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AN ELECTROPHORETIC STUDY OF SOLUBLE PROTEIN PATTERN, ADH AND MDH ISOENZYME BANDS IN F_1 HYBRIDS *HIBISCUS SABDARIFFA* L. x *H. CANNABINUS* L.

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ABSTRACT

An electrophoretic study of protein pattern, ADH and MDH isoenzyme bands of the seeds of *Hibiscus sabdariffa*, *H. cannabinus*, and F_1 progeny of their cross was carried out to prove the genuineness of the hybrids. The hybrid progeny differed from both male and female parents in respect of the number, length and intensity of the bands of the protein and isoenzymes. The results obtained indicated that the progeny that resulted from the above cross was comprised of genuine F_1 hybrids.

Key words: Interspecific hybrid, electrophoresis, isoenzyme.

Kenaf, a jute substitute, is obtained from two closely allied species of *Hibiscus*, namely, *H. cannabinus* and *H. sabdariffa* of family Malvaceae. Both the species have superiority in some characters over each other. *H. cannabinus* is fast growing with fine, lustrous fibre, but susceptible to root-knot nematodes; the stem wood is too soft to stand strong wind and the stem surface has fine prickles which create trouble in crop handling to the growers. On the other hand, *H. sabdariffa* is slow growing, its fibre is harder, stem surface smooth, and some strains are also resistant to root-knot nematodes. Kenaf breeders have stressed the need to hybridize these two species to evolve improved varieties combining the good qualities of both species, but so far they have not succeeded in achieving this goal. The failure is attributed mainly to the sterility of hybrid progeny and transmission of only a few male characters to the succeeding population. In other words, the F_1 progeny resembles closely the maternal parent raising doubt about the product of the cross being a true hybrid genotype.

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Abbreviations used: INT—3-(4-Iodophenyl) 2-(4-nitrophenyl)-5- phenyl-2H-tetrazolium chloride. PMS — Phenazine methosulphate. NAD — Nicotinamide adenine dinucleotide.

Since genetic differences are reflected in shifts of protein patterns, electrophoretic study of protein patterns is of special interest to the geneticists and plant breeders. Comparative electrophoresis of soluble proteins and isoenzyme analysis have been particularly useful in elucidating systematic relationships in groups where morphological data are inconclusive [1]. It is very precise in the identification of interspecific hybrids [2].

In this context, electrophoretic study of protein pattern and ADH and MDH isoenzymes in F_1 hybrids was undertaken in order to decide whether genuine hybrids are obtained from the cross between *H. sabdariffa* and *H. cannabinus* cultivated in Bangladesh.

MATERIALS AND METHODS

Dry seeds of *H. sabdariffa* cv. S-24, *H. cannabinus* cv. C-2 and their F_1 hybrids were used. Two hundred mg dry seeds of each sample were soaked in distilled water for 24 h and thereafter decoated, and then gently blotted dry. Extraction of the decoated seed sample was done by homogenizing with 1 ml 0.05 M Tris-HCl buffer (pH 7.6) containing 10 mM β-mercaptoethanol and 12.5% sucrose for 5 min in a prechilled mortar. The slurry was centrifuged at 10,000 g for 30 min at 4°C. The supernatant was filtered through Whatman No. 1 filter paper. This preparation was immediately used for polyacrylamide gel electrophoresis.

The protein concentration of crude extracts was determined according to the method of Lowry et al. [3]. Absorbance was measured at 750 nm on a LKB digital spectrophotometer (Model Ultraspec. 4050). Protein concentration in the experimental samples was calculated by using bovine serum albumin as standard.

Protein and isoenzyme separation was carried out by polyacrylamide gel electrophoresis according to the method of Bloemendaal [4]. Power supply and electrophoretic set (Model EC- 454 E.C. Apparatus Corporation, Florida) were used. 0.2M Tris-glycine buffer (pH 8.5) was used as running buffer. Electrophoresis was carried out at 2.5–3.0 mA/gel for 30 min and thereafter 50–60 min at 3–4 mA/gel for satisfactory separation of the bands. A small amount of 0.001% bromophenol blue with 10 µl of 20% bovine serum albumin was used as a marker during electrophoresis. Electrophoresis was done at room temperature under continuous water circulation around the buffer reservoirs. Freshly prepared 0.06% amido-black solution in 7% acetic acid was used for staining the gels for proteins. The gels were stained for 30 min and then destained by repeating rinsing with 7% acetic acid till clear blue bands became visible. Three replications were run for each plant sample.

The colour reagent for visualizing dehydrogenases was prepared by dissolving INT (20 mg), PMS (5 mg) and NAD^+ (20 mg) in 10 ml 0.2 M Tris-glycine buffer (pH 8.5). For incubating ADH isoenzyme, the reaction mixture for each gel consisted of 3.7 ml 0.2 M

Tris-glycine buffer (pH 8.5), 0.1 ml 90% ethanol, 0.3 ml fresh colour reagent. For incubating MDH isoenzyme, the reaction mixture for each gel consisted of 3.7 ml 0.2 M Tris-glycine buffer (pH 8.5) and 0.3 ml fresh colour reagent. The ADH and MDH zymograms were stained in a water bath at 37°C in dark for 15–25 minutes until the bands reached a desired intensity.

The results of electrophoresis are presented and electrophoretic bands numbered according to the recommendations of The Standard Committee of Enzymes [5].

RESULTS AND DISCUSSION

STUDY OF PROTEIN BAND PATTERNS

Fourteen protein bands were observed in the fractionated samples (Fig. 1). The female parent, *H. sabdariffa* cv. S-24, gave 13 and the male parent, *H. cannabinus* cv. C-2 gave 10 bands. Compared to this, the hybrid samples exhibited 11 bands.

A comparison of individual bands showed that band 1 was present in the female parent and absent in the male. This band was faint in the hybrid progeny. Band 4 of the female was divided into two subbands, which was not so in male and hybrid. In addition, the undivided band 4 of the hybrid and the male was darker. Band 5 was absent in the female but was

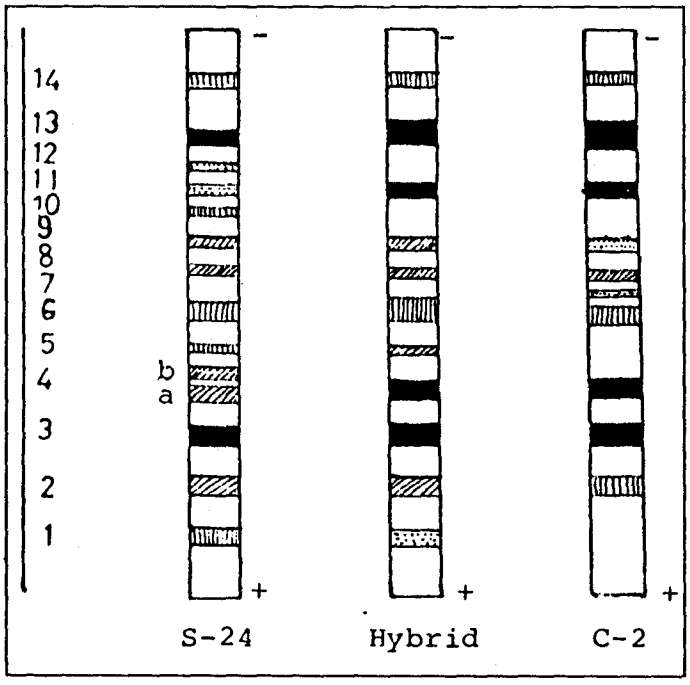

Fig. 1. **Electrophoregram showing comparative protein band patterns in *H. sabdariffa* cv. S-24 (left), *H. cannabinus* cv. C-2 (right), and their F_1 hybrid (middle), x1.0.**

darker in the hybrid as compared to the female parent. A faint band (No. 7) characterizing the male was absent in both the hybrid and female. Band 10 was faint in male and light in female, but absent in the hybrid. This is not unusual in the protein patterns of interspecific hybrids [6]. Band 11 was dark in the hybrid and male but light dark in the female. Band 12 was absent in both male and hybrid but present in the female parent. When the thickness of the bands was compared, bands 6 and 13 were thinner in the female parent as compared to those of the hybrid and the male. The banding patterns combining the main protein bands of the two parents have been reported in interspecific hybrids of *Gossypium* [7].

Homogeneity between the female parent and the hybrid was 69.2%, but 75% between the hybrid and the male. Two parents showed 54.3% homogeneity in respect of protein bands which was less than between the hybrids and parents. The overall analysis of protein bands in the parents and their crossed product confirms that the product of the cross *H. sabdariffa* x *H. cannabinus* was a genuine hybrid.

STUDY OF ADH ISOENZYMES

The study of ADH isoenzymes showed that the zymogram had total 4 bands (Fig. 2). The female and male parents had 3 and 2 isoenzyme bands, respectively. Unlike the male parent, band 1 was absent in the female parent. Bands 3 and 4 were absent in the male.

All the hybrid derivatives shared three bands (2, 3 and 4) with the female. However, band 2 was thicker in the hybrids and its intensity of staining was similar to that of the male parent. On a close look, the zymogram of the hybrid appeared quite distinct from the female parent because of difference in thickness and intensity of colour of the bands although the number of bands was the same in both.

STUDY OF MDH ISOENZYMES

The present investigation analysed the homology of MDH isoenzyme bands in the seed samples of *H. sabdariffa*, *H. cannabinus*, and the product of their cross (Fig 3). Five bands of MDH isoenzymes were observed in the zymogram. These bands had variable intensity. In *H. sabdariffa*, all the five bands were present. In addition, band 1 was divided into two subbands, the subband 1a being thicker than 1b. On the other hand, *H. cannabinus* had only four bands, band 5 being absent in this species. The hybrids exhibited all the 5 bands characteristic of the maternal parent. However, band 1 was undivided like the male parent. The band was also thinner than in the two parents. In addition, the intensity of band 5, missing in the male, was half of the band of *H. sabdariffa*. Since the male parent contributed no genes responsible for this product, the band intensity was less in the F_1. Thus, the isoenzyme studies provided an additional proof that the product of the cross *H. sabdariffa* x *H. cannabinus* was a true hybrid.

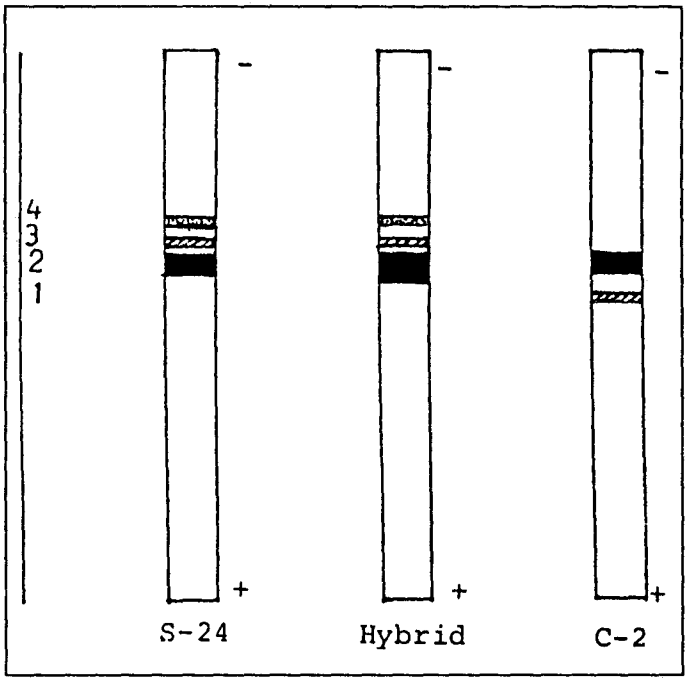

Fig. 2. Zymogram showing comparative band patterns of ADH isoenzymes in S-24 (left), C-2 (right), and their F_1 hybrid (middle), x1.0.

Dark Light dark Faint

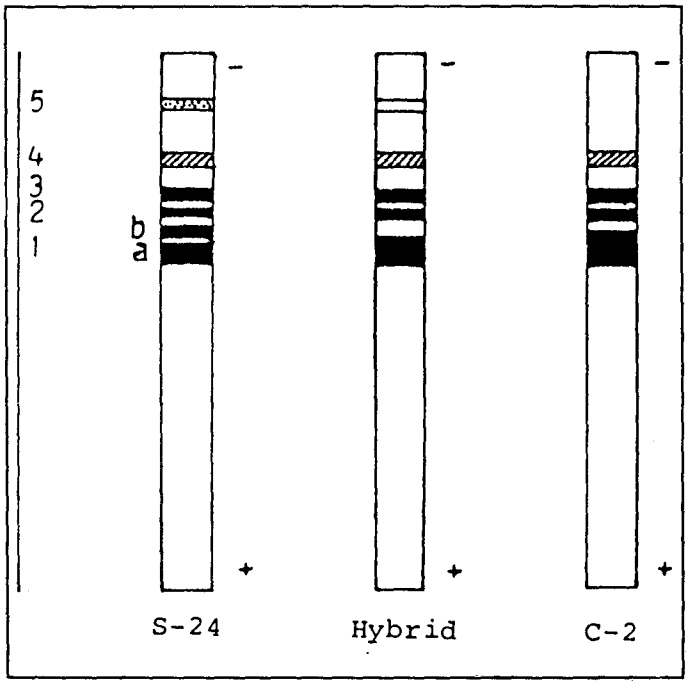

Fig. 3. Zymogram showing comparative band patterns of MDH isoenzymes in S-24 (left), C-2 (right), and their hybrid (middle), x1.0.

Dark Light Light dark Faint

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