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RESEARCH ARTICLE

Isozyme markers for identification of poplar (*Poplus deltoides*) bart clones

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SUMMARY

Isozyme markers for identification of ten prominent poplar clones namely G3, G48, S7C1, S7C4, S7C8, S7C20, L34, PP5, Fierelo and D121. Leaf extract used for polyacrylamide gel electrophoresis. On the basis of banding pattern all the genotype were grouped into four groups. All the genotypes were distinct in banding pattern and their relative mobility except G3 and L34.

Key Words : Isogyme markers, Poplar, Bart clones

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Poplar is the most important agro-forestry tree species in the states of north India. Poplar can greatly contribute towards production of wood for industrial and other commercial purposes, besides maintaining ecological balance and also as an excellent source of income to the farmer. This tree is a fast growing with a clean bole and reaches well over 25m in height and 100 to 130 cm in girth in a rotation period of 8-10 years. Poplar in their natural range occur throughout the forest of temperate regions of northern hemisphere between the southern limit of around latitude 30^0N and northern limits of 45^0N. There are 35 species of poplars currently recognized in the world. In India, poplar are widely planted above 28^0N latitude in Jammu and Kashmir, Punjab, Haryana, Uttar Pradesh, North Bengal and Arunanchal Pradesh along the roads, canals and in agriculture fields, parks, orchards and home gardens. There are six species (*P. ciliata*, *P. laurifolia*, *P. gamblei*, *P. ephratica*, *P. alba* and *P. jaquemontiana var. gluca*) indigenous to India growing along water courses in hills and valleys in the Himalayas.

P. delloides an exotic from North America is well adopted in northern plains of the country. The characteristics like straight clean bole, deciduous nature during winter, multiple uses, high value, soil enriching properties, compatibility with agricultural crops and high economic returns makes poplar versatile and most ideal species for planting on agriculture fields, fruit orchards, cattle sheads, farm roads and in home steads.

Many genetically improved poplar clones have been developed for growing in north-western part of India. Identification of specific clone on visual inspection and/or morphological and phenological description in quite complicated, difficult and time consuming and often created problems. Alternately, enzyme electrophoresis provides simply inherited markers as powerful tools for unambiguous identification of clones and cultivar and varieties of plant species (Adams, 1983; Nielson, 1985). Isozyme polymorphism have been extensively used for population genetic studies in forest trees but rarely for clone and cultivar identification (Cheliak and Pitel, 1984; Rajora, 1989).

MATERIAL AND METHODS

Ten poplar clones namely, G3, G48, S7C1, S7C4, S7C8, S7C20, L34, PP5, Fierelo and D121 were planted in nursery block of Agroforestry Research Centre, Govind Ballabh Pant University of agriculture and technology, Pant Nagar in a randomized block design with three replications. Each replication contained two row of each clone at 80 cm apart

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and each row had ten plants at 60 cm distance.

Leaf sample were collected in polythene bags directly from the field. After removing the petiole and midrib of leaf blades, one gram of leaf for each clone were weighed and homogenised in a pre-chilled mortar and pestal with one ml of chilled extraction buffer and one gram glass beeds. The mortor and pestal were constantly kept on ice during the grinding. Added the enzyme extract into appendorf tube, labelled and placed it on ice because several extract were being made.

Once all extracts of a sample group were ready centrifuged at 14000 rpm for 30 minutes at 4^0C in MB101 refrigerated centrifuge. Carefully blotted off any top layer that formed on top of the supernatant with a small strip of absorbent paper. Later the clear supernatant was carefully decanted into fresh eppendorf tubes. The sample was used immediately and remaining sample were kept at -20^0C in deep freezer for further use.

Preparation of enzyme extract buffer:

Prepared 100ml extraction buffer, pH 7.5 using following receipe described by Rajora and Dancik (1992). Tris based (w/v) 0.1 M, ascorbic acid (W/V) 0.2%, tween 1%, magnesium chloride 0.2%, calcium chloride 0.2%, sucrose 17.1%, 2-mercaptoethanol (v/v) 0.03%. Mixed in minimum volume and then adjusted pH 7.5 with concentrated HCl. After that 0.03 ml-2-mercaptoethanol was added and made up the volume upto 100ml.

Polycrylamide gel electrophoresis (PAGE) :

Apparatus- Two glass plate of size (22x16x0.4 cm) were taken. One of the plates had a U-shaped cut at the top so as to insert the comb. The plates were washed with soap solution, rinsed with distilled water and then wiped with methanol. The plates were dried with tissue paper. The two glass plates were joined parallel with one bottom and two sides spacers (0.1 mm in thickness) fixed in between a thin film of grease was applied on the spacer to prevent leakage of the unpolymerized gel during preparation. The plates were clamped together on the sides and then fixed on the stand by tightening the screws. Gel was poured in slowly. Any bubbles found in the gel were removed by titling the plates or with the help of curved syringe. Immediately, a plastic comb of thirteen teeth was inserted at its position and gel was allowed to polymerised for one hours. Afterwards, comb and lowerside spacer were smoothly stretched out.

The glass plates with the polymerised gel in between was placed upright on the stands in the bottom chamber with well on the top side, and clamped against the vertical plate of the electrophoretic assembly. Then electrode buffers was filled in both the chambers till it dips the gel on the corresponding side, *i.e.* top and bottom.

The upper electrode was the negative one and the lower one was positive. There were connected with the corresponding output in the blue line stabilized power supply.

Preparation of electrode buffer :

Tris 0.025 M, glycine 0.192 M, pH 8.3, dissolved 14.4 g glycine and 3.03 g tris in distilled water to make a final volume of 1000ml pH of the buffer comes nearly to 8.3. Buffer was kept at room temperature during winter.

Preparation of gel – The following reagents were prepared –

Solution A- Distilled water, solution B- 1M tris (pH 8.8), dissolved 12.14g tris (hydroacymethyl) admino methane ($C_4H_{11}NO_3$) in 80 ml of distilled water. Adjusted the pH to 8.8 and made up the volume to 1000ml.

Solution C- Acribis solution, dissolved 30g acrylamide monomer and 0.8 g bisacrylamide in distilled water to a final volume of 1000ml.

Solution D - 50 per cent (w/v) glycerol. Dissolved 50g glycerol in distilled water upto a final volume of 100ml.

Solution E – 50 ml tetra methyl ethylyne diamine used as such. Solution F – Ammonium persulphate 0.25% (w/v), dissolved 0.025 g APS in distilled water upto a final volume of 10ml. It was prepared fresh every time.

All the reagents were kept in the refrigerator and taken out only half an hour before use.

The gel was prepared by adding 3.4 ml of solution A, 12.6 ml of sodium B, 7.8 ml of solutionC (for 7% acrylamide), 2.6 ml of solution D, 50 ml of solution E and 6.6 ml of solution F. The mixture was gently shaken and immediately poured in between the glass plates. The gel took one hours to polymerize. After the gel had set, the comb and bottom spacer were pulled out gently. The glass plates were clamped to the assembly and buffer was filled in the buffer chambers. The bubbles, if any in the well were removed with the help of a syringe. Loading samples and running gel 0.5 ml of prepared samples were taken out in eppendorf tubes. To these tubes, small amount of sucrose (0.05-0.1 g) and a pinch of solid bromophenol blue were added. The tubes were capped and thoroughly shaken to dissolved the sucrose. Sucrose was added to increase the concentration of samples. The dye (bromopheonol blue) rendered deep blue colour to the solution. 30 ml of each samples were loaded under buffer in the well using Hamilton Microsyringe. A current of 15 MA was applied for 20 minutes and then increased upto 30 MA (3MA/sample). It took 4 hours for the dye to enter the lower buffer. The current was allowed till 15 minutes after dye elution. The assembly was then disconnected from the electric source. The plates were taken out and separated with the help of spatula.

Visualization of isoperoxidases:

The staining solution consist of the following reagents, prepared fresh each time :

Table 1 : Paraoxidase isozyme banding pattern in 10 clones of *Populus deltoides*

	Genotypes	Zone-I		Zone-II	Zone III							Total number of bands
		Ia	Ib	IIa	IIIa	IIIb	IIIc	IIId	IIIe	IIIf	IIIg	IIIh	
Group-I	S7C2O	-	-	+	-	-	-	-	-	-	+	-	2
Group-II	G3	+	-	+	-	-	-	-	-	-	+	-	3
	L34	+	-	+	-	-	-	-	-	-	+	-	3
	G48	-	-	+	-	+	-	-	-	+	-	-	3
	S7C4	-	+	+	-	-	-	-	-	-	-	+	3
	S7C8	-	-	+	-	-	-	-	-	-	+	+	3
	PP5	-	-	+	-	-	+	-	-	-	+	-	3
Group-III	S7C1	-	-	+	-	-	-	+	+	-	-	+	4
	D121	+	-	+	-	-	+	-	-	-	+	-	4
Group-IV	Fierelo	-	+	+	+	-	+	-	-	-	+	-	5

– 1% H_2O_2 in distilled water.
– 0.5% benzidine in 10% acetic acid.

Solution (a) and (b) were mixed in equal volumes *i.e.* 50 ml of each just before use and transferred to the glass tray. The gel was put into this stain. Blue bands appeared immediately where peroxidase isoenzymes were localized. The blue bands change to brown after around half an hour.

The gel was then transferred to 7% acetic acid for 3 minutes which ensures fixation of bands in the gel and washed in tap water for 3-4 min. This removes acetic acid as well as the extra strain from the gel. The gel was finally transferred to the tray containing distilled water and visualized on an illuminator. The band then appear quite clear and can be photographed. Zymograms of the isozyme were drawn. Position of the bands were recorded and electrophoretic mobilities calculated.

RESULTS AND DISCUSSION

Result obtained in isozymic studies are presented in Table 1. Results clearly indicate from the table that all the genotypes can be grouped into four groups on the basis of total number of bands. Genotype S7C20 has two band grouped in group 1, six genotypes (G3, G48, L34, S7C4, S7C8 and PP5) having three bands were grouped in group-II, two genotypes S7C1 and D121 having 4 bands were grouped into group-III and group-IV had one clone Fierelo with five bands. Group-II has six genotype with similar number of bands for each but these genotype were distinct from each other in relative mobility of isozymic bands migrated at different rates due to different molecular weight. Genotypes G3 and L34 showed similarity for both the numbers of bands and their relative mobility.

On the basis of relative mobility bands were grouped into three zone. Zone I had two bands 1a(0.315) and Ib (0.33), Zone-II had one band IIa(0.526) while zone III had eight bands IIIa (0.719), IIIb(0.771), IIIc (0.789), IIId (0.807), IIIe (0.842), IIIf(0.859), IIIg (0.877) and IIIh (0.929), band IIa present in all the genotypes followed by band IIIg present in seven genotypes namely G3, S7, C8, S7C20, L34, PP5, Fierelo and D121. Difference in the number of bands and electropheretic mobility of bands of zone I and zone-III, it was clearly established that there were differences between these genotypes in peroxidase isozyme except G3 and L34. Various enzymes studies have been carried in *Populus* successfully to identify the clones (Rajora, 1989; Rajora and Zsuffa, 1989, Malvolt *et al.*, 1991).

REFERENCES

Adams, W.T. (1983). Application of isozyme in tree breeding. In Tanksley, S.D. and Orton, T.J. eds. *Isozyme in plant genetics and breeding*. Part A. Amesterdam. Elsevier Science Publisher, pp. 381-400.

Cheliak, W.M. and Pitel, J.A. (1984). Electrophoretic identification of clones in trembling aspen. *Can. J. Res.*, **14** (5) : 740-743.

Malvolti, M.E., Baccone, A., Fineschi, S. and Paciucci, M. (1991). Biochemical markers in poplar taxonomy. *Monti-e-Boschi*, **41**(1) :49-56.

Nielson, G. (1985). The use of isozymes as probes to identify and label plant varieties and cultivars. In. Rattazzi, M.C., Scandalios, J.C. and Whitt, G.S. eds. Isozymes : *Current topics in biological and medical research*, Vol.2., New York, Alan, R. Liss Inc. pp. 1-32.

Rajora, O.P. (1989). Genetic structure and identification of *Populus deltoids* Mansh. Clones based on allezymes, *Genome*, **32** (3) : 440-448.

Rajora, O.P. and Dancik, B.P. (1992). Allozyme variation and inheritance in leaves of *Populus detoids*, *P. nigra*, *P. maximowiezii and P. Canadensis* in comparison to those in root tips. *Silv. Genet.*, **41** (4/5) : 289-292.

Rajora, O.P. and Zsuffa, L. (1989). Multilocus genetic structure, characterization and relationship of *Populus* x *canadonsis* cultivars. *Genome*, **32** (1) : 99-108.