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ISOZYME ANALYSIS OF *POPULUS DELTOIDES* AND A HYBRID POPLAR FOR THE EVALUATION OF GENETIC STABILITY IN THE CULTURE REGENERATED PLANTS

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MITRA, A. & PAL, A. 2000. Isozyme analysis of *Populus deltoides* and a hybrid poplar for the evaluation of genetic stability in the culture regenerated plants. Seventy-seven culture regenerated plantlets of two industrially important accessions of *Populus* species, viz. *P. deltoides* (clone D121) and a hybrid poplar (*P. deltoides* × *P. nigra* cv. 'Chile', clone 65/27) were analysed and compared with the respective donor explants

using isozyme profiles to obtain a better insight into genetic stability of the culture raised plantlets. Thirteen putative isozyme loci of acid phosphatase, diaphorase, phosphoglucose mutase and superoxide dismutase were scored. According to the data, the leaf raised plants of both the clones maintained their clonal fidelity, whereas the plants raised from petiole explants showed genetic variation suggesting leaf explants would be a better choice over petiole explants for micropropagation of *Populus*.

Key words: Genetic stability - hybrid poplar - isozyme - clonal fidelity - *P. deltoides*

MITRA, A. & PAL, A. 2000. Analisis isozim bagi *Populus deltoides* dan poplar hibrid untuk penilaian kestabilan genetik dalam tumbuhan terpulih. Tujuh puluh tujuh kultur anak pokok terpulih bagi dua penyesaran yang penting untuk industri bagi spesies *Populus* viz, *P. deltoides* (klon D121) dan satu poplar hibrid (*P. deltoides* X *P. nigra* cv. 'Chile', klon 65/27) dianalisis dan dibandingkan dengan eksplant donor masing-masing menggunakan profil isozim untuk mencapai penglihatan yang lebih baik untuk kestabilan genetik bagi anak pokok yang dibesarkan dengan kultur. Tiga belas loci isozim bagi asid fosfatase, diaforase, fosfoglukos mutase dan superoksida dismutase diperoleh. Mengikut data, tumbuhan yang dibesarkan daripada daun bagi kedua-dua klon mengekalkan ketepatan klonnya, manakala tumbuhan yang dibesarkan daripada petiol eksplant mempamerkan kepelbagaian genetik. Ini menunjukkan

1983, Lutz *et al.* 1985, Ahuja 1986, Locy 1989, Potter & Jones 1991). Furthermore, clonal identification using morphological and phenological criteria (Ronald *et al.* 1973, Eckenwalder 1977, UPOV 1981) is difficult, time consuming and is often subject to errors. Alternatively, the application of biochemical markers, especially those under Mendellian genetic control, such as allozymes, offers a powerful tool for such studies (Adams 1983, Bailey 1983, Brown & Weir 1983, Nielsen 1985, Rajora 1989a).

Isozyme polymorphism has been detected amongst the wild populations of

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poplars; however, little information is available regarding somaclonal variation in micropropagated poplar using isozyme profile as a biochemical marker. Rajora (1989b), in his elaborate experimental studies, has clearly shown that the clones of *P. deltoides* could be easily identified on the basis of their electrophoretically revealed allozyme genotypes. Isozyme analysis has been used extensively to screen for variations in organogenetically derived plantlets of a number of plant species (Brettell *et al.* 1986, Allichio *et al.* 1987, Wang & Holl 1988, Noh & Minocha 1990, Eastman *et al.* 1991, Heun *et al.* 1994, Charmet & Balfourier 1994).

The genetic uniformity of culture regenerated poplars has not been examined except for a few (Noh & Minocha 1990). The high incidence of mutations in plants regenerated from tissue culture (Larkin & Scowcroft 1983) has prompted us to look more carefully into the effect of *in vitro* treatments on the genetic stability/ instability of the tissue culture regenerated plants of cl. D121 and cl. 65/27.

Materials and methods

Plant materials

One exotic clone of *Populus deltoides* Marshall, cl. D121, and the hybrid clone 65/27 were collected from the WIMCO nursery at Alambazar, Calcutta. D121 is a male clone, selected by the Stoneville Experimental Station, USA, and introduced into India in 1976. This is a fast-growing clone both in girth and height and is highly appreciated for its wood quality and also for the suitability in local climatic conditions (Anonymous 1982).

The clone 65/27 is of Australian origin, derived from the controlled hybridisation between *P. deltoides* and *P. nigra* cv. 'Chile'. The hybrid clone was introduced into India in 1969. This evergreen clone is fast growing and resistant to *Melamspora* (Viart 1982).

These two clones are being maintained in the Experimental Farm of Bose Institute at Madhyamgram, India.

Direct regeneration was obtained from the leaf and petiole explants of *P. deltoides* cl. D121 and cl. 65/27 via adventitious shoot-bud formation, when explants were cultured *in vitro* on AC basal medium (Ahuja 1983) containing 0.5 mg/l BAP (6-benzylaminopurine) and 0.1 mg/l NAA (α-napthalene acetic acid) (Mitra & Pal 1990, Bhattacharya 1994). Leaf tissues of uniform size from the four-month-old *in vitro* grown plantlets were collected from randomly selected regenerants and young leaves and petioles of the similar physiological age were collected from the

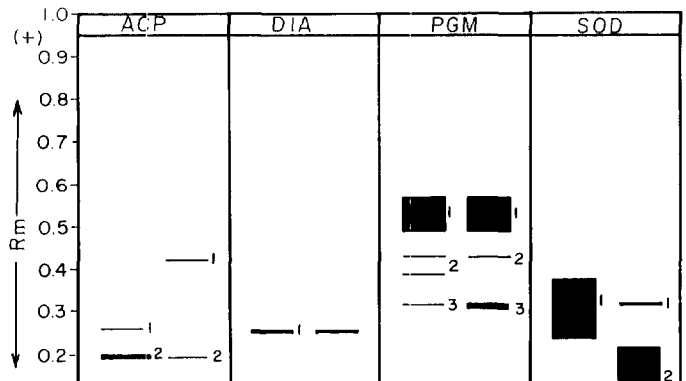
Genotypes of donor clones were determined from their isozyme profile, following the genetic interpretation for loci and alleles of ACP, DIA, PGM and SOD enzyme system as reported by Rajora (1986, 1989b) from the banding patterns in the clones and then verified by Mendellian inheritance studies in controlled crosses.

Electrophoresis for a particular enzyme of an individual regenerated plant was repeated three times and complete accuracy was maintained to score all loci.

During the evaluation of genetic stability, differentially expressed alleles of dimorphic or polymorphic locus were not considered as variant. However, if such changes were accompanied by any other variation of enzyme activity locus either of the same enzyme or in other enzyme system studied, then those regenerants were considered as genetic variants. Appearance of any new activity band/loci induced by *in vitro* methods, possibly due to codon modification, contributing to the genetic make-up of the material was considered as genetic variation. A culture regenerant with more than one variation in enzyme loci was also scored as genetic variant.

Results

Experiments with ACP, DIA, PGM and SOD enzymes from leaf and petiole explants of cl. D121 and cl. 65/27 revealed that all the loci of these four enzymes were uniformly present in leaf and petiole explants of the respective donor clone, whereas difference was noted between the *P. deltoides* cl. D121 and the hybrid clone 65/27 (Figure 1).



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But locus 2 was present only in Pt-R 10 and 11 and locus 4 was present only in Pt-R 5 and 6. A new activity locus was observed in Pt-R 10 and 11 at Rm 0.25, i.e. in between loci 1 and 2. On the other hand, Pt-R 17 exhibited two new zones of activity at Rm 0.54 and 0.64 (Figure 2).

Single DIA locus was uniformly present in all the 77 culture raised plants

Variation in the amount of PGM activity at locus 1 was exhibited by Lf-R 13 and

regenerants were scored as genetic variants (Figure 2, Table 1). Such genetic variations are usually induced under *in vitro* condition and the genetic make-up of the test material is altered even by codon modification that contributes to changes in amino acid sequences.

Polymorphic isozymes are those that exist in the form of one or more alleles and the alleles of protein coding genes are generally codominant, i.e. both the alleles are expressed in heterozygous organisms. The number of polymorphic alleles of isozyme contributes to the quantitative difference rather than any

polymorphic bands in a particular isozyme locus should not be considered as variations, as for example, PGM locus 1 in Lf-R 13, Pt-R 8, 17 and SOD locus 1 of Pt-R 5–10 of cl. D121, and SOD locus 2 of Pt-R 2, 8, 10 of cl. 65/27 (Figure 2). However, if such changes are accompanied by any other variation of enzyme activity locus either of the same enzyme or in other enzyme system studied, they were then scored as variants, e.g. Lf-R 13, Pt-R 4, 8, 10, 17 of clone D121, and Pt-R 2, 5–8, 10, 11, 17 of cl. 65/27 were considered as variants (Table 1). Potter and Jones (1991) also reported similar variants in potato.

Table 1. Culture regenerants (RO) showing variations in isozyme

The interpreted results of isozyme study amongst the 77 regenerated plants discussed above are summarised in Table 1. Leaf regenerated plants (R0) of both the clones showed clonal fidelity within the accepted level, i.e. ≤ 5%.

Establishment of clonal fidelity amongst culture regenerants is imminent for the adaptation of any micropropagation protocol for industrial use, where quality product is desirable. Moreover, poplar being a tree species requires at least 5 y to assess the industrial qualities based on morphological characters. Therefore, the present endeavour was made to evaluate the genetic stability /instability amongst the *in vitro* raised regenerants based on their isozyme profile.

Absence of any major change in isozyme pattern for the leaf-raised R0 regenerants of both the clones tested strongly implies that leaf raised plants of *P. deltoides* cl. D121 and the hybrid poplar cl. 65/27 maintained their clonal fidelity. Appreciably higher numbers, i.e. 20.0 and 38.1%, of variants were scored within the petiole regenerants of cl. D121 and cl. 65/27 respectively. Therefore, we strongly recommend *in vitro* propagation of elite varieties of *P. deltoides* using leaves as primary explants to raise true-to-type regenerants. Nevertheless, production of variants using petioles as explants might be useful for further selection of disease resistance.

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