CLONAL IDENTIFICATION OF *TECTONA GRANDIS* BY ISOENZYME STUDIES

By

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Introduction

Teak is an important commercial timber of India. Tree improvement work in Teak is in progress in many States and clonal seed orchards have been raised at different places. Characterization and identification of different clones in seed orchard will be of great value in Breeding works. By the use of isoenzyme technique, the relative contribution to the pollination by different clones and random mating, the proportion of plants originating from selfing following open pollination, contamination with pollen from surrounding stands, formation of provenance hybrids, correlation between economically important traits and the isoezyme genotype etc. could be studied.

Isoenzymes are multiple molecular forms of an enzyme with similar or identical substrate specificities occurring within the same organism. Isoenzymes are proteins consisting of Amino acids. The determination of amino acid is controlled by the genetic code. By gel electrophorosis, proteins which are very similar to each other can be separated and by this method, the presence of different genes (alleles) can be studied. Since the enzymes are the executive tools of the genes, this technique offers an opportunity to acquire information from the genome very close to the source of information.

Studies of proteins from pine pollen were reported by Bingham *et al*, as early as 1964. The intensive use of isoezyme study was first started in several research groups, during 1968-69. Lever and Burley (1974) have reviewed the application of Biochemical methods in Forestry. Scandalios (1969) has reviewed the genetic control of isoezymes in plants. Clonal identification has been carried out in conifers by the study of Peroxidase (Miyasaki & Sakai 1969; (Sakai *et al* 1974) and Esterase (Rasmuson *et al* 1971). So far, no such study has been undertaken in Teak. In the present study, the isoezyme *Esterase*, a hydrolysing enzyme with low substrate specificity, is used to characterise four clones of *Tectona grandis* from the clonal seed orchard of the Forest Research Centre, Coimbatore.

Materials and methods

Young emerging leaves (900 mg) of about 10 days old from four different clones (TNT 6; TNT 11; TNT 20 and KLS 1) were plucked, washed blotted and immediately homogenised in a 8 cm glass mortar in the presence of an extraction medium (2 ml.) which consisted of Tris HCl 0.1 m pH 8; Poly Vinyl Pyrrolidone (PVP) 0.1% Ascorbic Acid and

17% Sucrose. 1.0 gm. of Carburandom powder was used for grinding. All operations are carried out at 4° C to 5° C.

The homogenete was centrifuged at 20,000 RPM for 20 minutes in a refrigerated centrifuge. The supernatent was used for electrophorosis

Polyacralamide gel was used for electrophorosis. The method was essentially that of Davis (1964) and only small pore gel was used. Length of gel tube was 120 mm and dia. about 6 mm. 50 micro litre of extract per gel tube was loaded and current of 4 mill Amp./ Tube was passed for 2 hrs. Then the gel tubes were removed from the buffer tank and dewalled. The gels were immersed in phosphate buffer 0.1 M pH 6.8 for 15 minutes at 5°C.

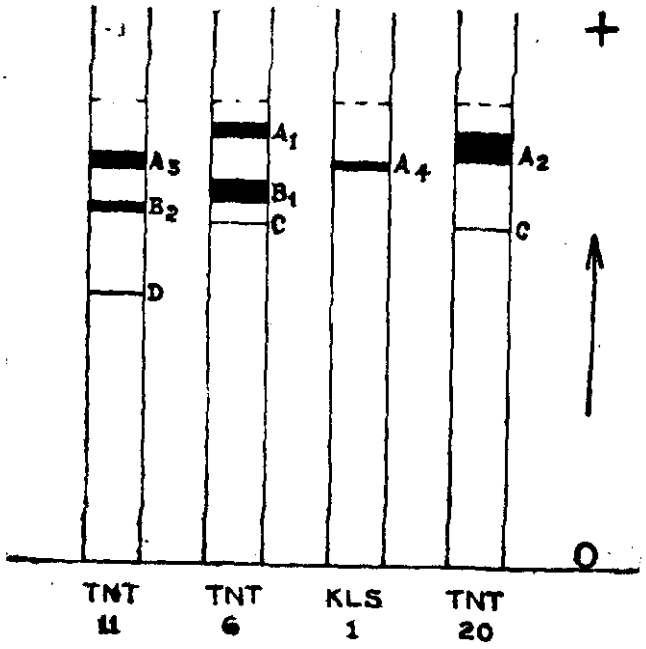
The gels were transferred to a staining solution containing 100 ml. 0.1 M. phosphate buffer pH 6.8, 15 mg of Alpha napthyle acetate dissolved in 1.5 ml of 50% Acetone, 75 mg fast blue R.R. salt and 10 ml 1-propanol for about 45 minutes at room temperature. The gels were fixed in 7% Acetic acid and photographed.

Results and discussion

Four regions can be identified in gels after staining for Esterase (*Fig. 1*). The fast moving bands A and B are clearly stained and C and D region bands are faint.

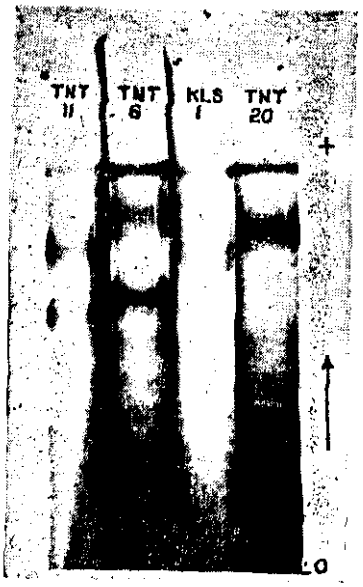
Region A shows four alleles A_1, A_2, A_3 and A_4, each of the four clones having one of the alleles; clone TNT 6—A_1, TNT 20—A_2, TNT 11—A_3 and KLS 1—A_4.

Fig. 1



Zymogram of the Teak leaf Esterase isoenzyme. 'O' indicates origin and the arrow indicates the direction of migration. Dotted lines indicate the marker.

Photograph



Photograph showing the leaf esterase bands of the four Teak clones.

Region B is represented by two alleles B_1 and B_2. B_1 in TNT 6 and B_2 in TNT 11. These band's are absent in the other two clones.

Region C is represented by a single band C which is faint but found in TNT 6 and TNT 20 and absent in the other clones.

Region D is alse faint but represented by a sing e band D in TNT 11.

The clone KLS 1 is represented by a single band A_4 only. TNT 20 could be identified by the presence of two bands. A_2 and C, A_2 being strong and characteristic. TNT 11 and TNT 6 have three bands each, but with different Rf values. Thus, the results indicate that there may be four gene loci codeing for different alleles of Esterase.

The study of all the clones in seed orchard at different seasons and in different age groups may throw more light on the dependability of the results for further breeding works.

Acknowledgements

The author is grateful to Shri K. Shanmuganathan, I.F.S., Head of Biological Research, Forest Research Centre, Coimbatore, for his encouragement and help at various stages.

SUMMARY

Identification of individual clones in a seed orchard will be of great value in breeding works. Four clones of Teak were analysed biochemically for the Esterase isoenzyme and the results indicate that it is possible to identify individual clones by this method.

समविकर अध्ययनों द्वारा *टेक्टोना ग्रांडिस* की पहचान

लेखक जी० कुमारवेलु

सारांश

वृक्ष प्रजनन कार्य के लिए बीजोद्यान में ही अलग अलग कुन्तकों (क्लोनों) की पहचान हो जाना बड़े मूल्य का है। एस्टरेस समविकर (आइसोएंजाइम) ज्ञात करने के लिए सागौन के चार कुन्तकों (क्लोनों) का जैवरासायनिक विश्लेषण किया गया जिनके परिणामों से संकेत मिलता है कि इस विधि से कुन्तकों को अलग-अलग पहचाना जा सकेगा।

Klonale Identifizierung der *Tectona grandis* mit isoenzymen Studien

G. KUMARAVELU

ZUSAMMENFASSUNG

Identifizierung der individuellen K'onen in einem Samengarten wird für Erzeugungarbeit auf großen Wert sein. Vier Klonen des Teaks waren für Estrase Isoenzyme biochemisch analysiert und die Ergebnissen zeigen an daß die individuellen Klonen zu identifizieren, bei dieser Methode möglich ist.

Identification des clones de *Tectona grandis* par les etudes isoenzymatiques

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Résumé

L'identification des clones individuels dans un verger à graines sera d'une grande valeur à l'égard de la selection et amelioration des plants. Quatre clones furent analysés biochimiquement pour rechercher l'isoenzyme estérase. Les résultats ont montré que l'on peut utiliser ce procédé pour identifier les clones individuels.

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