CLONAL IDENTIFICATION OF POPLARS BY ISOZYME ANALYSIS

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Introduction

Poplars are one of the fastest growing hardwood timbers in the world. The multipurpose wood is in great demand for manufacturing paper and pulp, plywood, match sticks and packing cases. Besides easy methods of propagation, they are economically harvested at short rotation of 8-10 years (Anon., 1983). In India, poplars are being increasingly planted in subtropical parts by farmers on their land as agroforestry tree.

Keeping in view their economical and ecological importance, a large number of Poplar clones (*Populus deltoides*) have been and are being developed or introduced from other countries by Research Institutes, Universities, State Forest Departments and private companies. As clonal material is regularly exchanged among themselves, different research organisations involved in Poplar research are facing problems regarding mixing of clones. It is difficult, laborious and time consuming to differentiate among different clones of a species due to their identical morphological characters. Forestry unlike agriculture is a long term proposition and mistakes committed once are reflected after several years. Hence it becomes imperative to maintain clonal identity so as to avoid their mixing during biodiversity conservation and in nursery as well as field plantations.

Isozymes are at present one of the tools and also cost effective as compared to DNA markers, offering excellent opportunity to identify different clones accurately in minimum possible time. Therefore, the objective of this study was to distinguish different Poplar clones using isozyme banding patterns.

Material and Methods

The present studies were carried out at Institute of Forest Genetics and Tree Breeding, Grosshansdorf, Germany during August-October 2000.

Tissue collection : Buds as well as leaf tissue were used for electrophoresis. Cuttings of different clones viz 11182, G48, A-124, S7C8, 109/86, G3, 3324, 421-2, S7C1 from stool bed nursery were collected in the month of August, 2000 from Regional

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Horticultural Research Station, Jachh, India. Half of the cuttings were stored at -70°C and the remaining half were rooted in potting mixture in greenhouse.

Electrophoresis : Isozyme analysis was carried out with the help of starch gel electrophoresis based on the methods given by Shaw and Prasad (1970), Conkle *et al.* (1982) and Cheliak and Pitel (1984) with slight modifications. During isozyme analysis 1-2 buds were transferred to wells of grinding block (kept on ice) containing 2-3 drops of extraction buffer. Two types of extraction buffers were tried (Rajora and Danick, 1992; Cheliak and Pitel, 1984). Better resolution was obtained with extraction buffer No.5 of Cheliak and Pitel. The buffer contained following chemicals in 0.05 M borate buffer (pH 7.1) : 2% Tergitol 15-S-9, 2% PEG (20 M), 8% PVP (7:1 of 40 M and 360 M), 50 mM ascorbic acid, 0.4 mM NAD, 0.1% Bovine Serum Albumin, 0.2 mM Pyridoxal-5-phosphate, 0.3 M Sucrose, 12 mM Cystein-HCl and 0.7% ß-mercaptoethanol.

The electrode and gel buffers (Cheliak and Pitel, 1984) used for different enzymes are given in Table 1.

Tronto starch used for this study was the product of Biomol (Hamburg). The gel concentration for both the types of buffers was kept at 12%. The gels were run for 3½ hours. Samples were electrophoresed three times. After the completion of electrophoresis, the gels were cut into three slices of about 2mm thickness. The top one was discarded. The remaining gel slices were then transferred in enzyme specific chemical solutions and kept at 37°C to undergo reaction.

Since no controlled pollinations were made to confirm the genetic control of the allozyme variants observed, therefore, only gel phentoypes were recorded.

Results and Discussion

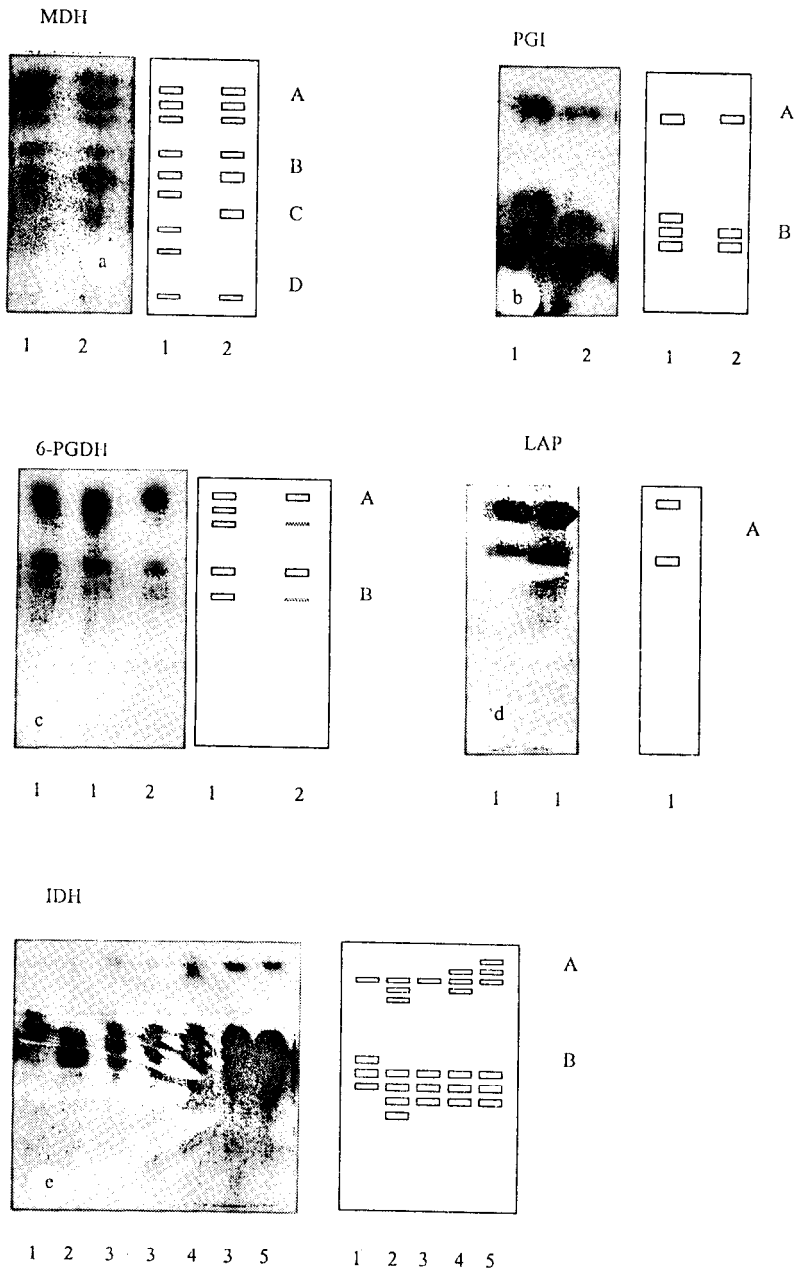
Eight enzymes were resolved both in bud and leaf tissues: Isocitrate

Table 1

Electrode and Gel buffers

Name of system	Gel Buffer	Electrode buffer	Enzyme system
Tris-citrate	Electrode buffer in the ratio of 1:2 with distilled water	18.0 g/l Tris 10.0 g/l Citric Acid pH 7.2	IDH MDH MNR PGI PGM 6-PGDH SKDH
Ashton	6.2 g/l Tris 2.0 g/l Citric Acid pH 8.3	11.8 g/l Boric Acid 1.0 g/l Lithium hydroxide	LAP AAT GDH

Fig. 1 (a-e)



Schematic illustration of five enzyme systems in *Populus deltoides*.
The numbers indicate gel phenotypes and the alphabets denote different zones

dehydrogenase (IDH, E.C. 1.1.1.42); Leucine amino peptidase (LAP, E.C. 3.4.11.1); Malate dehydrogenase (MDH, E.C. 1.1.1.37); Menadione reductase (MNR, E.C.1.6.99.2); Phosphoglucose isomerase (PGI, E.C. 5.3.1.9); Phospho-glucomutase (PGM, E.C. 2.7.5.1); 6-Phospho gluconate dehydrogenase (6-GPD, E.C. 1.1.1.41) and Shikimic acid dehydro-genase (SKDH, E.C. : 1.1.1.25). AAT and GDH did not show enzyme activity or produced faint/blurry bands hence are not presented. MNR, LAP, SKDH and PGM were monomorphic.

In MDH four zones of activity were observed i.e. MDH-A, MDH-B, MDH-C and MDH-D (after Malvolti *et al.*, 1991). All the clones exhibited similar banding pattern except the clone A-124 which showed extra band in MDH-C zone (Fig. 1a). The gel phenotype of clone A-124 is 1 where as rest of the clones have the phenotype 2.

PGI was found to be controlled by two loci as observed by Rajora, (1990) and Malvolti *et al.* (1991). Again similar banding pattern (Fig. 1b) was noticed in all the clones except clone A-124 which had two extra bands in zone B (gel phenotype 1). The remaining clones represented gel phenotype 2.

6-PGD exhibited two zones of activity, all the clones showed identical banding pattern except clone A-124 and S7C8 which had three bands in zone A thus having gel phenotype 1 (Fig. 1c). The rest of the clones have phenotype 2.

Two zones of activity were observed in IDH. Rajora (1990) and Rajora and Danick (1992) have reported three loci where as Malvolti *et al.* (1991) have observed two loci. In zone A, clones G3, S7C8, 421-2 and S7C1 had three bands but at different positions thereby indicating different phenotypes (Fig. 1e). Clones A-124, S7C8 and G3, represent gel phenotype 1, 2 and 4, respectively whereas phenotype of clones 421-2 and S7C1 was 5. Rest of the clones represent gel phenotype 3. In zone B, all the clones exhibited three bands except clone S7C8 which has four bands.

Table 2

Gel phenotypes of different clones of Populus deltoides

Clone Name	Gel Phenotype								Group
	MDH	6-PGD	PGI	IDH	LAP	PGM	SKDH	MNR	
11182	2	2	2	3	1	1	1	1	I
G48	2	2	2	3	1	1	1	1	I
A-124	1	1	1	1	1	1	1	1	II
S7C8	2	1	2	2	1	1	1	1	III
109/86	2	2	2	3	1	1	1	1	I
G3	2	2	2	5	1	1	1	1	IV
3324	2	2	2	3	1	1	1	1	I
421-2	2	2	2	4	1	1	1	1	V
S7C1	2	2	2	4	1	1	1	1	V

In LAP, PGM, MNR and SKDH all the clones had gel phenotype 1.

The combined analysis reveals that maximum polymorphism was observed in IDH where three out of 9 clones could be distinguished. For clone A-124, distinct banding pattern was observed in MDH and PGI. In both of these systems rest of the clones had similar banding pattern. Identical banding pattern was observed in clone A-124 and S7C8 in 6-PGD. But both the clone could be differentiated from each other in IDH, PGI and MDH where they had different banding pattern. Clone G3 exhibited distinct banding pattern in IDH. Clone 421-2 and S7C1 could be separated from rest of clones in IDH. Since they exhibited similar banding pattern in other systems hence they could not be distinguished from each other. Only three clones out of 9 could be distinguished. Angundez *et al.* (1999) were able to distinguish 5 out of 14 clones of poplars on the basis of isozyme markers where as Malvolti *et al.* (1991) could identify 10 out of 12 parent trees of *Populus deltoides* by the analysis of four enzyme systems.

The inability to distinguish all clones could be due to limitation in analysing large number of enzyme systems (Sanchez *et al.*, 1998). However, on the basis of present studies the clones could be divided into five distinct groups (Table 2). The clones within a group can be further distinguished with the help of DNA markers like RAPDS, AFLPS etc. (Angundez *et al.*, 1999).

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SUMMARY

Horizontal starch electrophoresis was used to distinguish different Poplar (*Populus deltoides*) clones. The enzymes; Malate dehydrogenase, Phosphoglucose isomerase, Isocitrate dehydrogenase and 6 phospho-gluconate-dehydrogenase were polymorphic. On the basis of isozyme analysis, the clones could be grouped into 5 groups. Three out of nine clones were identified by isozyme patterns.

समविकर विश्लेषण द्वारा पोपलर कृन्तकों को पहचानना

संजीव ठाकुर व जी॰फॉन व्यूलिश

साराशं

पोपलर (पोपुलस डेल्टायडिस) के विभिन्न कृन्तकों को अलग-अलग पहचानने के लिए क्षैतिज मण्ड वैद्युत कण संचलन विधि उपयोग की गई । इसमें मैलेट डिहाइड्रोजेनेस और 6-फास्फोग्लुकोनेट डिहाइड्रोजेनेस बहुरूपिक रहे । समविकर विश्लेषण के आधार पर कृन्तकों को पांच समूहों में वर्गित किया जा सकता है । नौ में से तीन कृन्तकों को समविकर सज्जाओं द्वारा पहचान लिया गया ।

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