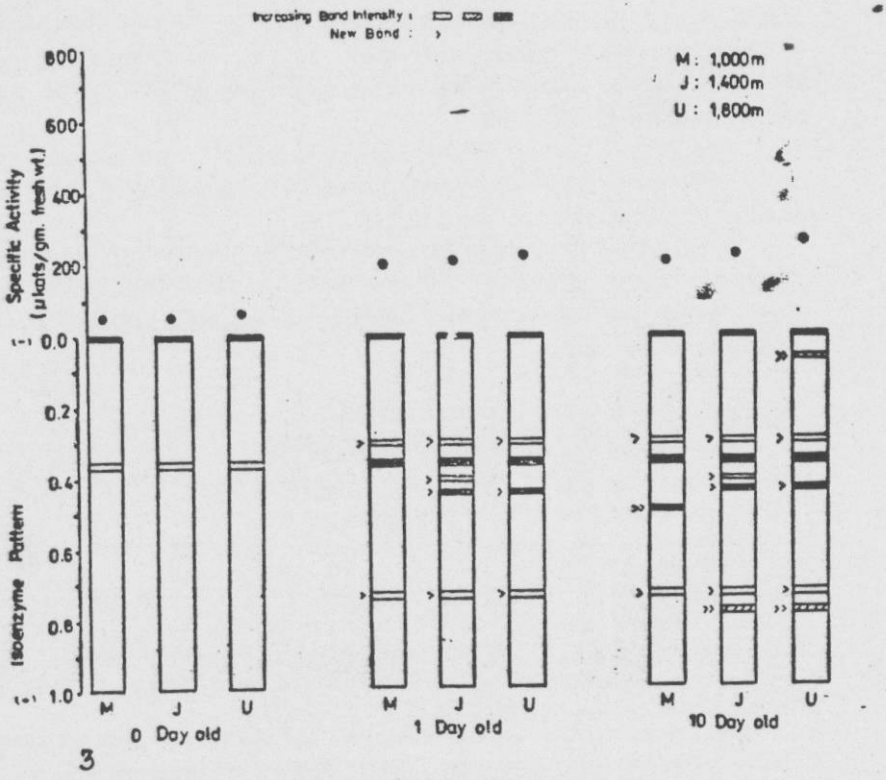

Fig. 3 : Specific activity of esterase and isoenzyme pattern.

essentially the same except for 2 bands which are different. Of the two new proteins synthesized during the initial seedling growth the one which was identical in all the three cases may be related to the effect of development and has nothing to do with the altitude effect. Whereas, the second new protein which is different in the three collections is due to the effect caused by difference in the altitudes. The appearence on the 10th day in Upper Shillong collection and its absence in other two collections is also atributed to its relation to the high altitude effect stored in the seed and expressed during development.

The study of the active gene products; oxidoreductase-Peroxidase and hydrolase-Esterase, as shown by many workers (Sandalios, 1974; Hadacova *et al.*, 1976) revealed that their specific activities increased with the active growth of the seedlings, in all the cases.

The isoperoxidases, though 4 in all the three collections were dissimilar even initially. The newly synthesized isoenzymes except for those at identical Rm values may be regarded as adaptive to the altitudinal effects since under the same genetic set up of the same plant species the functional attribute of the gene may differ under the different climatic conditions and this stored difference brought about in the functional gene is expressed in its product.

Unlike isoperoxidases, the isoestrases were identical initially. After 1 day of seed germination, new synthesis of 2 identical isoenzymes of esterase, in all the three cases may be the developmental function. However, the rest of the newly synthesized isoenzymes may be adaptive to the particular altitude.

The identical nature of some of the bands of peroxidase and esterase in the collections from Jowai and Upper Shillong may have some relation to their identical germination ability.

A perusal of the preliminary work clearly reveals that the altitudinal effects are expressed through the changes in the functional 'gene-products' at the basic biochemical level. However, extensive work is needed to study it in more detail.

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