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Peroxidase isozyme polymorphism in popular sugarcane cultivars

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Peroxidase isozyme was used to identify the variation among popular sugarcane cultivars of South India including the somaclones developed against biotic and abiotic stress tolerance. Electrophoretic polymorphism was surveyed for 12 diverse sugarcane cultivars and 11 somaclones. A total of 145 bands were scored with an average of 6.3 bands per cultivar. Presence of more number of isozymes reflected the genetic complexity of the genus *Saccharum*, a polyploidy of the tribe Andropogoneae. Variation in some of the somaclones was observed, but it was less polymorphic compared to other sugarcane cultivars studied. Peroxidase isozyme diversity among these variants in terms of similarity indices may be useful in identifying diverse cross-combinations for deriving hybrids of sugarcane.

SUGARCANE is an important commercial crop of India. The rapid progress in the sugar-industry sector is largely due to the release of high-yielding, early-maturing and promising cultivars by sugarcane breeders. However, in recent years the productivity has declined due to increase in stress conditions and other environmental factors associated with sugarcane agriculture¹. New genetic approaches like molecular marker technology have been adopted to map the sugarcane genome, in order to select better cross-combinations to develop popular hybrids. Isoenzyme markers are the oldest among the molecular markers. Isoenzyme markers have been successfully used in several crop-improvement programmes^{2–4}. Isoenzymes have proven to be reliable genetic markers in breeding and genetic studies of plant species⁵, due to consistency in their expression, irrespective of environmental factors.

In sugarcane, isozymes were first discussed in 1969 (ref. 6). Earlier studies have revealed variation among the relatives of sugarcane for peroxidases and other isozymes^{3,7,8}. In this communication an attempt has been made to study diversity in peroxidase for understanding the species interrelationship and variation among sugarcane somaclones for their biotic and abiotic stress tolerance, to provide further indications about their genetic relations.

The experimental material consisted of 12 diverse popular sugarcane cultivars of South India and 11 somaclones (GSBT, Gulbarga Selection Through Biotechnology), selected against biotic and abiotic stress^{9–11}. Leaf extracts of young shoots were homogenated in 0.1 N phosphate buffer (pH 7.2) and centrifuged at 12000 rpm for 15 min. The supernatant was subjected to electrophoresis as described by Laemli¹² on a non-denaturing polyacrylamide gel.

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For the detection of peroxidase on gels², staining solution was prepared by dissolving 500 mg of benzidine in 0.5 ml of ethanol, and 5 ml of acetic acid and 95 ml of water were added to it. The contents were mixed thoroughly and filtered through cotton. Then 250 μ l H_2O_2 was added to this just before staining. After electrophoresis, the gel was incubated in the staining solution for a few minutes till the clear bands appeared. The gel was washed with distilled water and photographed. The similarity indices were calculated between the cultivars, according to Nie and Liu¹³.

A phylogram depicting the relationship among the cultivars and somaclones was constructed using the neighbour-joining method of the phylogeny inference programme¹⁴. On staining the gel, blue bands were observed on a colourless background which later turned brown in colour. A total of 12 diverse isozymes were observed. The intensely-stained bands were used for scoring polymorphism.

Modern sugarcane varieties result from inter-specific hybridization and may contain more than 100 chromosomes contributed by up to five different species⁵. The electrophoretic banding patterns consisted of multiple bands of unequal intensities. This is consistent with the high level of polyploidy present in the various groups of sugarcane; a particular locus exists in many copies in the genome and also many alleles can coexist in the same plant and their dosage may be different. This leads to the complications in characterizing the different cultivars due to high number of bands which may migrate at close distances with faint bands, when the corresponding alleles have few copies in the genome. Sugarcane, being highly hetero-

zygous, produces more complicated banding pattern with multimeric condition (heteromeric or hybrid bands). The heteromeric bands stain darker or more intensely and are also present in larger quantities¹⁵. To avoid these consequences, strongly-lit bands were scored for polymorphism.

The zymogram (Figure 1 a) revealed the differences for peroxidases, indicating genetic diversity among sugarcane cultivars. The peroxidase isozymes displayed repeatable variation in migration. The one with faster migration displayed about eight isozymes with varying band intensity and the other with slow migration displayed four isozymes with high band intensity. However, only nine strongly-stained bands were scored for polymorphism. The presence and absence of bands were repeatable. More number of peroxidase isozymes may be attributed to their genetic complexity, as sugarcane shows polyploidy with as many as 80–100 chromosomes ($2n$).

Among eleven somaclones studied, GSBT 4, 5, 7, 8 and 12 displayed similarity with SI ranging from 0.50 to 0.92. The other somaclones were similar and some shared similar banding pattern with their parental clone Co 740. The 12 diverse cultivars displayed polymorphism with SI ranging from 0.50 to 1.00. Some of the sugarcane cultivars could easily be identified with their parents, as evident from the zymogram (Figure 1 a). CoC 671 could easily be identified with its parents Co 775 \times Q 63, which share similar banding pattern. CoC 86032 also shared a few common banding patterns with one of its parental clone, CoC 671. The phylogram placed these clones close to each other, with narrow genetic diversity.

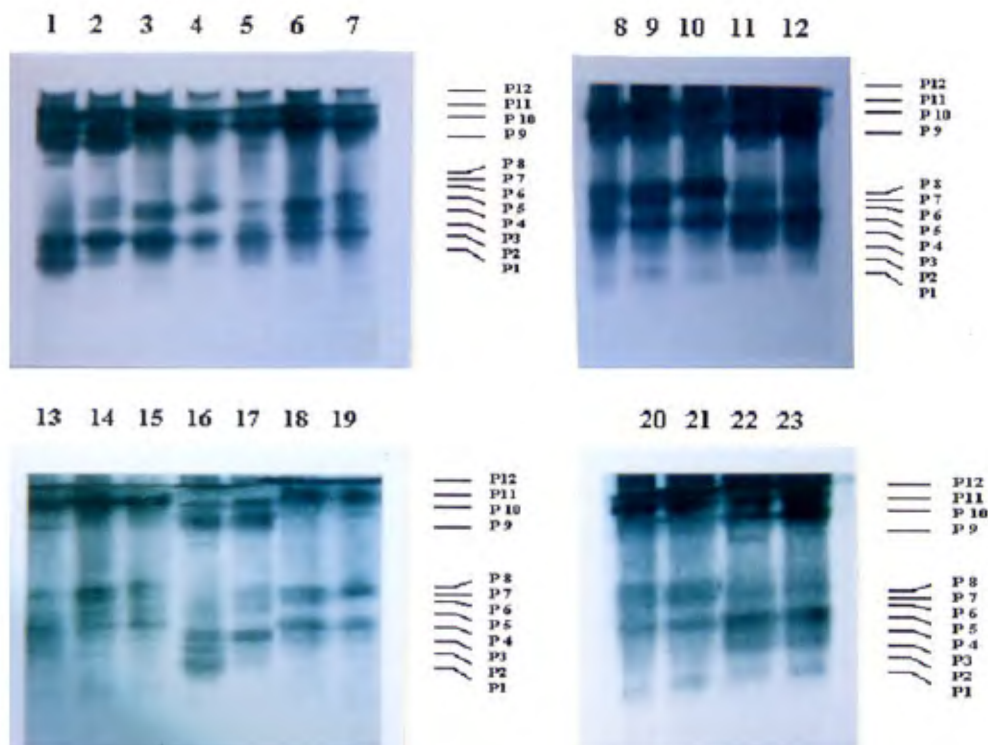


Figure 1 a. Zymogram of peroxidase isozymes in popular sugarcane cultivars.

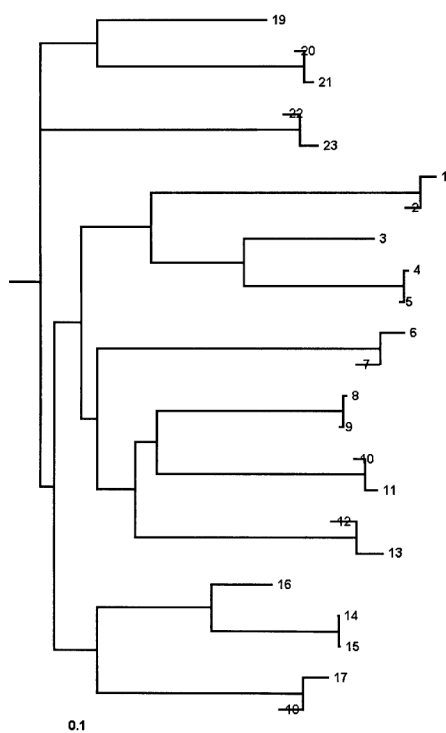


Figure 1 b. Phylogenetic tree constructed using pairwise distance matrix for peroxidase isozymes; 1, Co 419; 2, Co 62175; 3, Co 86032; 4, Co 62198; 5, CoC 671; 6, Co 775; 7, Q 63; 8, GSBT 5; 9, GSBT 7; 10, GSBT 8; 11, GSBT 9; 12, Co 740; 13, Co 95031; 14, Co 85061; 15, Co 8014; 16, Co 8011; 17, GSBT 14; 18, GSBT 15; 19, GSBT 16; 20, GSBT 3; 21, GSBT 4; 22, GSBT 12; 23, GSBT 13.

Low enzymatic polymorphism observed in somaclones indicates a narrow genetic base that may be explained as result of *in vitro* selection through embryonic callus cultures, which normally do not have large genetic differences compared to its parents¹⁶, and might have merely adopted themselves to combat stress conditions. But, there may be mutations at particular loci which gave different banding patterns that diverged when compared to the parental clone. However, thorough studies have to be carried out to understand the molecular basis of somaclones by adopting precise molecular markers. Hemaprabha and Rangaswamy¹⁷ claim 19 isozyme bands per cultivar for peroxidase in sugarcane clones, but we could observe only 12 isozyme bands per cultivar. Out of 12, only nine strongly-reacted isozymes were selected for data analysis. Our results are in concurrence with an earlier report³, where the presence of eight variable bands had been shown in *Saccharum* and its wild species.

The phylogenetic tree (Figure 1 b) revealed a more critical comparison among the commercial hybrid cultivars as well as some somaclones. The phylogram placed few somaclones close to each other depicting their genetic relatedness, since they were developed from a single parent. But some somaclones diverged from their parental clones, which may be due to induced chromosomal aberrations under *in vitro* conditions. The small genetic variation between the somaclones and closely-related species could

be attributed to polyploidy and the highly heterozygous nature of sugarcane.

The enzymatic polymorphism in sugarcane cultivars shows that this gene pool is still a good resource for breeding. Peroxidase isozyme diversity thus has useful utility in sugarcane-breeding programmes for selecting the desirable clones. This can identify cultivar variation which can be used for identifying diverse lines for use as parents in further studies. They can also be used toward a better understanding of phylogenetic relationships of different species. However, it is necessary to use molecular markers like RAPDs, RFLPs, AFLPs and ESTs to map the entire sugarcane genome, which could be used to generate novel cultivars through marker-assisted selection, map-based cloning and transgenesis.

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