ISOZYME BANDING PATTERN IN THE HYBRIDS OF *POPULUS CILIATA* x *MAXIMOWICZII*

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Introduction

In forest trees poplars have been extensively used in hybridization programmes. In the genus *Populus*, inter- and intra-specific hybridization, both natural as well as artificial is quite extensive and it makes a geneticist's or taxonomist's work herculean to identify the available clones. Various studies have been conducted to identify the cultivated clones on the basis of morphological characters or with the help of gene markers, but with limited success (Anon., 1979).

Now the clones/species are identified through molecular markers i.e. isozymes (Rajora, 1989) or DNA markers (Bradshaw, 1993). Out of these two methods, isozyme technique is easy and cost effective as compared to DNA technique and has been successfully used for the identification and classification of cultivars, varieties and clones by various authors (Adams, 1983; Rajora and Zsuffa, 1989; Toboloski and Kemery, 1992; Hains, 1994). Moreover the equipment and personnel training is more easily available and accessible in case of isozyme analysis.

Morphological analysis of F_1 progeny of the hybrids between *P. ciliata* and *P. maximowiczii* revealed that the leaf characteristics of the hybrids resembled the *ciliata* parent in its shape. The variation was in the leaf size, margin and the undersurface. The leaf margins of the hybrid was not ciliate and the undersurface of the leaf was whitish instead of being silvery white, which is characteristic feature of *P. ciliata*. At the same time some of the hybrid progeny had distinct rosette pattern of branching which resembled male parent *P. maximowiczii*, while rest of the progeny showed simple alternate branching pattern. The selected clones thus, showed a lot of variation with respect to growth characteristics amongst the F_1 progeny. To characterize them on the basis of molecular markers genetically, five of these selected clones were assayed for isozyme analysis.

Material and Methods

(a) *Tissue collection* : Dormant shoots of parents and selected hybrid plants (CM-3226, CM-3160, CM-3262, CM-3287 and CM-3195) were collected in the month of January, 1994 which were rooted in sand

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and soil mixture in the ratio of 1:1. Fresh leaves were collected in the month of March for further analysis.

(b) *Electrophoresis* : At the time of analysis 150 mg of leaf was crushed in 0.5 ml of phosphate buffer (pH 7.0) containing 20 mg of PVP and fine sand. Paper wick soaked crude extract were loaded on to the starch gel and allowed to pass through it under an electric influx. After the buffer front migrated a total of 7 cm, the gels were sliced and stained following methods of Conkle (1972) for five enzyme systems (Peroxidase, Leucine amino peptidase, Esterase, Catalase and Malate dehydrogenase). The analysis was repeated thrice for each assayed enzyme.

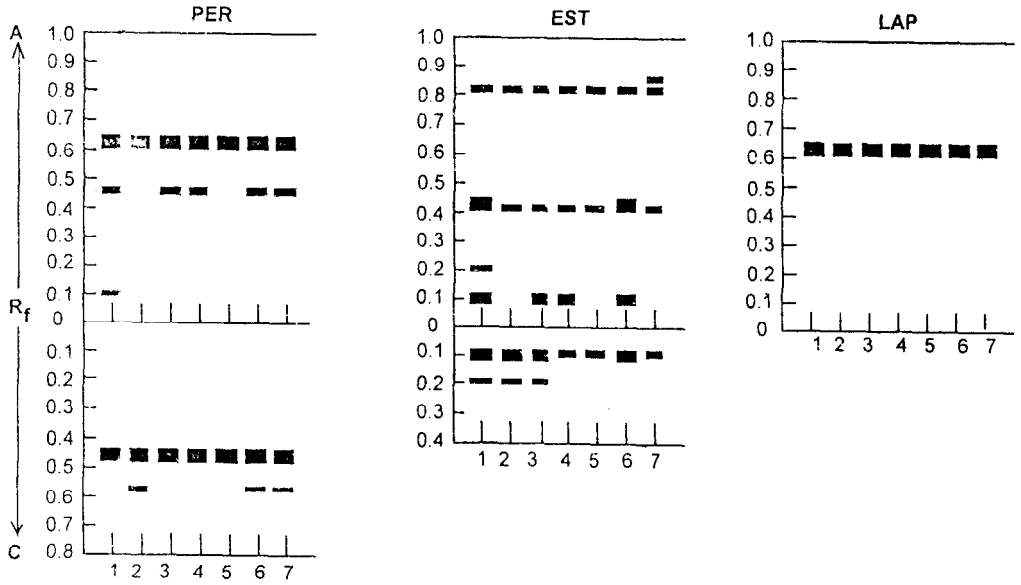
Results

Electrophoretic patterns

Out of the five different enzyme systems studied, three resolved well. However, no identifiable patterns were obtained for MDH and catalase. The distinct phenotypic banding pattern of the other three enzymes namely: PER, LAP, EST which resolved well gave following identifiable bands :

Peroxidase : The peroxidase banding pattern revealed a total of five bands. Three of the bands were common to both the parents i.e. *P. ciliata* and *P. maximowiczii*. The cloned hybrid progeny no. CM-3195

Fig. 1



1 : Populus ciliata, 2 : CM 3226, 3 : CM 3160, 4 : CM 3262 5 : CM 3287, 6 : CM 3195, 7 : P. maximowiczii.

Schematic illustration of phenotypic banding pattern of three enzyme systems in parents and hybrids of the cross *Populus ciliata* x *maximowiczii*

had similar banding pattern as that of the male parent i.e. *P. maximowiczii*. However, none of the clone hybrid progeny plants resembled the female parent in their banding patterns. Clone CM-3287 had only three bands, and therefore, least polymorphic in this clone out of the five tested clones (Fig. 1 PER).

Esterase : The esterase resolved in seven bands with male parent having four bands and the female parent having six bands. Both the parents differed by four bands. None of the cloned hybrid plants had the banding pattern similar to either of the parents (Fig. 1 EST). They showed a banding pattern intermediate to the banding pattern identified to male and female parents.

Leucine amino peptidase : The banding pattern showed LAP to be monomorphic depicting single band in the hybrids as well as the parents (Fig. 1 LAP).

Discussion

It was thus resolved from the phenotypic banding pattern of esterase and peroxidase enzyme that both the male and female parents are different from each other. The clone CM-3195 was more similar to male parent whereas, rest of the hybrid clones were different from parents. Esterases and Peroxidases have been successfully used to characterise different Poplar clones (Castillo and Padro, 1986; Bergmann, 1987) and Red Maple cultivars (Toboloski and Kemery, 1992).

The combined analysis of banding pattern for both the enzyme systems also revealed that clone CM-3195 has contribution from both male and female parents. Clone CM-3262, CM-3160 and CM-3226 had more contribution from female parent, whereas, clone CM-3287 has no contribution from either parent except common isozymes. The clone CM-3160 and CM-3262 had similar banding pattern for peroxidase.

Thus, from the present study, it can be concluded that all the selected five clones from the progeny of *P. ciliata* x *maximowiczii* are hybrids, but majority of them are more close to female parent than male parent.

SUMMARY

Isozyme analysis has been used to identify the hybrid plants of *Populus ciliata* x *maximowiczii*. Out of five enzymes tested, three resolved well in the electrophoresis. Peroxidase and esterase were polymorphic whereas leucine aminopeptidase was monomorphic.

पोपुलस सिलियाटा x मक्सीमोविजीआई संकरों की समविकर पट्टी सज्जा

अरूण कुमार हांडा, संजीव ठाकुर व डी०के० खुराना

साराश

पोपुलस सिलियाटा x मक्सीमोविजीआई संकर पादपों की पहचान करने के लिए समविकर विश्लेषण उपयोग में लाया गया है । परीक्षित किए गए पांच विकरों में से तीन विकर विद्युतकण-संचलन से भली-भांति विलय हो गए । पेरोक्सिडेस और एस्टेरेस पुरुरूपिक पाए गए जबकि ल्यूसाइन एमिनोपेप्टिडेस एकरूपिक पाय गया ।

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