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MORPHOLOGICAL, CYTOLOGICAL AND PEROXIDASE ISOZYME STUDIES IN DIPLOIDS, HYBRIDS AND AMPHIDIPLOIDS OF *VIGNA RADIATA* AND *V. MUNGO*

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ABSTRACT

Amphidiploids were developed from F_1 hybrids of *Vigna radiata* and *V. mungo*, which showed better performance for most of morphological traits compared with F_1s but not their parents. Better seed set in amphidiploids seems to have resulted from increase in bivalent formation and increased normal disjunction. The codominant expression of species-specific isoperoxidases was observed in interspecific hybrids and amphidiploids. The isoperoxidases can, therefore, be used as markers for the identification of hybrids and amphidiploids.

Key words: *Vigna radiata, Vigna mungo*, interspecific hybrids, amphidiploids, isoperoxidases.

To recombine the desirable characteristics of *Vigna radiata* (greengram, 2n = 22) and *V. mungo* (blackgram, 2n = 22), two important pulse crops, interspecific hybrids have been developed by different workers [1, 2]. These hybrids were found to be sterile or partially fertile. Therefore, amphidiploidy was induced to get fertile plants [1, 2]. In the present study, hybrids and amphidiploids of these two pulse species were developed, and characterised morphologically and cytologically.

It has been observed that variable expression of morphological markers has limited their use in hybrid identification. The low hybrid seed set and small size of chromosomes of these two species limit the hybrid identification by root tip analysis. Moreover, to raise amphidiploids, hybrid plants have to be identified at an early stage of plant development, for which some reliable marker is needed. Therefore, an effort was made to look for some isozyme marker for characterization of *V. radiata, V. mungo*, their hybrids, amphidiploids and autotetraploids. Isozymes have already been used for identification and characterization of some other plant species and their interspecific hybrids [3–5].

MATERIALS AND METHODS

Two cultivars each of *V. radiata* (ML 131 and SML 32) and *V. mungo* (T 9 and M 1-1) were used to make reciprocal crosses and obtain interspecific hybrids. The 10-15 days old hybrid seedlings were treated with aqueous colchicine solution in different concentrations (0.1, 0.25 and 0.4%). The colchicine solution was applied to the apical meristems of the hybrids and cultivar seedlings for different durations (4–8 h, or 1–3 days) to get amphidiploids and autotetraploids. For chromosome doubling, treatment of the 10-15 days old plants with 0.25% colchicine solution for 6 h daily for three consecutive days was most effective. The hybrids and amphidiploids so developed were analysed for morphological and cytological characteristics.

For meiotic studies, floral buds were fixed in Carnoy's fixative and the anthers were squashed in 2% acetocarmine. For stomatal studies, the lower epidermal layer of leaf was stained in iodine solution.

For isozyme analysis, leaf samples from the 15-day-old parent plants and putative hybrids, amphidiploids and autotetraploids were hand ground in 12% sucrose solution in a prechilled pestle and mortar. The homogenate was stored at 0°C till further use. The samples confirmed to be hybrids, amphidiploids and autotetraploids from chromosome counts and meiotic configurations were used for isozyme analysis. Starch gel electrophoresis method of Smithies [6] was used to study isozymes of peroxidase. The naming of isozymes was done according to the method of Pawar and Gupta [7].

RESULTS AND DISCUSSION

It was possible to make interspecific hybrids ML 131 x T 9 and SML 32 x T 9 using *V. radiata* as female in both crosses.

MORPHOLOGICAL STUDIES

Most of the workers have earlier shown amphidiploids to carry characteristics of both the parents [1, 2, 8]. In the present study, likewise, the seeds were bold but wrinkled, and plants showed indeterminate growth. The seed colour was intermediate. However, Subramanian [8] observed the seed colour of amphidiploids to be closer to *V. mungo*. Most of the morphological characters, viz. plant height, number of primary branches, petiole length, leaflet length and width, number of pods/plant, seeds/plant, pod length, and pollen fertility, increased in both the amphidiploids compared with their respective F_1 hybrids (Table 1). But when compared with the parents, each F_1 hybrid and amphidiploid showed a variable trend, unlike the consistent performance for all the traits observed by earlier workers [1, 2]. The amphidiploids had larger leaves and more pods/plant as compared with the parents. The amphidiploid SML 32 x T 9 showed better performance with respect to

Table 1. Morphological characters of *V. radiata*, *V. mungo* their hybrids and amphidiploids

Material	Plant height (cm)	No. of primary branches	Petiole length (cm)	Leaflet length (cm)	Leaflet width (cm)	Pods per plant	Seeds per plant	Seeds per pod	Pod length (cm)	Stomata per microscopic field	Pollen fertility (%)
ML 131	51.2 ± 1.1	3.2 ± 0.4	10.7 ± 1.0	8.5 ± 0.5	6.8 ± 0.2	45.0 ± 1.6	463.5 ± 2.6	10.3 ± 0.6	6.9 ± 0.3	46.2 ± 0.8	96.5
SML 32	43.8 ± 1.0	2.8 ± 0.2	10.8 ± 0.6	6.6 ± 0.2	4.9 ± 0.4	40.1 ± 0.9	389.0 ± 3.0	9.7 ± 0.6	6.7 ± 0.3	38.4 ± 0.6	95.8
T 9	35.4 ± 0.8	3.1 ± 0.2	7.4 ± 0.5	6.7 ± 0.5	3.5 ± 0.1	25.5 ± 0.8	130.0 ± 4.1	5.1 ± 0.4	3.7 ± 0.3	27.3 ± 0.6	95.1
M 1-1	67.5 ± 2.1	5.8 ± 0.4	7.4 ± 0.6	7.0 ± 0.3	3.9 ± 0.1	35.5 ± 1.1	149.9 ± 2.0	5.8 ± 0.3	4.2 ± 0.1	31.0 ± 0.6	95.6
ML 131 x T 9 (F_1)	30.4 ± 6.2	1.8 ± 0.4	6.9 ± 0.6	7.0 ± 0.3	5.0 ± 0.4	10.0 ± 1.1	31.7 ± 6.1	3.1 ± 0.3	2.8 ± 0.2	34.8 ± 0.4	11.0
ML 131 x T 9 (amphidiploid)	99.0	2.0	12.4 ± 0.8	10.8 ± 0.4	8.0 ± 0.3	66.0	79.0	1.2	3.5 ± 0.4	13.4 ± 0.4	68.6
SML 32 x T 9 (F_1)	23.3 ± 3.5	4.2 ± 0.1	4.8 ± 0.3	6.5 ± 0.8	2.2 ± 0.3	14.0 ± 1.8	21.5 ± 2.9	1.5 ± 0.1	2.7 ± 0.1	27.9 ± 0.7	14.5
SML 32 x T 9 (amphidiploid)	52.5	10.0	6.4 ± 0.8	12.1 ± 0.7	4.2 ± 0.7	56.0	151.0	2.7	4.0 ± 0.5	11.2 ± 0.4	76.3

number of primary branches and seeds/plant, pod length, and seeds/pod as compared with the amphidiploid ML 131 x T 9. The higher seed set in SML 32 x T 9 may be correlated with higher number of PMCs showing 22 II and normal chromosome disjunction as compared with the other amphidiploid (Table 2).

The number of stomata per microscopic field in F_1 hybrids was almost equal to one of the two parents, whereas it decreased considerably in the amphidiploids compared to parents and hybrids. This is in accordance with the earlier reports in various plant species [9, 10], where the reduction in number of stomata per microscopic field was clearly shown to be negatively related to increase in ploidy level.

CYTOLOGICAL STUDIES

Meiosis was normal in the parents with 11 bivalents at diakinesis or metaphase I and equal distribution of chromosomes at anaphase I and anaphase II. However, meiotic irregularities were observed in the F_1 hybrids and amphidiploids (Fig. 1). The average chromosomal configurations at diakinesis/metaphase I and distribution of chromosomes at anaphase I in F_1 and amphidiploids of ML 131 x T 9 and SML 32 x T 9 are shown in Table 2. Various chromosomal configurations like quadrivalents, trivalents, bivalents and univalents were observed in both F_1 and amphidiploids. Normal chromosomal pairing with 11 II was observed in 29–40% PMCs of the hybrids and 22 II in

Table 2. Chromosomal configurations at diakinesis/metaphase I and chromosome distribution at anaphase I in hybrids and amphidiploids of *V. radiata* and *V. mungo*

Material	Frequency per PMC at diakinesis/metaphase I				PMCs with 11/22 IIs (%)	PMCs at anaphase I (%) showing		
	IV	III	II	I		normal distribution	aberrant distribution	laggards
ML 131 x T 9:								
i) F_1	0.40	0.15	8.6	2.75	40.0	41.1	58.9	28.6
ii) Amphidiploid	0.10	0.10	20.8	1.70	55.0	57.1	42.9	14.3
SML 32 x T 9:								
i) F_1	0.29	0.53	8.5	2.30	29.0	28.5	71.4	28.6
ii) Amphidiploid	0.28	0.53	19.4	1.53	66.1	69.2	30.8	15.4

55–66.1% PMCs of the amphidiploids. Similarly, the frequency of PMCs showing aberrant distribution of chromosomes and laggards at anaphase I of amphidiploids was less than their hybrids. Therefore, meiotic irregularities in the amphidiploids were less as compared to the hybrids. Seed set in the amphidiploids was higher than in hybrids. Reduction in meiotic irregularities and increase in fertility in amphidiploids as compared to the hybrids were reported earlier [1, 2].

BIOCHEMICAL STUDIES

Four anodal and one cathodal isoperoxidases were observed in both cultivars of *V. radiata* (Fig. 2a, b). Frequently, an additional cathodal isozyme (C_1) showing very weak intensity was also observed in cv. SML 32. Both the cultivars of *V. mungo* showed five anodal and one cathodal isoperoxidases (Fig. 2a, b). In addition, two faint anodal isozyme bands (A_3, A_4) were also observed in cv. T 9. In *V. mungo*, all these isozymes, except A_9, were different from the bands of *V. radiata*. Thus, most of the isoperoxidases were species-specific and these differences were consistent. Interspecific variations in the isozyme patterns of different genera have been reported by various workers [5, 11]. Most of the cathodal and anodal isozyme bands present in the parents were expressed in the interspecific hybrid ML 131 x T 9 with increased intensity of some isozyme bands (A_1, A_5, A_6). This resulted in nine cathodal and two anodal bands in the hybrids (Fig. 2a, b). However, the isozyme A_7 from *V. radiata* did not express itself, while a new isoperoxidase (A_{10}) appeared. In the hybrid SML 32 x T 9, three isoperoxidases (A_5, A_{11}, C_3) were contributed by cv. T 9 and two (A_6, C_2) by cv. SML 32. Therefore, for quick screening of different hybrids, isoperoxidases A_5 and C_2 for *V. radiata* and A_6 and C_3 from *V. mungo* can be used as reliable genetic markers because of their easy detection and codominant expression in the hybrids. The codominant expression of leaf peroxidases was used by Carlson et al. [12] to identify interspecific hybrids of *Nicotiana*.

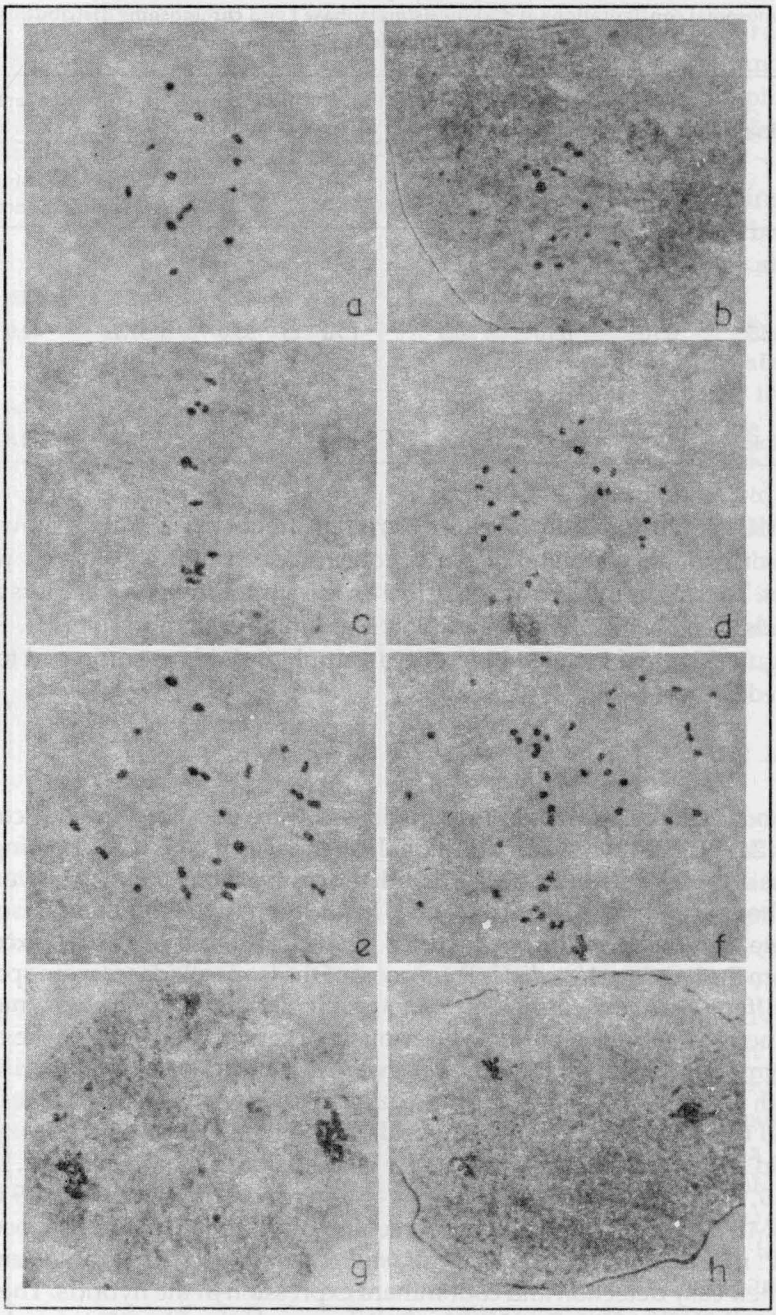

Fig. 1. Meiotic behaviour of interspecific hybrids of *Vigna radiata* x *V. mungo* (a–d) and their amphidiploids (e–h): a) diakinesis, 10II + 2I (ML 131 x T9); b) diakinesis, 1IV x 7II + 4I (SML 32xT9); c) metaphase I, 11II (SML 32 x T9); d) anaphase I, 10 + 12 (SML 32 x T9); e) metaphase I, 3IV + 13II + 6I (ML 131 x T9); f) metaphase – anaphase I, (SML 32 x T9); g) late anaphase I showing laggards (SML 32 x T9); and h) late anaphase II showing tripolar distribution of chromosomes (SML 32 x T9).

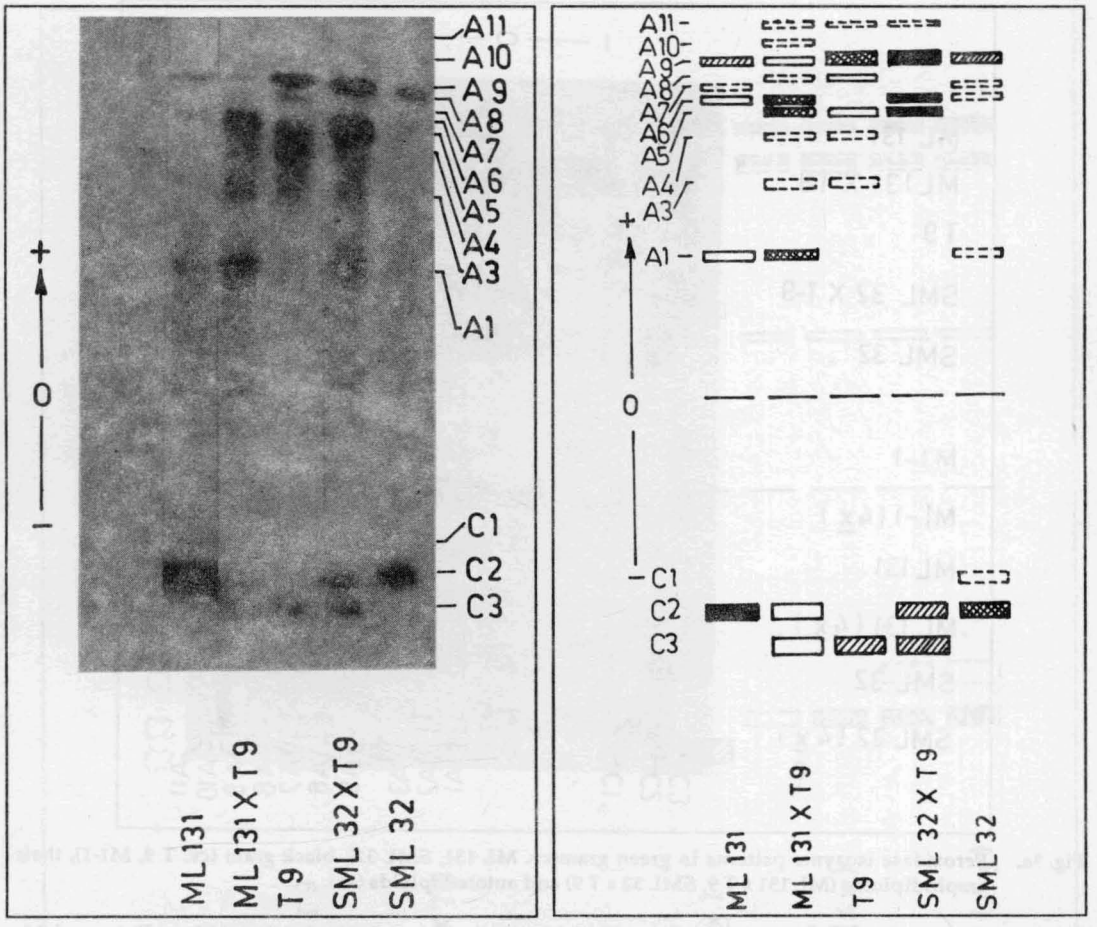

Fig. 2. **Peroxidase isozyme patterns (a) and their zymograms (b) in green gram (cv. ML 131, SML 32), black gram (cv. T 9), and their interspecific hybrids (ML 131 x T9; SML 32 x T 9).**

very light, light, medium, dark, and very dark intensity.

The isozyme pattern in the two amphidiploids (ML 131 x T 9, SML 32 x T 9) was nearly similar to their respective hybrids (Fig. 3a, b). The variations were primarily confined to increase or decrease in intensity of some of the isozyme bands. Prominent among these was the consistent reduction in the intensity of A_6 band in the amphidiploids as compared to the hybrids. This may serve as a marker to distinguish the amphidiploids from the hybrids. The isoperoxidase A_7 present in *V. radiata* did not appear in any of the hybrids or amphidiploids. Like hybrids, the various isozymes of two parents expressed codominantly in their respective amphidiploids. These results are in agreement with the results of isozyme studies of Smith et al. [11] on amphidiploids of different *Nicotiana* species and by Mitra and Bhatia [13] on amphidiploids of triticinae. Therefore, it is evident that the isoperoxidases

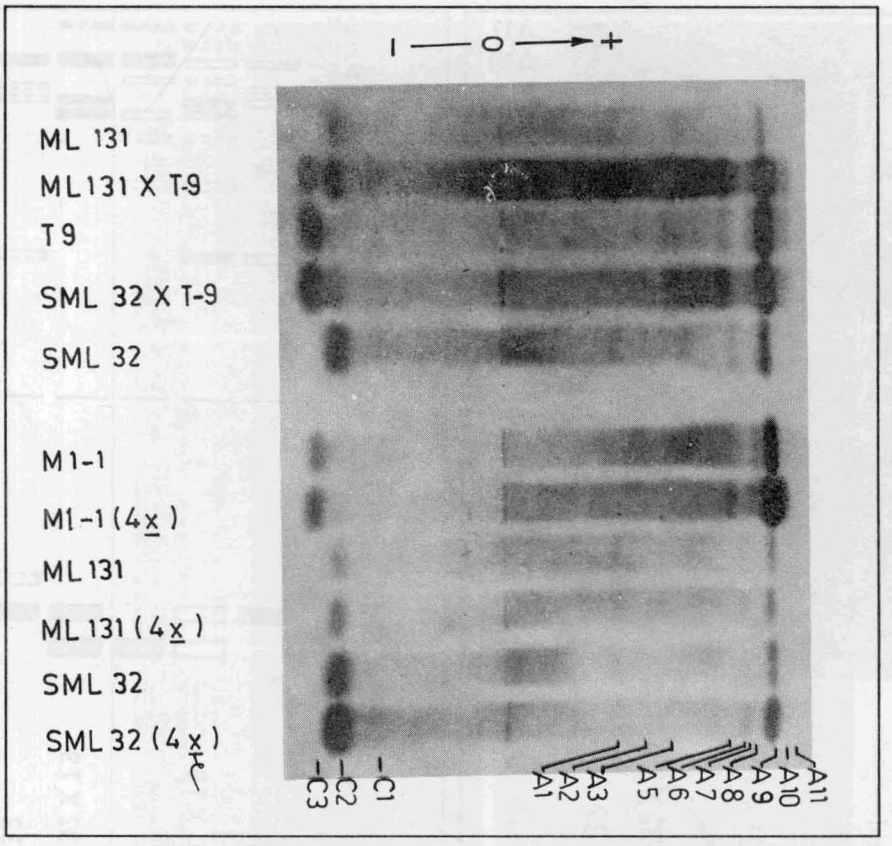

Fig. 3a. **Peroxidase isozyme patterns in green gram(cv. ML 131, SML 32), black gram (cv. T 9, M1-1), their amphidiploids (ML 131 x T 9, SML 32 x T 9) and autotetraploids (4x).**

express codominantly in the interspecific hybrids and amphidiploids of *V. radiata* and *V. mungo*, and can be used as genetic markers.

Autotetraploids of *V. radiata* (cvs. ML 131 and SML 32) and *V. mungo* (cv. M 1-1) also showed isozyme patterns similar to their respective diploids. However, the intensity of most isozyme bands increased in the autotetraploids (Fig. 3a, b). Increase in the intensity of bands may be attributed to increase in the number of structural genes due to doubling of chromosome number. This phenomenon, called gene dosage effect, where specific isozymes show an increased expression with rise in ploidy level, has also been observed by Nakai [14] in rice and Timko et al. [15] in *Ricinus*.

The results show that the amphidiploids are not only more stable meiotically but also show improvement in different desirable morphological characters in comparison to the hybrids. The variable expression of different traits in different amphidiploids, particularly

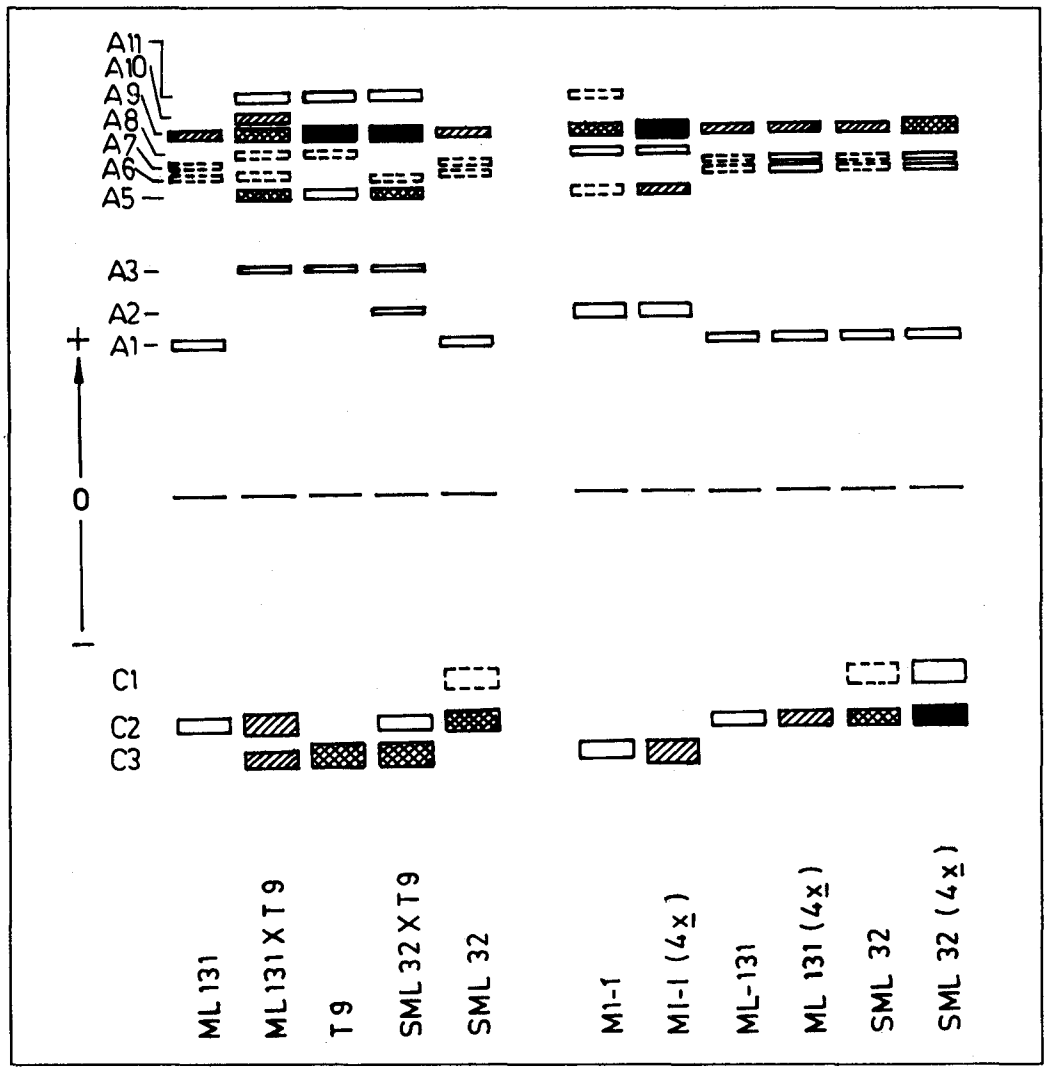

Fig. 3b. Zymogram showing peroxidase isozyme patterns in greengram (cv. ML 131, SML 32), blackgram (cv. T9, MI-1), their amphidiploids (ML 131 x T9, SML 32 x T9) and autotetraploids (4 x).

very light, light, medium, dark, and very dark intensity.

the ones contributing to economic value, indicates that the recombinants having desirable traits can be obtained by producing a large number of amphidiploids using different genotypes of moong and mash. By further selection, these can be improved to get more stable lines.

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