CHAPTER IV

BIOCHEMICAL STUDIES

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INTRODUCTION

Most of the varieties bear minor differences among themselves i.e. little differences make basis for new varieties; besides this, sometimes these differences are qualitative such as resistance to certain pathogens or environmental conditions etc. The classical taxonomic methods in such cases fail to cope up, therefore it was felt necessary to develop certain more precise markers.

Use of electrophoresis is well-established in the field of varietal identification. It owns tremendous success because of its independence on polymorphism of seed and seedling proteins and the fact that proteins are largely unaffected by environmental factors and are direct descendents of genome; make them more useful criteria for varietal identification.

Electrophoresis methods are also generally relatively easy to carry out and provide rapid analysis. The Electrophoresis is quite useful in varietal identification. It has got great potentials and the criterion for distinctness between varieties is taken as the presence or absence of a particular protein band or set of bands occurring at a defined position on the gel or as differences in the frequency of occurrence of protein-types. One may use proteins encoded at multiple loci (e.g. seed storage proteins) or at a single locus (isozymes or allozymes). The approach to decipher varieties depends on mode of reproduction in variety.

Because of the differences in genetic constitution of genotypes or parents and hybrids, biochemical methods can be the best judge for their correct and accurate identification. Enzymatic assessment is generally considered to be the most fruitful one in the biochemical methods as they are concerned with genome or make a direct product of genetic constitution. With this aim, castor, cotton hybrids and parents were subjected to biochemical estimation in 48 and 72 hr old seedlings and soluble seed proteins, as well as isozyme analysis which was done through electrophoresis.
MATERIALS AND METHODS

BIOCHEMICAL ESTIMATIONS:

Seeds of hybrids and parents of castor and cotton were germinated for 48 and 72 hrs in rolled filter paper towels and following enzymes were assayed in the total seedling:

1. Enzyme protein
2. Peroxidase
3. Polyphenol oxidase
4. IAA oxidase
5. Amylase
6. Protease
7. Invertase

PREPARATION OF ENZYME HOMOGENATE: 100 mg of seedling was weighed and homogenised in 5 ml of chilled acetone. It was left overnight in the refrigerator and centrifuged at 10,000 g for 18 min. The supernatant was discarded and the pellet was suspended in 5 ml of phosphate buffer (0.02 M, pH 6.4), recentrifuged at 10,000 g for 15 min. and the supernatant was collected. The resulting pellet was reextracted in another 5 ml of phosphate buffer, centrifuged and the supernatant so obtained was pulled with the previous one (final volume was made to 10 ml). From this homogenate various enzymes were analysed.

ENZYME (SOLUBLE) PROTEINS: (Lowry et al., 1951)

The enzyme protein was estimated for the calculation of enzyme activities on the basis of mg protein (specific activity).

1 ml aliquot was mixed with 5 ml of alkaline copper reagent [freshly prepared by mixing 50 ml of reagent A (2% Na₂CO₃ in 0.1N NaOH) + Reagent B (0.5% CuSO₄ in 1% sodium- potassium tartarate, prepared freshly by mixing stock solutions)]. After 30 min. 0.5 ml of 1N of folin ciocalteau reagent was added. The O.D. (Y) was read at 600 nm within, between 9th and 10th min. The amount of soluble protein (X µg) present in the seedling was obtained using the regression formula : X = 655.8Y - 9.9 and protein content was expressed as mg protein.
**PEROXIDASE:** (George, 1953) Peroxidase activity was determined by using guaiacol as an hydrogen donor, and hydrogen peroxide as a substrate. A 1 ml aliquot was mixed with 1 ml of phosphate buffer (0.2M, pH 6.4) and 1 ml of guaiacol (20 mM, i.e., 0.22 ml guaiacol diluted to 100 ml with distilled water). The optical density (O.D.) was recorded at 420 nm. 0.02 ml of $\text{H}_2\text{O}_2$ (20 vol) was added and incubated at room temperature for 1 min. O.D. was again recorded. Difference in the O.D. of the reaction and blank was calculated and the specific enzyme activity was expressed as $\Delta$ O.D./min/mg protein.

**POLYPHENOL OXIDASE:** (Mayer and Harel, 1979) Polyphenol oxidase, also referred to as phenolase, catechol oxidase, tyrosinase or catecholase, was assayed following the spectrophotometric method. In order to track the initial rate of the formation of quinone, 1 ml aliquot from the homogenate was mixed with 1 ml of phosphate buffer (0.2M, pH 6.4) and 0.5 ml of L-proline (5 mg/ml). The O.D. was recorded at 525 nm and 0.5 ml of catechol (0.01M) was added and incubated at room-temperature for 2 min. The O.D. was again recorded. A difference between the reaction and blank was calculated and the specific enzyme-activity was expressed as OD/3 min/mg protein.

**IAA OXIDASE ACTIVITY:** (Mahadevan, 1964) Residual IAA was determined with salkowski reagent. 1 ml of enzyme extract was mixed with 1 ml IAA (10 mg/100 ml) containing 0.5 mM MnCl$_2$ (9.89 mg MnCl$_2$ in 100 ml distilled water); 0.25 ml DCP (Dichlorophenol, 0.1 mM) and 3.5 ml phosphate buffer (0.05M, pH 6.5) kept for 1 hr. Reaction was terminated by mixing 2 ml reaction mixture with 2 ml Salkowski reagent (1 ml 0.5M FeCl$_3$ + 50 ml of 35% perchloric acid). This was incubated for 30 min in dark. Control was prepared similarly except that it had an inactivated enzyme. Another blank had 1 ml of distilled water instead of enzyme. O.D. was measured at 530 nm against blank and differences were worked out by referring to standard curve prepared with known amount of IAA. IAA activity was expressed as mg/IAA destroyed/hr/mg protein.

**AMYLASE:** (Paleg, 1962) A 1 ml of aliquot was mixed with 0.1% starch and 1 ml citrate buffer (pH 5.0, 0.025 M). Reaction mixture was incubated at room temperature ($30^\circ$C ± $2^\circ$C) for 30 min. Finally, 1 ml $\text{I}_2\text{KI}$ reagent (200 mg iodine dissolved in 2% potassium iodide in 0.5N con. HCl) was added. Tubes were thoroughly shaked and total volume was made upto 20 ml with distilled
water. O.D. was recorded at 600 nm and enzyme activity was expressed as mg starch degraded/30 min./mg protein.

**PROTEASE:** (Penner and Ashton, 1967 and as modified by Cruz *et al.*, 1970) 1 ml of enzyme aliquote was added to 3 ml of phosphate buffer (0.2M, pH 7.0) and 2 ml 0.5% casein solution (pH 7.0). Reaction mixture was incubated at 30°C for 1h, and was terminated by adding 2 ml of 15% TCA to 2 ml of the reaction mixture. After 20 min., the tubes were centrifuged and supernatant was used to determine the amount of tyrosine liberated using folin-ciocalteau reagent of Lowery *et al.* (1951). 1 ml of supernatant was mixed with 1 ml of distilled water and 4 ml of 0.5N NaOH. To this was added 1.2 ml of 1N folin-ciocalteau reagent and allowed to stand for 30 min. till the blue colour developed correctly. The O.D. was recorded at 600 nm. The activity was expressed in terms of mg tyrosine liberated/h/mg protein.

**INVERTASE:** (Hatch and Glasziou, 1963) 1 ml of aliquote was mixed with 0.1M sucrose in citrate buffer (0.1M, pH 5.4), and 1 ml citrate buffer. Reaction mixture was incubated at room-temperature for 60 min.; 2 ml of hot absolute alcohol was added for the reaction-mixture. The reaction mixture was further supplemented with 2 ml of 5% aq. sodium sulphate. The reaction mixture was incubated at 100°C to evaporate alcohol. The mixture was cooled and total volume was made upto 5 ml with distilled water. The mixture was centrifuged and supernatant was retained for enzyme activity using Nelson’s method (Wharton & MacCardy, 1972). 1 ml of supernatant mixed with 1 ml of Nelson’s reagent and reaction mixture was incubated at 100°C for 20 min. Tubes were cooled and 1 ml of arsenomolybdate reagent was added to it. Final volume of molybdenum blue was made upto 20 ml with distilled water. O.D. was recorded at 540 nm. Invertase activity was expressed as mg glucose liberated/20 min/mg/protein.

**ELECTROPHORETIC STUDY:**

**EXPERIMENTAL METHODS:**

The present investigation involved following studies:

I. Study of total soluble-seed proteins by vertical gel electrophoresis. The study was made on vertical gel following the sodium dodecyl sulfate-polyacrylamide gel electrophoresis using 14% gel (SDS-PAGE)
suggested by (Ashwani and Dwivedi, 1991).

II. Study of isozymes, viz. amyloses (AMY), polyphenol oxidases (PPO); peroxidases (PER); esterases (EST); acid phosphatases (ACP); alcohol dehydrogenases (ADH); malate dehydrogenases (MDH).
This study was made by SDS polyacrylamide vertical electrophoresis using 10% gel.

I. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins.

II. Vertical slab gel electrophoresis apparatus:
The apparatus consists of two buffer reservoirs, upper and lower. The upper one is notched, supported on an integral perspex stand. The gel is formed between two glass plates each about 0.4 cm thick. One plate is rectangular-shaped (17.0 x 24.5 cm) and the other is of the same size but with a notch 3.5 cm deep and 11.5 cm long cut in one of the 17.0 cm edges. The two plates are placed together with a perspex spacer (24.0 cm long, 1.0 cm wide and 0.15 cm thick) running down each vertical side of the sandwich so as to form space for gel. Sample wells are formed in the gel during polymerisation using a perspex sample comb. Details of slab gel preparations are described later in this chapter. The apparatus along-with the power supply unit (0 - 300V and 0-50 mA) were supplied by M/s.Scientific and Electronics Ltd., Bombay.

II. Preparation of the sample for soluble proteins: (Agarwal et al., 1988)
Cotton and castor hybrids and parent seeds were decoated and single seeds were crushed using chilled pestle-mortar in chilled acetone. The crushed seeds were defatted with three changes of acetone and one change of hexane over a period of 6-8 hrs and seed powder was extracted with 0.3 ml of 0.1M tris-glycine buffer (pH 7.5) containing 8 mM 2-mercapto ethanol. The homogenate was centrigued at 12000 g for 20 min at 4°C. The supernatant was either used immediately or stored at 0°C for maximum of 120 hrs.
III. Determination of proteins in seed sample: The protein content in the sample extract was determined by comparing the reading on standard curve.

An appropriate volume of the protein extract was diluted with the sample buffer (containing SDS, mercapto ethanol, glycerol and tracking dye) in such a way that the diluted sample contained about 300 µg of protein. The diluted sample was incubated in a hot-water bath (95°C) for 3 to 4 minutes followed by cooling at room-temperature. The samples were then ready for loading.

IV. Preparation of solutions:

**Stock Solution:**

1. **Extraction buffer**: (0.1M Tris-HCl, pH 7.5).

   1.21 g of Tris was dissolved in about 50 ml of water, to which dilute HCl (1:1) was added drop-by-drop to bring the pH to 7.5. To this, 0.08 ml of 2-mercapto ethanol was added and then the volume was made upto 100 ml by maintaining the pH at 7.5. The solution was stored in a dark bottle at 4°C.

2. **Sample buffer**:

   The sample buffer used to dilute and incubate the protein extract was prepared by mixing the following:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>4.00 ml</td>
</tr>
<tr>
<td>0.5M Tris HCl, pH 6.8</td>
<td>1.00 ml</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.80 ml</td>
</tr>
<tr>
<td>10% SDS (w/v)</td>
<td>1.60 ml</td>
</tr>
<tr>
<td>2-Mercapto ethanol</td>
<td>0.40 ml</td>
</tr>
<tr>
<td>0.05% Bromophenol blue (w/v)</td>
<td>0.20 ml</td>
</tr>
<tr>
<td></td>
<td>8.00 ml</td>
</tr>
</tbody>
</table>

3. **30% polyacrylamide solution**:

   29 gm of acrylamide and 1 gm of bisacrylamide dissolved in distilled...
water and made up to 100 ml and filter.

4. **1M Tris-Cl (pH 8.8):**
   12.1 gm of Tris base is dissolved in 80 ml of distilled water, pH adjusted to 8.8 with 1N-HCl and volume made upto 100 ml.

5. **1M Tris-Cl (pH 7.4):**
   12.1 gm of Tris base is dissolved in 50 ml of distilled water, pH adjusted to 7.5 with 1N HCl and volume made upto 100 ml.

6. **1M Tris-Cl (pH 6.8):**
   12.1 gm of Tris base is dissolved in 50 ml of distilled water, pH adjusted to 6.8 with 1N HCl and volume made upto 100 ml.

7. **10% Ammonium persulfate (APS):**
   0.5 gm of APS dissolved in 5 ml of distilled water. It was prepared just before use.

8. **20% Sodium dodecyl sulfate (SDS):**
   10 gm of SDS was mixed with 30 ml of distilled water and kept overnight. Next day, the volume was made up to 50 ml; slight heating was necessary to obtain clear solution, which was stable at room-temperature but precipitated in cold.

9. **N’N’N’N’ Tetramethylethylenediamine (TEMED):**
   All the solutions were stored at 4°C in dark.

10. **Electrophoresis buffer:**
    3.0 gm of Tris base, 18.8 gm of glycine and 1 gm SDS were dissolved in distilled water to make the final volume to 1000 ml.

11. **Staining solution:**
    Staining solution was prepared by dissolving 1.25 g coomassie brilliant blue R250 in 454 ml of 50% methanol and 46 ml of glacial acetic acid. The solution was filtered through Whatman no.1 filter paper.

12. **Destaining solution:**
    The solution used for removal of excess stain on the gel was prepared by mixing methanol, glacial acetic acid and water in the ratio of 3:1:6.
13. Solution for SDS-PAGE:

Plug gel:

- 30% Acrylamide solution: 4 ml
- H₂O: 8 ml
- APS: 0.1 ml
- TEMED: 0.1 ml

Total: 12 ml

Stacking gel:

- 10 ml of 5% gel volume
- 10% Acrylamide soln.: 1.7 ml
- H₂O: 6.8 ml
- 1M Tris HCl (6.8 pH): 1.25 ml
- 10% SDS: 0.05 ml
- 10% APS: 0.1 ml
- TEMED: 0.01 ml

Main gel:

- 30% Acrylamide solution: 10 ml, 14 ml
- H₂O: 8 ml, 4 ml
- 1M Tris Cl (8.8): 11.5 ml, 11.5 ml
- 20% SDS: 0.15 ml, 0.15 ml
- 10% APS: 0.3 ml, 0.3 ml
- TEMED: 0.02 ml, 0.02 ml

Total: 30 ml, 30 ml
V. **GEL PREPARATION:** The contents of 14% acrylamide concentration for the SDS-phosphate buffer system had been mentioned. TEMED was added just before pouring the gel mixture in-between glass plates.

A slab gel of 16.0 cm x 12.0 cm x 1.5 mm was prepared as follows:

Cleared glass plates were held apart at the correct distance by thin (1.5 mm) perspex spacers. The sealing of the glass-plate sandwich was done by placing rubber tubing on the 3 sides of glass plates, i.e. along-with the spacers. The clamped plate assembly was held vertically during pouring of the gel mixture by fixing to a vertical stand by metal-spring clips.

The gel mixture was prepared by adding the correct volume of all components. The contents were gently mixed and the gel solution was poured immediately between the glass plates by a wide-bore syringe upto 0.5 cm distance from the top. Immediately, a perspex comb was inserted between the glass plates and into the gel mixture. The teeth of the comb were smoothly between the glass plates. Special care was taken to ensure that air bubbles were not trapped beneath the comb. The assembly was left undisturbed for the gel to polymerise (30 to 40 min).

After polymerisation, the comb was carefully and slowly removed; the rubber tubing was also removed. The sample wells were cleaned and rinsed with reservoir buffer and the gel assembly was clamped on the electrophoresis-apparatus using metal clips. Reservoir buffer was added to the lower reservoir of the apparatus and air-bubbles, if any, were removed from bottom of the gel by slowly tilting the slab gel apparatus.

V. **SAMPLE LOADING:** Sample buffer containing about 200 µg of protein in µl solution was carefully loaded in the sample-well, using a micropipette. A total of 6 samples were loaded in the different sample wells of the gel. The remaining space in the sample-wells was slowly filled with the reservoir buffer. Then the upper reservoir was filled with the reservoir buffer.

**ELECTROPHORESIS:** The electrophoresis-apparatus was placed in a refrigerator and was connected to the power-pack with anode (+) connected to the lower reservoir and the cathode (-) to the upper reservoir. The power-pack was then connected to the mains, switched on, and adjusted to deliver 30 mA constant current until samples entered the gel. Then a constant current of 60 mA was applied until the tracking dye reached the bottom of the gel which
took about four hrs. After run, the power-supply was cut off.

VII. **RECOVERY OF GELS:** Slab gels were recovered by gently levering the glass plates apart, at the end, from the notch (to avoid damage to the fragile notched end). The gel resting on the unnotched glass plate was carefully slid into a glass tray by using a plastic spatula. The gel was first washed with distilled water, then marks were made on the gel to locate the origin as well as position of tracking dye.

VIII. **PROTEIN STAINING:** Gel-slab was placed in a glass tray containing about 500 ml of the staining solution and left overnight at room-temperature. After the staining was complete, the solution was collected for reuse. The gel was washed twice with distilled water. Excess stain was removed using the destaining solution. Gels were immersed in destaining solution in a tray which was frequently agitated. Destaining solution was changed in every 2 to 3 hours until the protein-bands were visible on clear background which took about 10 to 12 hrs. The gels were stored in trays containing a solution of 7% glacial acetic acid.

2. **SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS OF ISOZYMES:**

   I. Vertical slab-gel electrophoresis-apparatus, preparation of solutions and gel etc. remain the same as described for seed protein electrophoresis.

   II. **EXTRACTION OF ENZYMES:**

   Seeds were surface sterilised and germinated for 72 hrs on moist filter-paper towels. The enzymes were extracted from these seedlings by macerating decorticated single seeds with 1 ml of extraction buffer (0.1M Tris-HCl, pH 7.5) in a prechilled pestle-and-mortar in an ice bucket. The paste was transferred to a prechilled centrifugation tube.

   The contents were centrifuged in a refrigerated centrifuge at 15000 rpm for 30 minutes at 0-4°C. The supernatant was transferred to prechilled glass vials stored in a deep freeze at -10°C. In the seed extracts, proteins were estimated by following the method of Lowry *et al.* (1951) which has been described earlier, in the methods of this chapter, Preparation of gel. Loading of the sample and recovery of gel has also been described earlier in this chapter.

   III. **STAINING OF GELS:** Different gels were incubated for different
enzyme-activity staining systems as described below:

1. **AMYLASES**: (modified method of Brewer (1970)) The gels were incubated in 1% starch (soluble) solution prepared in citrate buffer (0.025 M, pH 5.4) for 1h at 25°C, stained with iodine solution (3 mM I$_2$ + 6 mM KI) for 30 min and washed with distilled water. Areas of enzyme activity appeared as clear zones against a black (dark) background.

2. **POLYPHENOL OXIDASES**: (Modified method of Shant et al., 1974) The gels were incubated with DL-DOPA solution (1.5 mg/ml, prepared in 80% alcohol) for 1 hr at 37°C in dark. Few bands with light-brown coloration were obtained indicating the enzyme-activities.

3. **PEROXIDASES**: (Hislop and Stahmann, 1971) The gels after the electrophoresis were incubated with the reaction mixture (70 ml of 20 mM guaiacol + 25 ml of 0.25M, pH 6.4 phosphate buffer + 20 Vol. H$_2$O$_2$) for 10 min. at the room temperature. The brown bands were obtained at the site of enzyme-activity.

4. **ESTERASES**: (Shaw and Prasad, 1970) Gels were incubated in 0.2M tris-HCl buffer pH 7.0 for 30 min and then gels were transferred in reaction-mixture containing α-napthyl acetate (3 mg/10 ml) and fast blue RR (1 mg/ml) in 0.025M Tris-HCl buffer (pH 7.0) at 37°C.

5. **ACID PHOSPHATASES**: (Sako and Stahmann, 1972) Gels were washed 3 to 4 times in 0.1M acetate buffer (pH 5.0). The buffer was changed every time as it is necessary to change (lower) the pH of gel from above 8.5 to 5.0. The gels were incubated for 3 to 4 h in solution containing 1-napthyl phosphate (1 mg/ml) and a few drops of 10% MgCl$_2$ in 0.1M acetate buffer pH 5.0. Greyish-black-coloured band appeared at the site of enzyme activity.

6. **ALCOHOL DEHYDROGENASES**: (Brewer, 1970) Gels were incubated at 37°C for 30 min in the reaction-mixture containing ethanol, 0.05 ml; phenazine methosulphate, 1 mg; Nitro BT, 10 mg; NAD, 40 mg; sodium cyanide, 2 ml (0.025M); and total volume, 20 ml, with Tris-HCl buffer 0.1M, pH 8.0. Light to dark purple coloured bands were observed.

7. **MALATE DEHYDROGENASES**: (Honold et al., 1966) Gels were pre-incubated for 20 min. in 0.2M Tris-HCl buffer, pH 7.5, and then transferred to staining-mixture containing 16 mM L-malate, 0.2 mM NAD, 0.25 mM
MTT-tetrazolium, 0.8 mM phenazine methosulfate, 4 mM MgCl$_2$ and 1.2 mM NaCN in 0.2M Tris-HCl buffer, pH 7.5. For staining purposes, purple-coloured bands were observed.

Note: Isozyme bands have a great tendency to fade away slowly, therefore, in all the cases, gels were washed twice with distilled water and fixed in 7% acetic acid and zymograms were traced immediately.

MEASUREMENT OF RM-VALUES:

The relative mobility (Rm) of protein/isozyme band(s) was measured with reference to the migration of tracking dye as follows:

$$Rm = \frac{\text{Distance migrated by the protein/isozyme band from the origin (cm)}}{\text{Distance migrated by tracking dye (cm)}}$$

Preparation of the banding patterns and detection of differences among hybrids and parents:

Through visual recording of gels, protein/isozyme bands were traced onto graph and mean Rm values were calculated for four independent runs made for each set of hybrid and parents and banding-patterns were drawn. Depending on the presence or absence of a particular band or the differences in the band size, intensity of the variation was considered qualitative or quantitative. The banding-patterns, thus obtained, were used to detect hybrids and parents.

RESULTS

BIOCHEMICAL ESTIMATIONS

PEROXIDASE (FIG. NO.1): (a) CASTOR HYBRIDS AND PARENTS:

Considerable high level of peroxidase activity occurred in 48 and 72 hr old seedlings of hybrid GAUCH-1 and its parents. Regarding discrimination of the enzyme activity, the male parent exhibited low enzyme activity than its hybrid and female; however the magnitude of variance was almost negligible in male and female parents.

Regarding hybrid GCH-4 and its parents, higher enzyme activities were detected in it's male parent followed by female and male parents. This hybrid produced favourable results and hybrid and parents could be distinctly identified in respect of this enzyme activity.
Enzymatic activities in 48 and 72 h old seedlings of hybrids and parents of castor and cotton.
Higher peroxidase enzyme activity is indicative of high resistance power towards toxins. Enzyme peroxidase is useful in identification of castor hybrids and parents.

(b) **COTTON HYBRIDS AND PARENTS:** Hybrid H-8 and its parents exhibited high peroxidase activities in which hybrid had maximum activity followed by female and male parents. The differences in hybrid and parents were remarkably clear.

Regarding hybrid H-6, the male parent, G.Cot-10 and the female parent G.Cot-100, the male and female were quiet distinct, and female showed higher enzyme activity than male. Hybrid and female were considerably close but then also the differences between hybrid and female were distinct.

Hybrid AHH-468 and its male and female parents produced maximum, medium, and low enzyme activity, respectively. Male and female parents were considerably close to each other but the differences were very apparent; however, the hybrid was highly distinct in its enzyme activity.

Peroxidase enzyme was highly suitable for identification of cotton as well as castor hybrids and parents.

**POLYPHENOL OXIDASE ACTIVITY (FIG. NO. 2):**

(a) **CASTOR HYBRIDS AND PARENTS:** Hybrid GAUCH-1 and its parents showed considerable high activity. The highest activity was measured in hybrid followed by female and male parents. The discriminative power of the enzyme polyphenol oxidase activity was higher than amylase enzyme but the differences were minor. In other words, the hybrid and its parents were approximately at the same level of polyphenol oxidase activity.

Hybrid GCH-4 and its parents exhibited greater resolution than hybrid GAUCH-1, and male parent 48-1 and it can be clearly identified from its hybrid, due to its very high activity of enzyme. Female parent can be clearly distinguished from male parent through its low level of enzyme activity, hybrid being near to female but medium in respect of male and female parents.

On these bases, polyphenol oxidase can be taken as enzyme of mixed importance for castor hybrids and parents as it is of low significance in discriminating first castor hybrid but it could differentiate hybrid GCH-4 and its male and female parents clearly. The level of enzyme activities...
remains constant from 48 h of growth to 72 hr.

(b) **COTTON HYBRIDS AND PARENTS**: Hybrid H-8 can be characterised by having maximum polyphenol oxidase activity than its male and female parents. However, male and female parents bear little differences therefore, only hybrid can be distinguished with confidence whereas male and female parents are not-so-easy to discriminate.

Hybrid H-6 and its parents bear minor differences in respect of enzyme activity; the differences are so minor that they often lead to ambiguous identification, therefore polyphenol oxidation is not important for discrimination of hybrid H6 and its parents.

Hybrid AHH-468 exhibits low enzyme activity in comparison to its parents. The parental lines show little or minor differences. Hybrid can be clearly distinguished from their parents. In the hierarchy of enzyme activity female is followed by male and subsequently by hybrid. Therefore polyphenol oxidase is of mixed importance for identification of castor and cotton hybrids and parents as some of the hybrids and parents respond in quiet unfavourable way. The constant enzymatic pattern in 48 or 72 hr old seedlings illustrates the activation of oxidative mechanism at early-growth stages.

**IAA OXIDASE (FIG. NO.3)**: (a) **CASTOR HYBRIDS AND PARENTS**: Castor hybrid GAUCH-1 and its parents showed little differential patterns. Higher enzyme activity was detected in female parent VP-1 followed by hybrid and male but differences were less in female and hybrid while male parent was quiet distinct from hybrid and female parent. IAA oxidase could differentiate only female parent and male whereas the differences between the latter, two were quiet minor. Hybrid GCH-4 and its female parent were found to be almost close to each other while their parents exhibited clearly low level of enzyme activity. This enzyme activity almost fails to distinguish female from hybrid. This enzyme is of mixed importance in discriminating castor hybrids and parents.

(b) **COTTON HYBRIDS AND PARENTS**: Hybrid H-8 exhibits higher enzymatic activity over male and female but male parent is quiet close to it and discrimination is not distinct. This trend was constant over increasing age of seedling.
Enzymatic activities in 48 and 72 h old seedlings of hybrids and parents of castor and cotton.
Hybrid H-6 was categorised by having extremely low level of enzyme activity followed by male and female. The level of genotypic discrimination was little, and careful observations could decipher hybrid and its parents.

Hybrid AHH-468 and its parents, on average, exhibited low enzymatic activity. Female gave feeble enzyme activity than hybrid and male parent. Male gave lesser activity than hybrid. The level of discrimination being very minor, the female parent of this hybrid could only be distinguished from male and hybrid. Therefore, over-all, the enzyme IAA-oxidase was of little importance in distinct identification of cotton and castor hybrids and parents. In both the crops, the level of enzyme activity was same as described above at two stages.

**Amylase Activity (Fig. No.4): (a) Castor Hybrids and Parents:**

Castor hybrid GAUCH-1 can be differentiated from its parents by its low amylase activity. Amylase activity is quiet feeble in hybrid. Amylase activity is slightly higher in female parent, but male parent can be clearly distinguished from female and hybrid due to its maximum and distinctly-high amylase activity, but it was not discriminative in hybrid and male parent of hybrid GAUCH-1. The second hybrid GCH-4 as well as parents can be categorised as genotypes having high amylase activity, but among these, female parent YW P-l is clearly below the mark. The differences between hybrid and female parent were highly prominent. Therefore amylase activity measurement was found to be of mixed importance in identification of castor hybrid and parents. In the case of first hybrid and its parents, only female can be separated with confidence, while in second hybrid, female was distinct from hybrid and male parent. The trend was constant over increase in age of seedling.

(b) **Cotton Hybrids and Parents:** The cotton hybrid H-8 is featured by medium amylase activity while male is the highest and female is the lowest in hierarchy of enzyme activity. The magnitude of mutual differences is very low, therefore hybrid H-8 and its parents can not be distinguished through amylase activity.

Hybrid H-6 and its parents exhibit comparatively similar sort of response in this hybrid and they can be categorised maximum followed by female and male. The feeble enzyme activity analysed at two stages of growth
does not contribute much in respect of this enzyme in hybrid H6. Regarding hybrid AHH-468 and its parents, the level of enzyme activity is very low though hybrid shows maximum enzyme activity followed by female, but extremely-low enzyme activity was detected in male parent DHY-286.1. Hybrid and its parents cannot be distinguished through amylase activity. Therefore, it appears that as cotton and castor are two oily-crops, carbohydrate degradative mechanism does not activate much immediately after germination and therefore it is of little use in deciphering hybrids and parents of castor and cotton.

**Protease: (Fig. No. 5):**

(a) **Castor Hybrids and Parents:** Male parent of hybrid GAUCH-1 was distinct in having the least enzyme activity while hybrid and female exhibited higher enzyme activity. Hybrid and male gave little differences therefore this hybrid can be taken for identifying male parent though female and hybrid can also be identified, but as resolution is lower, extreme care should be taken.

Regarding hybrid GCH-4, the male parent showed very low enzyme activity. Hybrid exhibited maximum while female gave intermediate activity. This hybrid and its parental response was better than the former. Only one genotype, mostly male, could be deciphered through this enzyme measurement.

(b) **Cotton Hybrids and Parents:** Hybrid H-8 and its parents responded in quiet different way and they could be identified separately. Hybrid was followed maximum extent, by male and female in respect of enzyme activity. The level of differences were satisfactory and useful.

Regarding hybrid H-6 and its parents, there was no differences between hybrid and female parent while male parent exhibited lower enzyme activity than female and hybrid. Therefore, only male could be distinguished through this enzyme activity.

Hybrid AHH-468 and its parents responded in such a discriminative way that it appeared to be most easy to be identified through this enzyme-estimation. Hybrid was recorded least while its male parent was far higher than it. Male parent was intermediate in respect of enzyme activity. Therefore, this enzyme was of mixed importance for discriminative purposes for hybrids and parents of castor and cotton, except hybrid AHH-468 and its parents. Regarding this hybrid, protease can be taken as an excellent marker
Enzymatic activities in 48 and 72 h old seedlings of hybrids and parents of castor and cotton.
in identification. Enzymatic recording did not alter with age of seedlings.

**Invertase: (Fig. No.6): (a) Castor Hybrids and Parents:**

Higher invertase activity in hybrid GAUCH-1 was followed by male and female parents. Male and female parents could be deciphered between themselves in respect of high enzyme activity in male while female gave very feeble response to the enzyme.

Regarding hybrid GCH-4 and its parents, the hybrid responded in intermediate fashion while male was maximum followed by female. Higher enzymatic activity deciphered male distinctly from female and hybrid, but low enzyme activity of female was also equally discriminative. Therefore, this enzyme was quiet useful in identification of castor hybrids and parents.

(b) **Cotton Hybrids and Parents:** Invertase acted as a good marker in identification of cotton hybrids and parents. The male parents of hybrid H-8 was measured very less in comparative measurement of the enzyme activity in hybrid and its parents while hybrid itself was medium and male parent responded more than both female parent and hybrid. The resolution was very easy to identify parents and hybrid distinctly.

Similarly, the hybrids and its parents also responded to enzyme invertase. In this, the male was recorded minimum while the female was intermediate. Hybrid gave maximum enzyme activity. The magnitude of difference was highly clear and therefore this hybrid and its parents could be easily identified through this marker enzyme.

High usefulness of the enzyme invertase was evident from discrimination of hybrid AHH-468 and its parents. The hybrid was measured medium while its male parent exhibited higher enzyme activity. The female patent was identified through its low enzyme activity. The distinct identification given by this enzyme was very clear and far from ambiguity, therefore the invertase enzyme marker can be recommended for identification of cotton as well as castor hybrids and their parents. The magnitude of differences remained constant over-age.
SOLUBLE SEED PROTEINS ELECTROPHORESIS (Fig. No. 7):

CASTOR: Hybrid GAUCH-1 developed more number of bands in comparison to its parental genotypes. Hybrid GAUCH-1 and its parents showed some common bands which were, band no.3 (Rm 0.14), 7 (Rm 0.46), 9 (Rm 0.58), 10 (Rm 0.68) while band no. 4 (Rm 0.27), 13 (Rm 0.85) were common between hybrid and male. Similarly band no.1 (Rm 0.03), 5 (Rm 0.34) were common in hybrid and female parent. These common bands could distinguish hybrid along with its either of parents however band no. 12 (Rm 0.76) was found to be specific for male parent and band no. 8 (Rm 0.53) and 14 (Rm 0.88) were found to be specific for female parent only.

Hybrid GCH-4 also showed similar band patterns. Although it developed total nine bands which were maximum among the three genotypes involved. However it did not develop any specific band(s). All the bands it had developed were found to be common with one or other parents. Band no.1 (Rm 0.03), 8 (Rm 0.53), 9 (Rm 10.58), 14 (Rm 0.88) were common between female and hybrid while band no. 4 (Rm 0.38), 11 (Rm 0.74) were common between hybrid and male. As in GAUCH-1, the band no.3 (Rm 0.14), 7 (Rm 0.46) and 10 (Rm 0.68) were common with the three genotypes involved. The band no. 2 (Rm 0.08) and 6 (Rm 0.38) were found to be specific for male 48-1 while band no. 5 (Rm 0.35) was specific for female parent only. Thus the differential band spectrum distinguished each genotype distinctly.

COTTON: The detailed description of band patterns in each hybrid and its parents is as follows:

Male parent Surat dwarf, of hybrid H-8 can be distinguished from rest of the genotypes in absence of band no. 1 (Rm 0.08) while band no.2 (Rm 0.09), 13 (Rm 0.38), 23 (Rm 0.59) and 29 (Rm 0.74) were seen in male parent only. The band no.15 (Rm 0.43) was clear in female parent, G.Cot-10 only. Female and hybrid had band no.3 (Rm 0.11), 8 (Rm 0.26), 9 (Rm 0.30) common on the other hand band no.19 (Rm 0.49), 21 (Rm 0.55), 30 (Rm 0.75) were common in hybrid and its male parent. Apart from this band no.4 (Rm 0.14), 6 (Rm 0.18), 7 (Rm 0.22), 27 (Rm 0.7), 33 (Rm 0.84), 34 (Rm 0.94) were present in all the three genotypes involved i.e. hybrid H-8 and its male and female parents.

Hybrid H-6 and its parents had many common bands such as no.1 (Rm
SCHEMATIC DIAGRAMS OF SOLUBLE SEED PROTEINS OF HYBRIDS AND THEIR PARENTS OF CASTOR AND COTTON

FIG. 7

- DARK BANDS
- MEDIUM COLOURED BANDS
- LIGHT COLOURED BANDS
0.08), 3 (Rm 0.11), 4 (Rm 0.4), 7 (Rm 0.22), 9 (0.30) and 30 (0.74) while hybrid could be characterized by presence of band no.5 (Rm 0.16), 26 (0.67), 32 (0.82) and 35 (0.94) on the other hand male genotype exhibited only four characteristic bands which were band no. 6 (Rm 0.16), 11 (Rm 0.34) and 21 (Rm 0.55) on the other hand female parent developed two special bands, number 12 (Rm 0.36) and 17 (0.46). Band no.8 (Rm. 0.26), 15 (Rm 0.43); 27 (Rm 0.70), 33 (Rm 0.84) were common in male and female parent. Similarly band no.2 (Rm 0.09) and 24 (Rm 0.62) were common in hybrid and female. Characteristic bands were greatly helpful in clear discrimination of hybrid H-6 and its parents.

Hybrid AHH-468 and its parents also showed many common bands such as no.1 (Rm 0.08), 5 (Rm 0.16), 7 (Rm 0.22), 14 (Rm 0.41) 22 (Rm 0.56), 24 (Rm 0.62), 28 (Rm 0.73), 33 (Rm 0.84) and 34 (Rm 0.94). Band no. 10 (Rm 0.31) and 25 (Rm 0.66) were found to be characteristic bands of hybrid AHH-468. While band no.3 (Rm 0.11) and 9 (Rm 0.30) were specific for male as well as female respectively. The characteristic bands of female parent were, no. 11 (Rm 0.34), 12 (Rm 0.36) and 16 (Rm 0.44) on the other hand band no. 8 (Rm 0.26) and 19 (Rm 0.53) were common between hybrid and female and band no. 18 (Rm 0.48) and 31 (Rm 0.75) were common in male and hybrid. In context of this hybrid also specific bands were found to be useful for differentiation purposes. Seed soluble protein band patterns were found to be the most important in the differentiation of cotton hybrids and their parents.

**Isozymes Electrophoresis:**

Schematic diagrams of various isozyme patterns have been depicted in Fig. No. 8 to 14. The castor and cotton hybrids as well as their parents were attempted to identify through their various isozyme banding patterns and mutual comparison of it within hybrid and its parents for each particular isozyme. Results described below were found to be constant for each hybrid and parent over change of season and in replication. The banding patterns were described on the basis of their comparative Rm values.
1. **AMYLASES (FIG. NO. 8): CASTOR**: Amylases isozymes patterns observed in castor hybrids and parents were as follows:

Hybrid GAUCH-1 and its parents produced a total of three bands differing in their Rm values. The three genotypes involved can be distinguished from one another in respect of bands other than no.3 (Rm 0.47) which is common in all the genotypes. VI-9, the male parent, can be differentiated from hybrid and female in presence of band no.1 (Rm 0.34). Hybrid and female can be categorised same in respect of band No. 2 (Rm 0.39) but the two genotypes can be identified in respect of band no. 4 (Rm 0.53) and 5 (Rm 0.059). Hybrid contains band no.4 (Rm 0.53) while female bears band no.5 (Rm 0.59). Band no.4 (Rm 0.53) is common in male while band no. 2 (Rm 0.39) is common in hybrid and female; band no. 2 (Rm 0.39) is absent in male while band no. 4 (Rm 0.53) is absent in female. The second hybrid GCH-4 and its parents can be identified distinctly on the basis of band no. 4 (Rm 0.53) and 5 (Rm 0.59) as band no 2 (Rm 0.39) and 3 (Rm 0.47) are common in three genotypes. Hybrid can be differentiated from female in the presence of band no.4 (Rm 0.53) while female can be deciphered from hybrid in respect of band no.5 (Rm 0.59). The two bands (no. 4 and 5) are absent in male.

**COTTON**: The isozymes patterns observed in cotton hybrids and parents were as follows:

Male parent S.D. developed total 5 bands while hybrids and female parent produced only four bands. Band no.1 (Rm 0.04), 3 (Rm 0.39), 5 (Rm 0.51) were present in hybrid H-8 as well as parents where-as there was staining intensity difference in band no.5 (Rm 0.51) in female. Male parent carried band no.4 (Rm 0.44) extra than hybrid and female while band no.7 (Rm 0.81) was extra in female G.Cot-10. Band no.6 (Rm 0.71) was common in hybrid and male but absent in female.

Therefore hybrid H-8 and its parents could be deciphered on the basis of amylase isozyme pattern. The second hybrid H6 and its male and female parents developed, in total 3,4,4 bands respectively. Band no. 1 (Rm 0.04) 3 (Rm 0.39), 5 (Rm 0.51) were common in hybrids and its parents but female G.Cot-100 was distinct in presence of band no.6 (Rm 0.71) which was absent in hybrid and male parent G.Cot 10. Male parent G.Cot 10 was easy to be identified due to the presence of band no.7 (Rm 0.71). Band no. 7 was absent
SCHEMATIC DIAGRAMS OF AMYLASES ISOZYMES PATTERNS OF HYBRIDS AND THEIR PARENTS OF CASTOR AND COTTON

FIG. 8
in hybrid and female. Thus hybrid H-6 was easy to be distinguished from its parents through amylases isozyme pattern.

Third hybrid AHH-468 and its parents developed total 4 bands each. Among them, band no.1 (Rm 0.04), 3 (Rm 0.39), 6 (Rm 0.71) were common in all the three genotypes. All the bands, i.e. band no. 1 (Rm 0.04), 2 (Rm 0.21), 3 (Rm 0.39) and 6 (Rm 0.71), were common in female and hybrid. Male parent DHT-286-1 was specific in the presence of band no.5 (Rm 0.51) which was absent in hybrid and female, thereby, facilitating their identification.

**POLYPHENOL OXIDASES: (FIG. NO. 9); CASTOR:** Polyphenol isozymes pattern in castor hybrids and parents are as follows:

Regarding hybrid GAUCH-1 and its parents, band no.3 (Rm 0.21), 5 (Rm 0.42) and 9 (Rm 0.74) were common in hybrid and parents. Male parent VI-9 was distinct from hybrid and female in the presence of band no.6 (Rm 0.48). Male parent had got band no.2 (Rm 0.17), and 10 (Rm 0.78) common with hybrid. Female parent differed from hybrid and male parent in the presence of band no.1 (Rm 0.07) and 8 (Rm 0.71). Band no.10 (Rm 0.78) was absent in female.

The second hybrid GCH-4 also showed band no.3 (Rm 0.21), 5 (Rm 0.42) and 9 (Rm 0.74) common with its parents. Hybrid bore band no.1 (Rm 0.6) common with female whereas band no.2 (Rm 0.17) was common with male. Hybrid possessed band no.4 (Rm 0.26) and as extra bands than its parents. Female parent differed from male and hybrid in the presence of band no.7 (Rm 0.56) whereas male differed from hybrid and female in the presence of band no.10 (Rm 0.8) and 11 (Rm 0.86).

**COTTON:** Polyphenol oxidases isozymes patterns present in cotton hybrids and their parents were as follows:

Total 7 bands were developed by hybrid H-8 while male and female parents had 6 and 5 bands respectively. Band no.1 (Rm 0.08), 2 (Rm 0.13), 5 (Rm 0.52), 7 (Rm 0.76) and 8 (Rm 0.86) were common in hybrid and parents while band no.9 (Rm 0.89) was specific for male and hybrid. Hybrid could be easily identified from male and female in the presence of band no.4 (Rm 0.47).

Hybrid H-6 and its male and female parents had 6, 5, and 5 bands each, respectively. In this hybrid also, band no. 1 (Rm 0.08), 5 (Rm 0.52), 7 (Rm
SCHEMATIC DIAGRAMS OF POLYPHENOL OXIDASES ISOZYMES
PATTERNS OF HYBRIDS AND THEIR PARENTS OF CASTOR
AND COTTON

FIG. 9
0.76) and 8 (Rm 0.86) were common in three genotypes. Hybrid and female had band no. 6 (Rm 0.71) common but hybrid was different in presence of band no. 9 and these could easily differentiate hybrid (Rm 0.89) from its parents. Regarding hybrid, AHH-468 had 7 bands while male and female had 6 and 5 bands respectively. The band no.1(Rm 0.08), 4 (Rm 0.47), 5 (Rm 0.52) and 8 (Rm 0.86) were common in hybrid and parents. Band no.3 (Rm 0.28) and 7 (Rm 0.76) were common in hybrids and male while band no.9 (Rm 0.89) was common in hybrid and female. Therefore, reactive position of bands determined identification of this hybrid.

**PEROXIDASES: (FIG. NO. 10): CASTOR:** Peroxidase isozymatic patterns of castor hybrids and parents were as follows:

Hybrid GAUCH-1 and its parents exhibited minor differential patterns in respect of Rm values. Hybrid GAUCH-1 can be clearly distinguished from its male and female parents in the presence of band no.1 (Rm 0.16). Band no.1 is absent in female and male. Band no. 3, 4, and 7 show minor changes of Rm values in respect of hybrid and its parents where Rm values were 0.22, 0.23, 0.24 for band no.3, 0.33, 0.33, 0.34 for band no.4; and 0.48, 0.49, 0.49 for band no.6, in male, hybrid and female respectively. Band no.5 (Rm 0.4) and 8 (Rm 0.7) were common in hybrid and parents. Band no.6 (Rm 0.59) was absent in female but common in hybrid and male parent. Hybrid GCH-4 and its parents show total of 5 bands which differentiate hybrid and its parents in respect of different Rm values, therefore hybrid and their parents can be identified on the basis of relative band position in gel.

Band no.2 (Rm 0.18), 5 (Rm 0.4), 6 (Rm 0.49) and 7 (Rm 0.59) were common in hybrid and male. Hybrid can be differentiated from male in the presence of band no.4 (Rm 0.34). This band of hybrid was common with female parent. Hybrid was also different from male in the presence of band no.3 (Rm 0.23) which was common with female parent. Band no.7 (Rm 0.49) was common with three genotypes. Band no.7 (Rm 0.59) was absent in female, whereas band no.8 (Rm 0.7) was present in female but absent in hybrid and male.

**COTTON:** Peroxidases isozymes appeared in cotton hybrids and parents were as follows:

Regarding hybrid H-8 and its parents, a total of 6,7,5 bands were observed respectively. The band no. 2 (Rm 0.16), 5 (Rm 0.42), 6 (Rm 0.52)
SCHEMATIC DIAGRAMS OF PEROXIDASES ISOZYMES
PATTERNS OF HYBRIDS AND THEIR PARENTS OF CASTOR
AND COTTON

FIG. 10
and 9 (Rm 0.71) were common in hybrid and its parents. Hybrid was distinct in presence of band no.1 (Rm 0.05) and band no.11 (Rm 0.88). The band i.e. band no.4 (Rm 0.29), was common with female parent and absent in male S.D. Male was distinct in respect of band no.7 (Rm 0.61) and 10 (Rm 0.88) which were absent in hybrid or female. Therefore, all the bands which female contained were common with hybrid. Hybrid contained two extra bands no.1 (Rm 0.05) and 11 (Rm 0.88) than female whereas male contained only one extra band no.4 (Rm 0.29).

Peroxidases banding pattern was useful in identification of cotton hybrids and parents. Hybrid H-6 contained total 6 bands whereas its male and female parents developed 5 bands each. In this hybrid, only band no.6 (Rm 0.52) and 9 (Rm 0.71) were common with all the 3 genotypes involved. Male and hybrid were distinct in the presence of common band no.5 (Rm 0.42) which was absent in female. Band no.3 (Rm 0.22) and 7 (Rm 0.61) were common in hybrid and female and absent in male. Male was specific in the presence of band no.1 (Rm 0.5) which was absent in hybrid and female. Thus, peroxidase could distinctly identify cotton hybrid and its parents.

Hybrid AHH-468, the male parent DHY-286-1 and the female parent AK-32 has 6,5,4 bands totally out of which band no.5 (Rm 0.42) and 6 (Rm 0.52) were present in hybrid as well as in its parents. Female had all the bands identical with hybrid, i.e. band no. 2 (Rm 0.16), 5 (Rm 0.42), 6 (Rm 0.52) and 9 (Rm 0.71) were common in them. Band no.3 (Rm 0.22) and 7 (Rm 0.61) were at the same position in hybrid and male. Male parent was distinct in presence of band no.1 (Rm 0.5) which was absent in hybrid and female. Peroxidase could decipher hybrid and its parents clearly.

**ESTERASES (FIG. NO. 11): CASTOR:** The banding pattern of esterases isozymes in castor hybrids and parents were as follows:

Maximum number of bands observed in hybrid GAUCH-1 were 9 followed by male 8, then female 7. Regarding banding patterns, band no. 2 (Rm 0.19), 6 (0.51) and 11 (0.87) were common in three genotypes involved whereas band no.4 (Rm 0.36), 7 (Rm 0.58) and 9 (Rm 0.79) were common in hybrid and male parent. Band no.8 (Rm 0.7) and 12 (Rm 0.91) were common in hybrid and female
parent. All the bands present in hybrid were found common with one of the
two parents except band no.1 (Rm 0.11) which was absent in hybrid.

Female parent differed from hybrid and male parent in presence of band
no.3 (Rm 0.26). Regarding hybrid GCH-4, total number of bands were maximum
in hybrid (8); female bore 7 while male parent contained only 5 bands. This
hybrid possessed band no.1 (Rm 0.11), 6 (Rm 0.52) and 11 (Rm 0.87) in common
with both the parents. Band no.4 (Rm 0.36) and 7 (Rm 0.58) were common in
hybrid and male. Regarding extra bands present in hybrid over male parent,
it was band no.10 (Rm 0.83). Band no.12 (Rm 0.91) present in hybrid which
was common with female parent while female parent differed from hybrid and
male in presence of band no.2 (Rm 0.19) and 3 (Rm 0.26)

COTTON: Esterases isozymes banding patterns observed in cotton hybrids and
parents were as follows:

Hybrid H-8 had total 11 bands while its male and female parents
exhibited total 10 and 11 bands each. The band no.2 (Rm 0.09), 4 (Rm 0.18)
and 5 (Rm 0.24) were common in three genotypes, i.e. hybrid and its parents.
Band no.7 (Rm 0.32) and 12 (Rm 0.62) were at a minor higher position in
female than in hybrid or male parent.

Band no.8 (Rm 0.46), 10 (Rm 0.53) and 15 (Rm 0.74) were common in
hybrid and male but absent in female. Similarly, band no. 19 (Rm 0.91) was
common with hybrid and female but absent in male parent. Hybrid could be
specified in presence of band no.13 (Rm 0.67) which is absent in either of
the parents. Female could be discriminated from male and hybrid in presence
of band no.9 (Rm 0.51), 11 (Rm 0.61) and 18 (Rm 0.91). These bands were
absent in hybrid and male.

Esterases isozymes analysis was of great use in identification of
cotton hybrid H8 and its parents. Second hybrid H6 produced only 8 bands
while its male parent had 11 and female gave 8 bands. Band no.7 (Rm 0.32)
and 19 (Rm 0.91) were the only bands common with all the genotypes involved
in this hybrid formation. Band no.4 (Rm 0.24), 9 (Rm 0.51), 12 (Rm 0.63) and
18 (Rm 0.91) were common in hybrid and male G.Cot 10, but absent in female
parent. Band no.3 (Rm 0.18) and 14 (Rm 0.72) were at the same position in
hybrid and female. Male could be identified distinctly from hybrid and
female in presence of band no.2 (Rm 0.09), 5 (Rm 0.26), 17 (Rm 0.77), and 20
SCHEMATIC DIAGRAMS OF ESTERASES ISOZYMES
PATTERNS OF HYBRIDS AND THEIR PARENTS OF CASTOR AND COTTON

FIG. 11
(Rm 0.96). These bands were absent in hybrid and female while female could be discriminated from male and hybrid in presence of band no. 6 (Rm 0.32), 8 (Rm 0.46) and 16 (Rm 0.76). Band no. 11 (Rm 0.61) was a common band in male and female parents. Hybrid did not contain any specific bands and all of its bands were on common position with either of the parents. Hybrid AHH-468 and its male and female parents were characterized by presence of 9, 9 and 8 bands respectively. Band no. 4 (Rm 0.24), 7 (Rm 0.32), 16 (Rm 0.74) and 19 (Rm 0.91) were common in hybrid and both of its parents while band no. 1 (Rm 0.03) and 8 (Rm 0.46) were common in male and hybrid only. Similarly, band no. 6 (Rm 0.32) and 10 (Rm 0.53) were common in female and hybrid. Band no. 2 (Rm 0.09) and 18 (Rm 0.84) were at the same position in male and female. Male had one distinct band no. 11 (Rm 0.61) and it was not present in female and hybrid. Similarly, hybrid had band no. 14 (Rm 0.72) which was absent in male and female parents. Esterases isozyme had great resolution and was fully capable of identifying hybrids and parents.

ACID-PHOSPHATASES: (Fig. No. 12): Castor: Acid phosphatases banding patterns in castor hybrids and parents were as follows:

Male parent of hybrid GAUCH-1 bore maximum number of bands (8) followed by female parent (7) and hybrid (6).

Band no. 1 (Rm 0.12), 4 (Rm 0.31), 7 (Rm 0.53), 10 (Rm 0.76) and 11 (Rm 0.86) were commonly present in hybrid and its parents. Band no. 6 (Rm 0.49) was common in hybrid and male parent. Male parent differed from hybrid in presence of band no. 2 (Rm 0.18) and band no. 8 (Rm 0.59) whereas female parent differed from hybrid in presence of band no. 3 (Rm 0.24) and band no. 9 (Rm 0.64). These bands were absent in hybrid and the male parent. The second hybrid and its male and female parents produced 5, 4, 7 bands respectively. The second hybrid GCH-4 and its parents also exhibited band no. 1 (Rm 0.12), 7 (Rm 0.53) and 10 (Rm 0.76) commonly in all the three genotypes. Band no. 5 (Rm 0.42) was common in hybrid and male. All the bands present in male parent were common with hybrid bands. Female parent produced band no. 3 (Rm 0.24), 4 (Rm 0.32) and 9 (Rm 0.64) extra than hybrid and male parent. Band no. 11 (Rm 0.86) was commonly exhibited by female and hybrid.

COTTON: Isozymes acid phosphatases produced following band patterns in cotton hybrids and parents:
SCHEMATIC DIAGRAMS OF ACID PHOSPHATASES ISOZYMES
PATTERNS OF HYBRIDS AND THEIR PARENTS OF CASTOR
AND COTTON

FIG. 12
Total 8 bands were observed in hybrid H8 while its male and female parents produced 9 and 7 bands respectively. Band no.3 (Rm 0.24), 6 (Rm 0.44), 9 (Rm 0.61) and 10 (Rm 0.73) were present in hybrid and parents but differential staining intensity was seen for band no. 3 (Rm 0.24) and 9 (Rm 0.61) in respect of female parent. Hybrid could be distinguished from male and female parents in presence of band no.2 (Rm 0.16) and 5 (Rm 0.39). Female G.Cot 10 could be identified from male and hybrid in presence of band no.7 (Rm 0.51). Band no.8 (Rm 0.57) deciphered male from hybrid female. Band no.1 (Rm 0.06) was absent in hybrid while band no.4 (Rm 0.29) and 11 (Rm 0.81) was common in male and hybrid. Band no.1 (Rm 0.06) and 12 (Rm 0.91) were common in male and female.

Acid phosphates could easily distinguish hybrid H8 and its parents in mixtures. Hybrid H6 and its male and female parents developed 9,7,6 bands respectively. Band no. 3 (Rm 0.24), 6 (Rm 0.44), 9 (Rm 0.61) and 12 (Rm 0.91) were found to be common in hybrid and its both parents. Band no.9 (Rm 0.61) had light staining-intensities, and on this basis, in hybrid which helps in identification of hybrid H6. There was no distinguishing band for pinpointing the genotypes involved. All the bands were common with one or the other genotype. Band no.1 (Rm 0.06), 7 (Rm 0.51) and 10 (Rm 0.73) were common in male and hybrid while band no. 5 (Rm 0.39) and 11 (Rm 0.81) were common in hybrid and female. Therefore, this marker could group either male hybrid or female hybrid but could not distinguish each separately. It was considered to be of little help in identification of hybrid H-6 and its parents.

Hybrid AHH-468 and its male parent developed 7 bands each while the female parent had only 5 bands. Band no.3 (Rm 0.24), 6 (Rm 0.44), 9 (Rm 0.61) and 12 (Rm 0.91) were common in hybrid and its parents, however, band intensities were different in respect of band no.6 (Rm 0.44), 9 (Rm 0.61) and 12 (Rm 0.91). Male parent could be distinguished on the basis of extra band no. 11. Hybrid and female were lacking in it. On the other hand, band no.1 (Rm 0.06), 10 (Rm 0.73), and 7 (Rm 0.51) were common in male hybrid and female hybrid; in other words, female Ak-32 could be identified in absence of band no.1 (Rm 0.06) and 10 (Rm 0.73) while male and hybrid can be deciphered from female in absence of band no.7 (Rm 0.51). Acid phosphatases
are useful in identification of cotton hybrids and parents.

**ALCOHOL DEHYDROGENASES (FIG. NO. 13): CASTOR:** Alcohol dehydrogenases isozymes patterns observed in castor hybrids and parents were as follows:

Regarding hybrid GAUCH-1 and its parents, total 2 bands were produced by male while 3 bands were developed by hybrid female parent. Band no.4 (Rm 0.46) was commonly developed by male, female and hybrid, while band no.2 (Rm 0.36) was at equal Rm position in hybrid and male. Similarly, band no.5 (Rm 0.54) was common in hybrid and female. Female differed from male and hybrid in presence of band no.1 (Rm 0.32). The second hybrid GCH-4 and its male parent 48-1 developed four bands which were measured out to be at the same Rm positions. These bands were no.1 (Rm 0.32), 3 (Rm 0.43), 4 (Rm 0.46) and 4 (Rm 0.54).

**COTTON:** Isozymes alcohol dehydrogenases developed following band patterns in cotton hybrids and parents:

Hybrid H-8 produced total 4 bands while its male parent S.D. and female parent G.Cot 10 developed only 3 bands each.

Band no.3 (Rm 0.58) and 4 (Rm 0.66) were observed in hybrid and parents commonly. Band no.1 (Rm 0.36) was common in hybrid and female parent while band no.2 (Rm 0.48) was common in hybrid and female parent. Specificity of band was lacking. Hybrid and female can be distinguished from male on the basis of their common band no.1 (Rm 0.36). Similarly, male and hybrid can be differentiated from female in presence of their common band no.2 (Rm 0.48).

Regarding hybrid H-6 and its parents, each of the three genotype developed only total 3 bands. In this set, only band no.3 (Rm 0.58) was common in hybrid and parents. Band no.1 (Rm 0.36) was common in male and female parent while band no.2 (Rm 0.48) and 4 (Rm 0.66) developed at a similar position in hybrid female and hybrid male respectively. In other words, hybrid can be specified by lack of band no.1 (Rm 0.36). Male G.Cot 10 could be distinguished from female and hybrid in absence of band no. 2 (Rm 0.48); on the other hand, female could be identified on the basis of absence of band no. 4 (Rm 0.66).

DHY-286-1, the male parent of hybrid AHH-468, and hybrid itself represented 4 bands while female developed only two bands.
SCHEMATIC DIAGRAMS OF ALCOHOL DEHYDROGENASES
ISOZYME PATTERNS OF HYBRIDS AND THEIR PARENTS
OF CASTOR AND COTTON

![Diagram showing isozyme patterns of hybrids and their parents of castor and cotton.](image-url)
Band no.3 (Rm 0.58) and 4 (Rm 0.66) were seen at same positions in gel and no.2 (Rm 0.48) and 5 (Rm 0.76) were common in hybrid and male but absent in female. Therefore, regarding this hybrid, it can be said that female can be distinguished from male and hybrid in possessing only two bands. Male and hybrid did not differ in respect of alcohol dehydrogenases isozyme banding pattern. Thus, alcohol dehydrogenases were not much useful in distinguishing cotton hybrids and parents.

MALATE DEHYDROGENASES (FIG. NO.14): Following band patterns of isozymes malate dehydrogenase were observed in castor hybrids and parents:

Hybrid GAUCH-1 and its male and female parents developed total 3, 4 and 3 bands respectively. Band no.2 (Rm 0.33) was common in three genotypes while band no.3 (Rm 0.42) and band no.5 (Rm 0.59) present in hybrid were common in male and female parents respectively. Male parent differed from hybrid and female in presence of band no.4 (Rm 0.49). Band no.1 exhibited minor differences with respect to hybrid and female. In female, it was recorded at 0.27, while in hybrid, at 0.26. The second hybrid GCH-4 produced total 4 bands the band no. 2 (Rm 0.33), 3 (Rm 0.42) and 5 (Rm 0.59) were found to be common with male parent which produced only 3 bands, while only band no.2 (Rm 0.33) was common in all the hybrids and parents. Female produced 3 bands and all of them were at equal Rm values of that of hybrid. Their numbers were 1 (Rm 0.27), 2 (Rm 0.33) and 5 (Rm 0.59).

COTTON: Malate dehydrogenases banding patterns of cotton hybrids and parents were as follows:

Total 3, 4 and 3 bands were developed by S.D., H-8 and G.Cot 10. Out of these, three bands, band no.2 (Rm 37) and 3 (Rm 0.44) were common to hybrid H8 and its parents. Band no.1 (Rm 0.26) and band no.4 (Rm 0.69) were common in hybrid female G.Cot 10 and hybrid male respectively. There were no specific bands to decipher each of the genotype involved distinctly. Hybrid H-6 and its male parent G.Cot 10, each, developed total 3 bands while its female parent G.Cot 100 exhibited 4 bands. Band no.1 (Rm 0.26) and 2 (Rm 0.37) were common to hybrid and parents. Band no.3 (Rm 0.37) was absent in hybrid, while band no.4 (Rm 0.69) was observed in hybrid and female only. Hybrid AHH-468 and its female parent were characterized by presence of total four bands while male parent DHY-468 developed only two bands. Band no.2 (Rm
SCHEMATIC DIAGRAMS OF MALATE DEHYDROGENASES
ISOZYME PATTERNS OF HYBRIDS AND THEIR PARENTS
OF CASTOR AND COTTON

FIG. 14
0.37) and 3 (Rm 0.44) were developed at the same position in gel while band no. 1 (Rm 0.26) and 4 (Rm 0.69) were specific for female and hybrid. Female and hybrid could not be distinguished on the basis of malate isozyme pattern of this particular hybrid and parental genotypes. Overall malate dehydrogenases did not prove much useful in identification of cotton hybrids and parents as most of the bands made common appearance either with one or both the parents.

DISCUSSION

The biochemical identifying methods i.e. estimation of enzyme activities and electrophoresis provided easy and simple techniques to differentiate hybrid and parents of castor and cotton. However, in mutual comparison the estimation of enzyme activities for crude extracts were found to be little less sensitive and precise than electrophoresis of seed soluble proteins and isozymes.

The electrophoresis of proteins and enzymes is a widely favoured method owing to its capability of providing distinct, stable and reproducible profiles in respect of genotypes (Cooke, 1988, 1992). Due to its remarkable capabilities the technique of electrophoresis has been accepted for certifying crop cultivars in several countries such as Germany (Bundesortenamt, 1990), however, in India, the crop cultivars are still certified on the basis of grow-out tests based on floral and vegetative traits of mature plants which take up long duration. The biochemical methods provide an unique opportunity to deliver results within few days. The potentialities of the two techniques i.e. enzyme activity measurement and electrophoresis, in respect of identification of hybrids and parents of castor and cotton are discussed below.

The biochemical method, however are required to be standardized thoroughly before their application in varietal identification.

The enzyme activities, when considered in progeny and parental lines, always show three possibilities: either hybrid exceeds the two parents due to inheritance of better characters, or metabolism from parental genotypes as has been depicted by Mitra (1977) in cotton hybrid H-4 and parents or does not show any response of heterosis, i.e., non-heterotic hybrids. Roos
and Sarkissian (1968) did not obtain any heterotic effect about iso-citrate dehydrogenase enzyme during the period of 2 to 6 days following water-imbibition. While only heterotic hybrids responded to iso-citrate lyase in heterotic manner, non-heterotic hybrids failed to show such responses.

The cotton and castor hybrids showed different levels of enzymatic activities, in comparison to their parents. All the hybrids in two crops showed a better metabolic machinery in terms of enzymes playing a major role such as peroxidase, polyphenol oxidase, IAA-oxidase, amylase, protease and invertase. The heterosis influenced the hybrids in a way for better growth and vigour. Ghosh et al. (1974) found a better amylase activity in sorghum hybrids than their parental lines. This observation is in agreement with Sharma and Mani (1992) who obtained a higher peroxidase activity in hybrid than either of the parental lines. Castor hybrids showed enhanced hydrolytic enzyme activities with exception of polyphenol-oxidase activity and the parental lines showed higher oxidative enzyme activities. Similarly, cotton hybrid H-8 had high oxidative enzyme activity in comparison to its parents SD and GCot-10. Hybrid H-6 had increased hydrolytic enzyme activities over oxidative enzymes. Similar findings were recorded in hybrid AHH-468. The different patterns of enzyme inheritance were apparent in the growth-responses of the genotypes.

Bhatt et al. (1979) showed mid-parental and better-parental heterosis about nitrate reductase activity in Sorghum hybrids and parents. Fleming and Paliner (1975) suggested that some inbreds have better specific combining ability than others causing better heterosis.

Variatel identification can be achieved through determination of enzymatic activities in different varieties as well as hybrids and parents.

The method can be highly advantageous in differentiating heterotic hybrids over non-heterotic ones. This method of biochemical estimation has got a few advantages. It can predict the genotypic differences on the basis of enzymes in a single seedling and the method is quite routine over other biochemical methods such as electrophoresis. The biochemical estimations do not require special instruments but routine laboratory equipments and chemicals can be utilized during the enzymatic estimation. Apart from these
few advantages, the method of biochemical estimation has got several disadvantages also.

The method is highly non-reproducible in terms of the results obtained from different sources. The different instruments, brands of chemicals and temperature bring about a lot of changes in final results. Therefore, the method for each enzyme and each hybrid/parent seed-lot need to be standardized on different instruments and with different chemicals before it is applied in varietal identification. All these disadvantages make biochemical estimations less significant. However, once the procedure, the instrument etc. have been standardized, the method becomes as useful as other tests which are applied for varietal identification.

**SEED PROTEINS:** The proteins are directly copied from genetic information stored inside the nucleus. These fingerprints, therefore, make a reliable source of knowledge about genomic status of the system. The seed proteins, or rather the electrophoresis of seed proteins, provide an ideal marker to decipher genotypes for varietal identification.

A large number of workers have approved the seed protein profiles as the important criteria differentiating various crop cultivars such as Spoor and Hay (1979); Orf *et al.* (1980); Blogg and Imrie (1982); DePrins Van DeWeghe (1983); Kapse and Nerkar (1985); Koranyi (1989); Konarev *et al.* (1987); Gardiner and Forde (1987); Onokpise *et al.* (1988); Agrawal *et al.* (1988); Ohms *et al.* (1987); Tomer *et al.* (1990) and Rao *et al.* (1990) suggest proteins as such offer a very convenient method as these substances can be directly extracted from intact-seeds which, according to Mc Donald (1980), represent a model for consistent electrophoretic differences as they are characterized by a state of suspended metabolic activity in a plant life-cycle. Later, these views were also supported by De Prins and Van De Weghe (1983).

Konarev *et al.* (1987) stated that protein hydrophobicity and charges present on their molecules serve as important criteria in separation of cultivars, and they should be preferred to isozymes owing to above-mentioned properties.

Electrophoresis of a single seed is an important technique, however, there exists considerable contrasting reports about its usage in
differentiation of cross-and self-fertilized crop cultivars. According to Cooke (1990), proteins are coded by multiple gene loci; therefore, protein profiles make an ideal character for identifying self-types which do not involve any foreign pollen interactions. He discouraged the usage of proteins for cross-pollinated crops but Konarev et al. (1987) stated that electrophoresis of storage-proteins is a very promising method for monitoring seed production of cross-pollinated cultivars as it allows estimation of heterogeneity for cultivars. Similarly, Gardiner and Forde (1987) also found SDS-PAGE of proteins to be more sensitive in cultivar differentiation in out-breeding grasses than in inbreeding species.

Castor and cotton hybrids and parents presented quite differential patterns for soluble seed proteins and the method appears to be simple as dry seeds were to be extracted directly. Gardiner and Forde (1987); Tomer et al. (1990); Aiken and Gardiner (1991), owing to quickness of protein electrophoresis, pronounced it as the most potential technique. The results obtained in cotton are in harmony with studies of Kapse and Nerkar (1985); Agrawal et al. (1988); Rao et al. (1990). The minor variation among the different work results could be due to differences in pore size, pH of buffer and techniques; similar sorts of variations have been reported. These authors, while working on soybean cultivar identification, found differences in results with Larsen (1967). Leaving this apart, protein electrophoresis alone offers an attractive package of varietal identifications.

**Isozyme Electrophoresis:** Isozymes represent multiple forms of a single enzyme, mainly differing in molecular weights. These isozymes provide a highly significant feature for differentiation of genotypes. These isozymes represent specific gene-products and they are neither supposed to be influenced by the growing conditions of the plant nor do they get influenced by mechanical treatments of seeds which can affect morphological characters (Anderson, 1982).

Isozyme-spectrum provides an ideal index of genotypic differences. Cooke (1990) considered isozyme markers more than seed proteins for cross-pollinated varietal identification as the isozymes are coded by single gene therefore they can eradicate environmental and natural crossing-induced variations. Freeling (1983); Markert and Moller (1959) also found them
better markers to analyse inter-genomic interactions, and similar results were obtained in castor and cotton also.

The isozyme-banding patterns in hybrids and parents of castor and cotton revealed the genetic diversity through the total number and position of bands and their intensities. The band-patterns were found to be constant over changing environmental conditions. Similar facts were advocated by Zillman and Bushuk (1979); Sarkar and Bose (1984). As stated by Blogg and Imrie (1982), in context with soybean varietal identification, several variants are required to achieve an accurate and reliable identification of hybrids and parents. Polyphenol oxidases, acid-phosphatases and peroxidases in castor; and esterases, peroxidases, polyphenoloxidases and acid-phosphatases in cotton worked out the desirable task in a mentioned-order of series.

Although alcohol dehydrogenases and amylases were also useful, Isozyme malate dehydrogenases were mostly unresponsive for tagging phenotypes. Lakhanpaul and Babu (1985) did not get much varietal differences in crude extracts while refined extracts showed varietal variability. Mitra (1977) found malate dehydrogenase to be useful as he got the highest percentage of heterosis in it while studying enzyme-complementation in cotton hybrid H-4 and its parents. alcohol and malate dehydrogenases mostly induced common bands in three genotypes involved in hybridization; therefore, the two enzyme-patterns were not found to be suitable for identifying hybrids and parents in castor and cotton.

Workability of different isozymes, peroxidases and esterases has been extensively employed in varietal identification (Spoor and Hay, 1979; Sharma and Mani, 1992), while Agrawal et al. (1988) rejected the usage of peroxidases while they had accepted capabilities of esterases in identification of cotton hybrids and parents. Sharitz et al. (1980); Greneche et al. (1991) found esterases, acid-phosphatases and amylases to be highly sensitive for identification of Typha, rye-grams and soyabean cultivars. Isozyme variants depend on the type of crop but malate dehydrogenases have been found to be less co-operative in varietal identification (Sharitz, 1980); Greneche (1991) recommended extensive study of the enzyme before using it for discrimination of varieties. Spoor and Hay
(1979) ascribed this fact to substrate-specificity of the enzyme. Mashburn et al. (1978) demonstrated that electrophoretically identical isozymes in malate dehydrogenases which differ among species in respect of their thermolabilities McNaughton (1965) examined enzyme kinetics of malate dehydrogenases and stated that the enzyme-system may differ even when the band-patterns are same.

Mitra et al. (1970) found that substrate-specific enzymes, such as malate dehydrogenases, did not exhibit intra-specific enzyme-variation, but enzymes having a broad substrate-specificity, such as esterases and peroxidases, show large inter- and intra-specific variations. Besides other facts, isozyme analysis provides a unique opportunity to identify genotypes immediately after germination. Cardy and Beversdorf (1984) recommended a computer-sorting programme. The adoption of these techniques as a routine one, however, depends upon several other considerations such as cost of analysis, time needed to conduct the test and the expertise required.

Universal application of Isozyme differentiation techniques in varietal identification, however, is not recommended by Anderson (1982). Owing to species-polymorphism, the isozymes and other biochemical characters are not recognized by the International Union for Protection of New Varieties of Plants (UPOV) as descriptive characters, but this, of course, does not prevent the usage of biochemical factors in varietal identification, but polymorphism should be analysed perfectly for use as a rough guide. Overall, the technique of electrophoresis can be assigned as one of the quickest and most reliable techniques for varietal identification purposes as it can deliver results within short periods. Smith and Wych (1986) and Orman et al. (1991) preferred electrophoresis to morphological characters owing to environmental factors. However, Kaizuma and Hymowitz (1981) were of opinion that electrophoresis can divide cultivars into broad groups, especially, in soybean, but seed-protein electrophoresis should be used along-with several other chemical, biochemical and morphological techniques so that dichotomous identification-keys can be prepared. In the present context any of the isozyme, as has been mentioned earlier, showing variations in band-spectrum, can be used, but coombing of various variants always improves the rate of accuracy. Therefore, a single banding-pattern of esterases or peroxidases
can not be used to 'type' a genotype or hybrid/parent. Occasionally genetically-unstable variations may also come up, and which should be analysed out thoroughly by using the isozyme present in high frequencies or by using an increased amount of seeds (Spoor and Hay, 1979).

The technique of electrophoresis requires a well-equipped laboratory and a trained personnel having knowledge of genetics. Though the technique provides one of the quickest devices to explore the genotypes, the same technique also suffers from certain disadvantages such as cost per run (Smith and Wych, 1986), cost of chemicals and equipment, etc.

Regarding application of electrophoresis, one is required to know the identity of isozymes, frequencies of isozyme alleles for a particular region (Cardy and Kannenberg, 1982; Smith, 1984) and can thus leave the necessity of testing the loci, which are unlikely to reveal genotypic identity, thereby also increasing preciseness, specificity and sensitivity of the technique. Though the technique of electrophoresis constitutes one of the most useful tool in varietal identification but is fully capable of providing accurate discrimination, some times, it exhibits variation in results obtained through different sources and creates a question-mark about reproducibility of the technique. These variabilities have been found due to differences in pore size, pH of buffers and other chemicals, i.e. through usage of chemicals from different brands; therefore, a large number of samples must be studied to eliminate confusions which are likely to influence reliability of the results.

**CONCLUSIONS**

The enzyme estimation, protein and isozyme electrophoresis provide a quick, routine and reliable method for identifying hybrids and parents of castor and cotton.

The enzyme estimations differentiate hybrids and parents on the basis of heterosis but the method is largely problematic as it depends on many factors such as temperature, chemicals, sensitivity of instruments, vigour of lots and type of crop. The seed soluble band profiles provide a sensitive marker index for differentiating the hybrids and parents of castor and cotton without any environmental interference.
Esterases, peroxidases, polyphenol oxidases and acid phosphatases help to screen the hybrids and parents of castor and cotton while amylase, alcohol dehydrogenases and malate dehydrogenases are not much useful in identification of parents and hybrids.

Soluble seed proteins and isozyme electrophoresis can be recommended for screening the hybrids and parents of castor and cotton.