GEL ELECTROPHORETIC STUDY OF SEED PROTEINS AND ISOZYMES OF <u>GOSSYPIUM</u> SPECIES WITH SPECIAL REFERENCE TO DETERMINATION OF GENETIC PURITY OF CULTIVARS

BY
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IN MEMORY OF MY

BELOVED MOTHER

CANDIDATE'S DECLARATION

I hereby declare that the dissertation or a part thereof has not been previously submitted by me for a degree of any University.

T. Nageshwar Rao

(T. Nageshwar Rao)

PARBHANI

Date : 7th January, 1988.

CERTIFICATE I

Shri Thalla Nageshwar Rao has satisfactorily prosecuted his course of research for a period of not less than four semesters and that the dissertation entitled "GEL ELECTROPHORETIC STUDY OF SEED PROTEINS AND ISOZYMES OF GOSSYPIUM SPECIES WITH SPECIAL REFERENCE TO DETERMINATION OF GENETIC PURITY OF CULTIVARS" submitted by him is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the dissertation or part thereof has not been previously submitted by him for a degree of any University.

Date : 7th January, 1988.

(Y.S. Nerkar)
Guide

CERTIFICATE II

This is to certify that the dissertation entitled "GEL ELECTROPHORETIC STUDY OF SEED PROTEINS AND ISOZYMES OF *GOSSYPIUM* SPECIES WITH SPECIAL REFERENCE TO DETERMINATION OF GENETIC PURITY OF CULTIVARS" submitted by Thalla Nageshwar Rao to Marathwada Agricultural University in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in the subject of Agricultural Botany (Genetics and Plant Breeding) has been approved by the Student's Advisory Committee after oral examination in collaboration with the external examiner.

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T. Nageshwar Rao
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CONTENTS

INTRODUCTION

1. INTRODUCTION

Cotton is the most important commercial crop playing a key role in the economic and social affairs of India. India, with an annual cotton production of 8.71 million bales has 7.58 million hectares of area under cotton crop, which is 25% of the world's cotton area (Anonymous, 1987). Besides providing the means of livelihood to several lakhs of farmers growing it, cotton sustains the prestigious textile industry in India. Cotton is grown in almost all the states of India. Nearly 90% of the country's cotton is produced in Maharashtra, Gujarat, Karnataka, Andhra Pradesh, Punjab, Madhya Pradesh and Tamil Nadu. The area under cotton in Maharashtra state is about 2.76 million hectares, which is 36.4% of the total area under cotton in the country (Anonymous, 1987). The State has large area under hybrid cotton both under irrigated and rainfed conditions, accounting for 10% of the total cotton area.

The hybrid H4 of <u>hirsutum</u> cotton became very popular in the seventies in irrigated as well as rainfed areas of the State. In recent years new hybrids and varieties of American cotton have been developed and released for commercial cultivation. Seed production of H4 and other hybrids has become a commercially viable agro-industry. The farmers are becoming increasingly aware of the importance of quality seed of not only hybrids but also of varieties of American and '<u>Deshi</u>' cottons. Hence the quality seed of cotton is in great demand.

Hybrid seed is produced at present by hand emasculation and pollination employing the rural labourers. There is a chance of getting selfed seed on the female plant. Owing to the high premium for the hybrid seed, the traders are occasionally tempted to adulterate the hybrid seed with the morphologically similar seed. To ensure quality and purity of seed, the certification agencies are following field plot techniques for cultivar identification. But these techniques are time consuming since the key characters are to be observed until after flowering. Moreover, these tests require field facilities on large scale. Seedling vigour tests though less reliable can also be employed. Contrary to this, biochemical tests such as electrophoresis of soluble seed proteins and enzymes are easy to carry out and a large number of samples can be handled within a short span of time in the laboratory (Kahler and Allard, 1970; Wrigley and Shepherd, 1974; Hayward and Mc Adam 1977; Arus, 1983; Cooke <u>et al</u>., 1986 and Suurs, 1987).

Several investigators have emphasised the importance of protein and enzyme electrophoresis for the identification of individuals and cultivars of different species such as wheat (Shewry et al., 1978b), barley (Mc Daniel, 1970), oats (Cooke and Draper, 1986), rice (Iwasaki et al., 1982), maize (Goodman and Stuber, 1980), soybean (Larsen and Benson, 1970), Brassica (Wills et al., 1979) and cotton (Cherry et al., 1970 and Kapse and Nerkar, 1985). Such studies authenticate the genotype basis of qualitative variation and validate the use of protein/enzyme variation in varietal identification (Mc Kee, 1973 and Douglas, 1983).

Proteins and enzymes are the primary products of the genes and hence are most suited for genetic purity determination (Niejenhuis, 1971). Changes in coding base sequence will result in corresponding replacements in the amino acids and thus in the primary structure of proteins and enzymes (Cherry et al., 1970). In the presence of an electric field and while passing through a semiporous gel medium these differences cause dissimilar forms of a protein/an enzyme i.e., isozymes (Markert and Moller, 1959) to migrate at different rates of speed, ideally producing one distinct band in the gel medium for each dissimilar protein/isozyme form. Depending on the type of protein and the enzyme under investigation an appropriate histochemical staining procedure is employed to resolve within the gel the relative position of each protein/isozyme band. These stained bands together constitute the zymogram under standardised laboratory conditions. The banding variation between varietal zymograms is attributable to genotypic differences between varieties under comparison (Tanksley and Orton, 1983a).

For comparison of varieties, the tissues sampled must be of similar physiological age and condition. A simple way of achieving this will be to use seeds (Buttery and Buzzell, 1968). Seeds are least influenced by the fluctuations in the environment and hence are most suitable for such studies. Many enzymes are expressed in seeds and the biochemical tests can be done as soon as the seed is available (Arus, 1983). The genetic purity determinations can be made even on single seeds (Wrigley and Shepherd, 1974; Shewry _et al_., 1978b; Dalling _et al_., 1979 and Gebre _et al_., 1986).

The protein and enzyme species are also least affected by the plants' growing environment (Adriaanse _et al_., 1969; Zillman and Bushuk, 1970; Fedak and Rajhathy, 1972; Sarkar and Bose, 1984 and Hussain _et al_., 1986) thus imposing no serious limitation on the use of protein/isozymes in varietal identification.

Fingerprinting of seed proteins and isozymes using the electrophoresis technique thus holds lot of promise for cultivar identification in cotton. It is in this context that the present investigation on cotton seed electrophoresis was undertaken with the following objectives :

1. To study the profiles of water soluble proteins in the seeds of selected cotton varieties, hybrids and their parents.

2. To study the profiles of salt soluble seed proteins of the selected cotton varieties, hybrids and their parents.

3. To study the isozyme patterns of some enzymes in the seeds of selected cotton varieties, hybrids and their parents.

4. To critically evaluate the seed protein and isozyme fingerprints as a substitute to field test for cultivar identification and determination of genetic purity.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Use of gel electrophoretic technique by the plant breeders in identification of cultivars started in the late 1950's after the development of starch gel electrophoretic (SGE) technique by Smithies in 1955. However, with the development of polyacrylamide disc gel electrophoretic (PAGE) technique by Davis (1964) and Ornstein (1964) its use has been increased. Further, the slab gel electrophoretic technique has eased the discrimination of cultivars under similar chemical environmental conditions.

Limited research work has been reported on the use of gel electrophoretic technique in the identification of cultivars of *Gossypium* species. Hence, an attempt has been made to review the research work done on gel electrophoresis of seed proteins and isozymes in identification of cultivars in *Gossypium* species as well as other crops.

2.1 Study of soluble seed proteins for species discrimination and varietal identification

2.1.1 *Gossypium* species

2.1.1.1 Species discrimination

After studying the seed protein banding patterns, Thein (1968) concluded that the diploid species having A genome were the parents of new world's tetraploids with *G. raimondii* as the D genome donor. Although *G. arboreum* and *G. herbaceum* were identical in protein banding pattern, *G. herbaceum* appeared the more likely A genome donor.

Cherry *et al*. (1970) analysed by PAGE the proteins from dormant seeds of wild and cultivated species and varieties within the six genome groups of *Gossypium*. There were overall similarities in the major banding patterns exhibited by varieties of the A1 and A2 genome groups. It was also found that band variations were greater between diverse species than closely related species. The differences in the banding patterns were attributed to small genetic changes in the genomes which had been selected for in the different environments. Such genetic differences would result in slight changes in amino acid composition of the proteins which in turn could lead to a variability in their mobility.

Johnson and Thein (1970) studied the electrophoretic patterns of seed extract of 25 *Gossypium* species and found them consistent with the conventionally classified diploids into six genomes (A to F).

In a comparative study of seed proteins of artificially synthesised triploid F1, colchicine induced hexaploid and the naturally occurring allotetraploids of *Gossypium* species, Cherry *et al*. (1971) found a differential additive banding pattern.

Electrophoretic study of leaf proteins of four cultivated *Gossypium* species indicated the presence of a characteristic spectrum of components at identical stages of growth. The spectrum differed during the various developmental stages (Shadmanov and Nikokiris, 1973).

Genome analysis in six *Gossypium* species was carried out by Choi and Chang (1973). Seed protein spectra were consistent with reference to genetic, cytological, morphological and phenogenetic investigation. It was also concluded that the possibility of *G. herbaceum* being one of the progenitors of the new world cotton cannot be set aside on electrophoretic evidence.

In their studies on seed proteins of three species of sub-section *integrifolia*, Shadmanov and Briskman

(1974a) opined that G. klotzschianum should be classified not as a separate species but as a botanical variety of G. davidsonii. They also concluded that G. raimondii differs markedly from the other forms in its protein spectrum. Shadmanov and Briskman (1974b) further concluded that G. hirsutum and G. glabrum shared a common genotype and that G. tricuspidatum is a geographical race of G. hirsutum.

In the tetraploid AADD genome species and some selected diploid species of cotton, Johnson (1975) found identical electrophoretic patterns of seed proteins. He was also of the opinion that the protein pattern and morphological types favour the hypothesis that G. barbadense (AADD) originated in northern South America from the cross G. herbaceum x G. raimondii and that the cultivated races of G. hirsutum represent various degrees of introgression involving G. barbadense and the Mexican G. hirsutum complex.

Cherry et al. (1977) detected seed protein variability among wild species of Gossypium. Their gel patterns also confirmed the taxonomic arrangement (genomes A to F) of Gossypium species. The protein gel patterns of cotton seed extracts from various polyploid species was very similar to the additive pattern of the parental species formed by combining preparations of the latter in a synthetic mixture prior to gel electrophoresis.

Rakhmankulov _et al_. (1980) found all the major protein bands in six different genomic taxa that were found in the parental forms except for the absence of certain minor components.

2.1.1.2 Varietal Identification

Castleberry and Coleman (1972) classified four storm proof and non-storm proof varieties of cotton into four groups on the basis of their globulin patterns.

In the studies on water, salt and alkali soluble seed proteins of various cotton cultivars Ibragimov _et al_. (1973) found differences in albumin fractions in respect of the amount of electrophoretic zones and their mobility.

The electrophoretic spectrum of soluble seed proteins of 30 _G. hirsutum_ cultivars showed marked differenes between fuzzy seeds and genetically fuzzyless seeds and also between long fibred and short fibred cultivars (Zapruder _et al_., 1980).

In an electrophoretic analysis of the soluble protein fractions in the seed of a mutant variety 59041 of _G_. _barbadense_, Yunuskhanov _et al_. (1982) found the protein markers H 0.13 and B 0.18. To this they opined that the gene for protein B 0.18 has been replaced in the mutant by the gene for H 0.13. This was further substantiated by the presence of fuzz on the chalazal section and lateral surface of the mutant seed like that in _G_. _hirsutum_ seed controlled by the gene Fifi which evidently was linked with the gene for protein marker H 0.13.

Kapse and Nerkar (1985) studied disc electrophoretic patterns of soluble seed proteins in four intra _G_. _hirsutum_ hybrids, two _G_. _hirsutum_ x _G_. _barbadense_ hybrids, parents of the hybrids and two varieties each of _G_. _hirsutum_ and _G_. _arboreum_. They observed unique differences in banding patterns of the cultivars. They opined that this technique could be useful to identify cotton cultivars and can be complementary, if not a substitute, to field test for the determination of genetic purity of seed.

2.1.2 Wheat and related species

Based on the electrophoretic pattern, Chen and Bushuk (1970) reported that the proteins of triticale are inherited from its parents - wheat and rye. Further, they observed differences in the pattern of gliadins and glutenins in a durum cultivar and a cultivar of hard red spring wheat and opined that these qualitative difference might account for the difference in their bread making quality and provide additional evidence in support of the hypothesis that genes for bread making quality factors are in the D genome.

Zillman and Bushuk (1970) observed that seed source had no effect on gliadin electrophoregrams of 5 Canadian wheat cultivars grown at 10 locations in Manitoba and Saskatchewan nor the 5 Australian cultivars grown in Canada and Australia. Their results confirm that the gliadin electrophoregram is independent of environment and substantiate the suitability of the electrophoregram for wheat cultivar identification.

Ellis (1971) reported that SGE of grain proteins made it possible to place a number of wheat cultivars in groups and 17 cultivars could be identified by a combination of their electrophoretic pattern, phenol reaction, coleoptile anthocyanin and grain hardness.

Three lab procedures, namely, phenol reaction, hardness testing and electrophoresis were examined by Wrigley and Shepherd (1974) for identification of about 50 wheat cultivars grown in Australia. They reported that the most discriminating of these methods was SGE of gliadin proteins extracted from single grains.

Dhaliwal (1977) electrophoresed albumins, globulins, gliadins and glutenins of several species of Triticum and Aegilops on SDS-PAGE. He observed that electrophoretic patterns differed from one species to another only in a few minor components or density of certain components.

Shewry et al. (1978b) reported that the SDS-PAGE technique is more sensitive in distinguishing gliadin of different wheat varieties than SGE.

Du Cros and Wrigley (1979) compared several electrophoretic methods in terms of their reliability, convenience and ability to distinguish between varieties of wheat, barley, rye and triticale using proteins extracted from the endosperm. Gradient gel electrophoresis and isoelectric focussing (IEF) offered improvement over the use of SGE and uniform pore polyacrylamide gels.

Zillman and Bushuk (1979) obtained 85 distinct gliadin electrophoregram formulae for the 88 Canadian wheat cultivars studied and observed only 3 pairs of formulae essentially identical which they attributed to very close genetic relation among these 3 cultivars.

Fullington _et al_. (1980) observed that each of the 5 wheat varieties exhibited at least one outstanding feature in its densitometric pattern when the total protein extract from single seed was studied by SDS-PAGE.

Jones _et al_. (1982) determined and catalogued 88 United States wheat cultivars based on PAGE patterns of their gliadins. Most of the cultivars were readily differentiated by their electrophoregrams. However, some closely related cultivars gave them ideal patterns and were thus not uniquely identifiable by PAGE.

Damania *et al*. (1983) examined prolamins in 3168 single seeds of 64 land races of wheat and barley from Nepal and Yumen Arab Republic by vertical slab PAGE method. They reported that this technique is easy and rapid in assessing the extent of variation present within the population of land races.

Mansur-Vergara *et al*. (1984) used the technique of SDS-PAGE of total protein extraction to estimate the storage protein genetic variability among 841 accessions of wild emmer wheat collections from various ecological regions in the Middle East. The computerised densitometer scanning of protein bands indicated that the greatest genetic variability occured for proteins in the high molecular weight region (above 70,000 MW) followed by those in the medium range (70,000 to 33,300 MW). Comparatively little variability was revealed for protein sub-units of below 33,300 MW.

Studies of Virdi and Larter (1984) revealed that polymorphism can exist in the protein banding patterns of any given population such as the triticale amphidiploids and their parents when prolamins were extracted and analysed by PAGE. The degree of polymorphism was greatest in triticale populations synthesised from open pollinated heterozygous rye strains.

Burgoon et al. (1985) devised a method to detect non-durum wheat gliadin proteins in pasta using aluminium lactate-PAGE at pH 3.1. Pastas were prepared from various mixtures of durum and hard red winter wheat flours and gliadins were extracted. Zymograms of pasta extracts matched those of the corresponding flour extracts. Durum wheat extracts lack certain slow moving gliadin protein bands present in other flours. Pasta adulterated with as little as 5% hard red winter flour could be detected with this method.

Damania (1985) analysed gliadins of tetraploid and hexaploid wheats of Nepal by Al-lactate PAGE method. The tetraploids lacked slow moving bands in the 'omega' region found in hexploid wheats. These bands also aided in identifying different hexaploid wheat cultivars.

Khan et al. (1985) investigated the PAGE procedure to determine its suitability for wheat variety identification by analysis of gliadin proteins. Gels of varying firmness were obtained by varying relative amounts of catalysts. Gel firmness and pore size affected resolution of the gliadin proteins.

Sapirstein and Bushuk (1985) described a computer aided methodology that facilitates identification and comparison of wheat cultivars based on gliadin relative mobility and band density data. The data was used in an equation to quantify electrophoretic pattern homology that determines the order of cultivar ranking. A separate computer programme evaluates the uniqueness of the unknown electrophoregram and identifies diverse genotypes. Their results (Sapirstein and Bushuk, 1986) provided strong evidence of the influence of gliadin composition to discriminate wheat cultivars according to functional type.

Cooke *et al*. (1986) described two methods of electrphoresis which enabled seeds of bread wheat, durum, wheat, rye and triticale to be characterised and distinguished from each other. The variation in prolamin and estarases composition both within and between different cultivars of the species was examined and generalised protein pattern for each species was presented. Thus they concluded that different electrophoretic techniques can provide a very rapid, definite way of characterising and distinguishing between seeds of various cereal species and opined that this is another example of the increasing utility of electrophoresis in the areas of plant genetics, breeding and testing.

Zawistowska *et al*. (1986) observed that PAGE and SDS-PAGE patterns were similar for both Neepawa and Chile varieties of wheat but reversed phase - high performance liquid chromatography (RP-HPLC) patterns were quite different for low molecular weight(s) proteins.

2.1.3 Hordeum species

Mc Daniel (1970) characterised individual seed protein of selected Hordeum cultivars by disc gel electrophoresis procedure and reported that cultivars related by descent, breeding or geography were more similar in protein phenotype than relatively unrelated cutivars. Variations in proteins between cultivars of H. vulgare were greater in some cases than protein variations between H. vulgare, H. spontaneum and H. irregular. Further he opined that this procedure may be particularly important as an initial screening tool in the effective production of heterotic barley hybrids.

Mc Causland and Wrigley (1977) examined endosperm proteins of 19 barley cultivars. They obtained 7 different patterns for hordeins by SGE and 13 different patterns by flat gel IEF of water soluble and hordein proteins, which aided in cultivar identification in barley.

Shewry et al. (1978a) compared the polypeptide composition of the hordein fractions from 29 varieties of barley on SDS-PAGE and IEF. A total of 11 different patterns were recognised using the former system while further small differences were revealed by IEF and 2-dimensional analysis. They reported that if these techniques are considered along with other grain charcters it would be possible to identify all the 29 varieties.

Shewry et al. (1978c) compared the polypeptide composition of hordein polypeptides of single seeds of 88 varieties of barley on SDS-PAGE. They reported that this technique is of great potential value both for the commercial identification of grain samples and as an aid to existing techniques for establishment of varietal distinctness.

Burbidge et al. (1986) established methods of barley identification such as visual examination and gel electrophoresis. However, they reported that high performance liquid chromatography (HPLC) offers speed, automatic sorting of results and distinction between some similar varieties.

Gebre et al. (1986) used PAGE of hordein polypeptides to identify and catalog 40 barley cultivars. An electrophoregram formula was prepared for each cultivar using relative band mobility. Only 10 cultivars showed unique hordein band patterns and rest were separated into 17 groups, each group consisting of several cultivars. Some differences mainly due to presence or absence of faint bands were observed among cultivars within groups. In general, they reported that single kernels gave better resolution than meal samples.

Nielsen and Johansen (1986) opined that the hordein PAGE pattern was clearly the most powerful single system for identifying barley cultivars.

2.1.4 *Avena* species

Murray *et al*. (1970) studied seed proteins of 3 synthetic ampidiploids, 1 autotetraploid and 8 species that include representatives of all ploidy levels and karyotypes of *Avena*. They reported that A and C genome diploids were readily separated by band differences. The A genome protein patterns were essentially identical while the C genome patterns differed primarly in band number. The tetraploid protein patterns differed but the hexaploid had high homology. However, intra-specific polymorphism was detected in the hexaploid cultivars.

Cooke and Draper (1986) examined the soluble proteins of single seeds of different species of *Avena* and cultivars of *A. sativa* by PAGE using lactate buffer. Considerable polymorphism was observed by them in the avenin composition of the different species. It was possible to identify the species of wild oats unequivocally and to distinguish them from cultivars of *A. sativa*. The method was applicable to both whole and broken caryopses. Hence they opined that this technique provides a definite reference technique for the identification of *Avena* species in seed testing laboratory.

2.1.5 Rice

Studies of Siddiq *et al*. (1972) revealed that there were marked variations in the seed protein banding pattern within and between three sub-species of *Oryza sativa*. Variability for number and intensity of protein bands was wider in *indica* than in *japonica* and *javanica* sub-species.

Iwasaki _et al_.(1982) studied proteins extracted from milled rice of long, medium and slender grain varieties by SGE. Twenty bands were resolved for both albumin and globulins, which supported the idea of classifying rice varieties into groups on the basis of electrophoregrams.

Chauhan and Nanda (1984) tested the efficacy of certain physio-chemical characters of grain and electrophoretic methods in rice varietal identification. They observed a wide variation in the pattern of protein bands, their electrophoretic mobility and intensity. However, they reported that the electrophoretic spectrum of salt soluble seed proteins exhibited an ambiguous picture and resulted in controversy in classification to that of physio-chemical characters. Hence they suggested that instead of total salt soluble seed proteins, the variations in the specific proteins (enzymes) may be screened to explore their possible use in varietal identification.

On the contrary Sarkar and Bose (1984) observed both qualitative and quantitative differences in electrophoregram tracings in salt soluble protein fractions in a number of rice varieties. Analysis of variance was found to be useful in estimating the quantitative differences. The tracings of pattern appeared to be unique for each of the varieties investigated and remained constant under different environmental conditions. They suggested that this technique could be conveniently used for variety identification in rice.

Similarly Guo *et al*. (1986) examined the prolamin fractions by electro-focussing and categorised 25 varieties of Chinese rice into 4 major groups. Four major protein bands allowed differentiation of *indica*, *japonica* and glutinous rices and their hybrids. Minor bands allowed further differentiation within each group.

2.1.6 Sorghum

Chavan *et al*. (1980) investigated the soluble proteins of high and low tanin sorghum cultivars by disc gel electrophoresis. They observed that while in the low tanin cultivars IS4129 and IS3441 17 and 16 bands were resolved respectively, not a single band was resolved in the high tanin cultivars. Further they opined that tanin forms complexes with proteins during extraction and alters their properties, i.e. binding of tanins will increase the molecular weight of protein which may block the entry of such complex into the gel.

Tripathi et al. (1981, 1983) made comparison of protein patterns in anthers and seeds of 6 different male steriles, maintainers and fertility restorer lines of sorghum by disc gel electrophoresis. They observed both qualitative and quantitative differences in soluble protein patterns from sterile and maintainer lines.

Using vertical slab PAGE and IEF, Tripathi et al. (1982) studied the soluble protein patterns from seeds and anthers of 24 different male steriles, maintainers and fertility restorer lines of sorghum. They observed consistent qualitative and quantitative differences between the lines and opined that these techniques are useful in characterising male steriles.

Sastry et al. (1986) investigated the genetic variability of storage proteins in the grain of several inbreds, hybrids and varieties representing different races of sorghum by IEF and HPLC. It was observed that grains of different inbreds usually contained different kafirin and alcohol soluble proteins. The hybrids contained proteins inherited from both parents with those from the female predominating. Representatives of the different sorghum races varied in their component alcohol soluble proteins, those most widely removed from the geographical centre of origin showing great differences.

2.1.7 Maize

Wall et al. (1984) observed that the electrophoretic patterns of zeins from extracts of different inbreds have different components and hybrids contain polypeptides from both parents, but the female parent's contribution was quantitatively greater. They also recorded that inbreds which were derived from crosses usually contained some zeins from both parents. They concluded that electrophoretic patterns can characterise zeins in corn inbreds and can aid in relating corn hybrids and inbreds to parental lines.

Wilson (1985) studied endosperm zeins of different corn inbreds by IEF on agarose gels, which were resolved into 30 components. Each inbred exhibited 7 to 12 bands which were useful for comparing inbreds, varieties and hybrids. A nomenclature based on IEF patterns of 6 inbreds permitted comparisons of many possible patterns. Closely related inbreds were often very similar but some differences occured.

Smith and Wych (1986) used electrophoretic and morphological traits to estimate the percentage of female selfed plants in seed lots of 4 hybrid maize cultivars at contamination levels of 0, 3, 6 and 12%. Field determinations of contaminants were carried out at seedling, anthesis and maturity stages while electrophoretic tests were performed on coleoptile tissue from 5-day old seedlings. Results based upon the morphological comparisons at seedling and anthesis stages were highly inaccurate. Field comparison at maturity were generally accurate. However, electrophoresis results were consistently more accurate with added advantages of speed and ability to determine the percentage of female sibs prior to harvest.

2.1.8 Soybean

Seed proteins of 61 soybean varieties were analysed by Larsen (1967) on disc gel electrophoresis. The studies revealed two components of stained proteins that separated the varieties into two major groups. Component A was present in 13 varieties and component B in 48 varieties. In no instance were A and B observed in a single variety. Further Larsen and Caldwell (1968) reported that when a variety with A seed protein was crossed with a variety with B seed protein, the seed from the cross contained both proteins but at lower concentrations of each than when occuring alone. Selfed F1 plants produced F2 seed of A, AB and B types in a ratio of 1:2:1 respectively. The data indicated that the proteins are controlled by a pair of codominant alleles at a single locus.

By electrophoresis of seed proteins on gradient polyacrylamide slab gels, Lowry et al. (1974) detected differences between Lee and Pickett varieties of soybean and mutants.

Orf et al. (1980) studied the PAGE patterns of seed protein extracts from 3338 soybean accessions. They reported that the technique was useful in classifying soybean cultivars into broad categories.

Chauhan et al. (1985) investigated electrophoretic variations in proteins and enzymes in dry and germinated soybean and barley seeds by PAGE. Their results indicated that electrophoretic profiles of proteins and enzymes are species specific and can act as markers in assessing seed quality.

2.1.9 Beans

Characterisation of 32 cultivars of *Phaseolus vulgaris* was done by densitograms of water soluble proteins separated by SGE (Adriaanse et al. 1969). This study also indicated that the protein patterns are unlikely to be affected by external conditions such as nitrogenous fertilization, climatic conditions and soil properties.

Ziegenfus and Clarkson (1971) reported that PAGE of salt soluble seed proteins was more sensitive than immuno electrophoresis and double diffusion in detecting differences among the 7 taxa investigated in the genus *Acer*.

Blagrove and Gillepse (1978) examined the extracts from 80 pure lines of winged bean (*Psophocarpus tetragonolobus*) by PAGE and observed differences in the proportion of one seed globulin polypeptide among the lines studied.

Barratt (1980) examined the globulin fraction from *Vicia faba* seeds of different cultivars and different lines of the same cultivar by SDS-PAGE. The technique separated the globulin polypeptides into distinct groups termed as A, B and C. Distinct differences between cultivars and the different lines of the same cultivar were observed in group A and B polypeptides with little variation in group C. Variation in polypeptide composition of different cultivars was also detected in respect of virilin and legumin by SDS-PAGE.

Wolff (1980) investigated the seed albumins of several members of the family Papilionaceae by gel electrophoresis. He found relationships between mutants, varieties and sub-species. More distant related once did not show similarities in banding pattern. He also observed that the environment had no influence on the albumin pattern if genetically identical material of Pisum sativum was grown in different places and different vegetation periods.

Hussain et al. (1986) studied seed proteins by SDS-PAGE to discriminate 7 different cultivars of field beans. Their results indicated that sufficient variation was present among the 7 cultivars to afford their unambiguous identification.

Przybylska (1986) reported that the electrophoretic patterns of the legumin fraction obtained by means of urea-PAGE and IEF and the isoenzyme phenotypes were useful in discriminating between and within collection samples of Pisum.

2.1.10 Groundnut

Dawson and Mc Inston (1973) analysed arachin and conarachin fractions extracted from 8 samples of groundnut varieties grown in different locations. They observed marked variations in the electrophoretic patterns especially of conarachin fractions. Amino acid composition of the fractions showed highly significant differences.

2.1.11 Forages

In their studies on 30 alfalfa varieties and 26 species accessions of *Medicago sativa* and *M. falcata*, Bingam and Yeh (1971) observed that protein patterns, in general, were basically similar in cultivated and wild materials and at diploid and tetraploid levels. One major band was present in *M. falcata* and absent in pure *M. sativa*. Further they separated the cultivated varieties into 4 major groups on the basis of the absence or presence and density of one main band.

Wilkinson and Beard (1972) could distinguish 6 creeping bent grass (Agrotis palustris) cultivars by their leaf protein banding patterns. Out of the 10 Kentucky blue grass (Poa pratensis) cultivars, 6 could be placed into two groups and 2 could be identified singly, while 2 showed no characteristic banding pattern. Hence they suggested that cultivar identification need not rely on electrophoretic pattern alone.

Dalling et al. (1979) separated seed globulin proteins of 17 sub-terranian clover (Trifolium subterraneum) cultivars using PAGE. They observed that each cultivar had a unique banding pattern and suggested that the technique could be useful to the plant breeder for cultivar identification and determination of genetic purity of a seed sample.

On the basis of the disc electrophoretic analysis of seed proteins and enzymes of diploid and tetraploid species of Italian rye grass (Lolium multiflorum) and perennial rye grass (Lolium perenne), Nakamura (1979) recommended esterase analysis for the separation of rye grass species.

De Prins and Van de Weghe (1983) characterised some Italian rye grass (Lolium multiflorum) and perennnial rye grass (L. perenne) cultivars by seed protein and esterase banding patterns on pH gradient polyacrylamide gels. They suggested that the technique can be used for rapid varietal identification. Further they opined densitometric scan of the gels may make identification much easier and possibly allow detection of mixtures of cultivars.

2.1.12 Poinsettia

Soluble leaf proteins from 18 cultivars of Poinsettia were analysed by Werner and Sink (1977) using disc gel electrophoresis. They reported that the soluble protein banding pattern was the same for all the 18 cultivars.

2.2 Study of isozymes for species discrimination and cultivar identification

2.2.1 *Gossypium* species

In the PAGE analysis of the esterase (EST) isozymes of dormant seeds of 29 species and 29 varieties in the genus *Gossypium*, Cherry and Katterman (1971a) observed that species and/or varieties within each genome had banding patterns more similar to one another than to members in the other genome groups. Only minor differences distinguished the A and B genome species whereas band variations were greater between more distantly related than closely related species in the C, D and E genomes. *G. longicalyx* (formerly considered an E genome species) showed an overall banding pattern unique to itself. These results further supported the classification of the diploid species into the presently recognised six genome groups A to F). Variation was observed also for leucine aminopeptidases (LAP) and catalases (CAT) between the species of different genome groups (Cherry *et al.*, 1972), though the within species polymorphism noted for EST was not observed for LAP and CAT.

Cherry and Katterman (1971b) observed six different EST zymograms for the single seed samples of four natural populations of *G. thurberi* and suggested that intraspecific variation should be evaluated before any interspecific analyses are undertaken.

Hancock (1982) studied electrophoretic variation with respect to alcohol dehydrogenase (ADH) in the tetraploid species *G. hirsutum* and the diploid species *G. raimondii*, *G. herbaceum* and *G. arboreum*. He observed both four and six band patterns in *G. hirsutum* while only three bands in all the diploids. The patterns observed were *G. raimondii*- A, *G. hirsutum* - B and C, *G. arboreum* - D and E and *G. herbaceum* - D. Significant differences in the activity in response to change in reaction conditions were found between several isozyme patterns in C, D and E but not in A or B.

2.2.2 Wheat and related species

Within a single species of *Triticum*, Kobrehel and Gautier (1974) observed that the electrophoretic patterns of peroxidase (PER) may differ from one variety to another.

Thus two classes of *T. aestivum* can be distinguished. Further they reported that growing conditions have no influence on isozyme pattern.

Cubadda *et al*. (1975) studied the EST isozyme banding pattern in caryopses of *Triticum aestivum*, *T. durum* and *T. timopheevi* by gel electrofocussing. In *T. aestivum* 17 bands were ascertained in the pH 5-8 range. 11 were of higher intensity, 4 were weak and 2 very weak. Using Chinese spring nullitetrasomic lines it was possible to locate the genes controlling several isozymes on the chromosomes of the homoeologous group III.

Fejer *et al*. (1979) resolved 6 ADH bands in different hexaploid wheat sub-species and varieties by IEF - PAGE. Further they reported that this is in full accordance with the hypothesis that the active ADH isozymes in hexaploid wheat are dimers composed of 6 possible combinations of sub-units coded by triplicate structural genes.

Characteristic amylase (AMY) banding patterns were obtained by Mosoji (1984) for each of the 4 inbred lines of rye during different stages of grain development.

Ramirez and Pisabarro (1985) studied isozyme patterns of PER, AMY and alkaline phosphatase (AKP) to characterise and classify 6 rye seed samples. The different electrophoretic patterns allowed them to distinguish each sample.

Smith (1986) reported that the morphological characters cannot accurately describe or portray relationships among cultivated germplasm of rye and opined that biochemical data (isozyme electrophoresis and RP-HPLC) provided detailed and highly discriminative varietal profiles for wheat, barley, maize, sorghum and oats.

By SGE, Vapa and Hart (1987) determined zymogram phenotypes of 14 enzymes for 80 Yugoslav wheat cultivars. For 6 enzymes the zymogram phenotypes were observed to be different from the one produced by _Triticum aestivum_ cv. Chinese spring.

2.2.3 Barley

In barley, Kahler and Allard (1970) observed that banding pattern for EST isozymes in the 30 parents of a composite cross are governed by 7 loci and that the allelic status of individual seedling can be determined precisely for each locus by SGE. Further they reported that large number of individuals can be scored rapidly and inexpensively.

Fedak and Rajhathy (1972a) analysed 55 Canadian barley cultivars and some parental genotypes by SGE for EST isozyme patterns. Each cultvar was assigned to one of the 9 different patterns observed. They reported that the polymorphism was a varietal characteristic and apparently not associated with age of the cultivar or area of adaptability.

Fedak and Rajhathy (1972b) studied isozyme patterns in hybrid barley and found the hybrid plant to contain the sum of parental bands for PER, EST and AMY isozymes. Genetic studies of EST and AMY isozymes revealed the additive type of gene action governing band variation between parents, while combination of epistasis and over-dominance type of gene action were observed in the hybrid.

Out of the three isozyme systems viz., EST, PER and acid phosphatase (ACP) studied in shoot extracts of barley by Bassiri (1976), the EST alone could be used to differentiate between most of the cultivars.

Andersen (1982) examined 47 barley cultivars for AMY isozymes in sprouted grains and EST in the first developed leaf. He concluded usefulness of the AMY and EST isozymes as a valuable supplement in identification of barley cultivars in the laboratory.

2.2.4 Oats

Singh et al. (1973) investigated ten cultivars of oats for the isozymes of EST, LAP and PER. They observed that each variety had a stable and characteristic isozyme pattern which makes this technique useful for varietal identification.

2.2.5 Rice

Isozyme polymorphism for EST was studied by Nakagahra _et al_. (1975) in 776 native varieties of _Oryza sativa_ collected from Asian countries. Horizontal agar gel thin layer electrophoresis was used to separate the enzymes from leaf extracts. The EST isozymes occured in altogether 14 bands, of them 9 were easily distinguishable and their combination composed 27 different zymograms indicating the presence of substantially complex variation. Each isozyme differed in its frequency of occurence in each of the 8 areas extended from Sri Lanka to Japan.

Pai _et al_. (1975) analysed statistically the data taken from densitometric tracings of ACP zymograms of leaf blade extracts of _Oryza_ species. Their results indicated that hybrids between strains with different mobilities would produce three major and three minor hybrid bands each occurring between two corresponding parental bands.

Chern and Katayama (1982) studied the zymogram pattern of ACP isozymes in the leaf blade extracts of 3747 varieties and strains of _O. sativa_ collected from all over the world. A total of 9 bands were observed, one band was common to all the material examined. The cultivars studied by them were classified into 4 groups based on the ACP zymograms.

Second (1982) studied isozyme patterns of 13 enzymes including EST, MDH, ADH, CAT and ACP in the leaf blade extracts of 1948 strains of *O. sativa*, *O. glaberrima* and *O. breviligulata* by SGE. The polymorphism of the banding pattern was high in *O. sativa*.

Enzymatic variation detected by SGE was used by Glaszmann (1987) to investigate the genetic structure of *Oryza sativa* L. species. He observed 15 polymorphic loci coding for 8 enzymes in the 1088 traditional rices from Asia. The data resulted in the identification of 6 varietal groups of which group I is considered as *indica* type and group VI as *japonica*. He further suggested that isozymes permit early and fast assessment of the nature of a variety which is independent of environment and hence recommended this technique for varietal identification.

Tomar and Choudhary (1987) analysed disc electrophoretically 3 each of dormant and non-dormant varieties of rice for ribonucleases in embryo and endosperm of the presoaked seed, leaf and root tissue of 7-day old seedlings. The isozyme variations for ribonucleases I & II were observed only in the embryo. The bands 3 & 4 were specific and associated with varieties showing grain dormancy.

2.2.6 Sorghum

To compare different male sterile, maintainer and restorer lines of sorghum, Tripathi et al. (1981, 1983) studied EST, PER, ADH and GDH isozyme patterns by PAGE of anther and seed extracts. Based on EST isozyme patterns and densitographs, the diverse male steriles with different cytoplasms were classified into three groups. Each group was further differentiated on the basis of minor differences in EST isozyme pattern. PER patterns showed characteristic but not clearcut differences. ADH and GDH isozyme patterns in general were similar in both sterile and maintainer lines.

Using PAGE and IEF, EST isozyme patterns of seed and anther extracts of 24 cytoplasmic genic male sterile sorghums were studied by Tripathi et al. (1982). EST isozyme patterns of milo based male steriles differed from male steriles of diverse Indian origin and thus served as aid in characterising male sterile sorghum stocks.

A study of PER and ACP by SGE of anther extracts was made by Phul et al. (1987) to characterise 5 sorghum male steriles and their maintainers. Differences in presence/absence and intensity of bands were observed for the isozyme patterns of all male steriles and their corresponding maintainers. ACP did not show as much divergence as observed for PER. Thus they opined that isozyme patterns play important role in characterising such stocks.

2.2.7 Maize

Hunter and Kannenberg (1971) used isozyme variability as an indicator of genetic diversity in maize. 15 inbred lines of maize were characterised for EST, AMY, LAP, CAT, ADH and PER isozymes. Differences in these isozyme systems were used to calculate diversity index.

Mac Donald and Brewbaker (1972) reported that two maize transaminase isozymes Ta_1^1 and Ta_1^2 were found to be controlled by a single locus Ta_1. Ta_1^1 homozygotes proved to be the slower variant than Ta_1^2 homozygotes. Heterozygotes displayed three isozymes i.e. Ta_1^1 , Ta_1^2 and a third isozyme intermediate in migration between the two parental types.

Using SGE, Goodman and Stuber (1980) could fingerprint most of the maize inbred lines surveyed by them in United States and Canada by analysing 13 isozyme systems including EST, MDH, ADH, CAT and ACP in coleoptiles of 5-day old seedlings. Further they opined that laboratory genotyping or isozyme fingerprinting can be used for establishing cultivar identity or uniqueness and can complement, if not substitute, field test of various seed lots.

By analysing 12 isozyme systems on SGE, Cardy (1981) could obtain 146 unique fingerprints of the 155 Canadian maize hybrids (single cross, double cross and 3-way cross) studied by him.

2.2.8 Soybean

Buttery and Buzzell (1968) discovered varietal differences for PER activity following electrophoresis of seed extracts of soybean. They detected presence of one main band and at least two minor bands of isoperoxidases in 'Harosoy 63' which were absent in 'Black hank'.

Variety specific zymograms of PER isozymes were obtained by Larsen and Benson (1970) in the extracts of dry soybean seeds, which indicated usefulness of the technique in characterisation of soybean varieties. Gorman and Kiang (1977) examined a number of commercial soybean varieties and found them to be displaying 1 to 3 banding patterns for each of the four enzymes studied by them.

Payne and Koszykowski (1978) analysed seed extracts of 44 soybean cultivars by PAGE for EST activity. They consistently detected five EST isozymes in all the cultivars. Quantitative differences were observed among the cultivars in the activity of two isoesterases which could serve as an aid in identifying soybean cultivars.

Wagner and Mc Donald (1982) examined several rapid laboratory tests to determine their usefulness in characterising 36 soybean cultivars. The tests which successfully differentiated the cultivars included the electrophoresis of amylase and urease in unimbibed seed.

2.2.9 Beans

Based on the study of EST and PER isozyme pattern by SGE, Bassiri and Rouhani (1977) could identify 40 broad bean cultivars and reported that the differences in banding patterns among cultivars of the same region were as great as those of the cultivars from unrelated regions.

Using SGE, Bassiri and Adams (1978a) studied EST, ACP and PER isozyme patterns in primary leaves, stems and roots of 13 species of the genus Phaseolus. Most species were observed to exhibit unique banding patterns in different tissues for each isozyme system.

Among the isozyme systems studied in primary leaf, stem and root tissue from seedlings of 34 United States common bean cultivars by Bassiri and Adams (1978b), PER and EST were found suitable for cultivar identification while ACP could not be considered as a good system for such purpose.

Mahmoud _et al_. (1984) examined three isozyme systems, namely AMY, EST and GOT in the seeds of Pea. Clear variation was observed in the banding patterns between different lines. Their investigations revealed that each of the isozyme systems studied is genetically controlled by codominant alleles at a single locus.

Janardhanan _et al_. (1986) investigated changes in isozyme profiles of 5 enzymes that occur during seed development and germination in wild pulse (_Mucuna utilis_) by PAGE. Significant observation was that, for all the 5 analysed enzymes, the isozyme patterns towards the end of seed maturation and early germination were more or less identical and can be used effectively in the cultivar identification.

Isozymic variation was used by Mittal et al. (1987) for estimating the genetic diversity in green gram. Eight varieties were characterised for EST, AMY, ACP, CAT and PER isozymes. ACP and EST exhibited more polymorphism than others. Differences in these systems were used to calculate the diversity index (DI).

2.2.10 Safflower

Bassiri (1977) utilised the ACP and PER isozyme patterns of seedling roots to identify 9 introduced and 5 local cultivars of common safflower. Based on the similarity of the banding patterns for both the systems, he opined a close genetic relationship exists between *Carthamus oxyacantha* and *C. tinctorius*.

2.2.11 Tomato

Tanksley and Jones (1981) subjected ADH in seed extracts of a number of tomato cultivars by SGE. The allozymic variation was used to determine genetic purity of F1 hybrids. Of the tested varieties, 46% proved amenable to the test.

2.2.12 Brassica species

Acid phosphatase (ACP) isozyme patterns in the seed of Brassica oleraceae and Brussels sprout cultivars were analysed by Nijenhius (1971). The ACP isozyme located in the 15-20 mm region of the electrophoregram proved to be controlled by one locus consisting of 4 alleles acting without dominance. As a result, the ACP pattern in pure inbred lines was characterised by a single band in this region, while crossing the inbred lines with a different allelic constitution gave hybrid seed with the related double band pattern. Thus it was possible to determine the proportion of inbred seed in the hybrid seed sample.

Woods and Thurman (1976) observed three ACP isozymes in the F1 hybrid seeds of Brussels sprouts. They reported that ACP are suitable for the determination of sibs in F1 hybrid seed, provided one of the parent possesses the slow moving ACP and the other fast moving one.

Wills et al. (1979) studied 14 enzymes in the seed extracts of B. oleraceae cultivars by PAGE. Half of the enzymes studied gave discrete bands which were utilised to determine sib frequencies in hybrid cultivars.

Yadava et al. (1979) carried out comparative electrophoretic studies of protiens and enzymes of different Brassica species and naturally occuring amphidiploids. They observed considerable variation with respect to soluble protein and EST isozyme patterns, contrary to the PER and CAT isozyme patterns.

Wills and Wiseman (1980) separated by PAGE the ACP isozymes in crude extracts of cotyledons of 2-day old seedlings in 19 hybrid B. oleraceae cultivars. At least 22 bands distributed over 7 zones were recognised. Analysis of zone I allowed the estimation of sib frequencies in 12 out of the 19 hybrids. By the analysis of zone I in either seedlings or seeds, sib estimates could be made in 15 of these hybrids.

Arus et al. (1982) developed statistical models to estimate the seed purity based on the isozyme phenotypes of the parental inbreds and corresponding F1 hybrid seed lots. While working with _B_. _oleraceae_, estimates of contaminations ranged from 1.5 to 40.1% which were comparable with those from commercial grow out tests.

Arus _et al_. (1985) analysed six isozyme genes in seed samples of 65 commercial F1 hybrids of 4 horticultural groups of _B_. _oleraceae_. Their results indicated that by the electrophoretic test, it was possible to determine purity in 91% of the hybrids analysed and 74% of the hybrids were individually distinguishable by their isozyme phenotype.

Kumar and Gupta (1985) made a study of seed PER and EST isozyme patterns by SGE to characterise 12 genotypes of Indian mustard. Their study showed same number of bands for PER and varying number for EST isozyme. They ascertained that these patterns especially the staining intensity of bands can be used successfully to characterise different Indian mustard genotypes.

Schenck and Wolf (1986) obtained three somatic hybrids by fusion of protoplasts of _B_. _oleraceae_ and

B. campestris. Gel electrophoretic patterns of the fusion product were compared with their respective parental species. By comparing multiple forms of EST and phosphorylases they demonstrated that in all the cases the hybrid plants contained one or more enzymes from each parent.

Suurs (1987) proposed a routine method of large scale electrophoresis for use in plant breeding which does not require sophisticated apparatus. The method was used to test the percentage of sibs in a hybrid seed lot by separation of ACP isozyme in F1 seed of B. oleraceae. He further reported that a skilled lab technician can handle 768 samples a day.

2.2.13 Forages

Out of the 15 Kentucky blue grass cultivars studied for the PER isozymes, Werner et al. (1976) could characterise 11 of them individually while 4 were grouped into two groups.

Spoor and Hay (1979) investigated the use of PAGE for identification of cultivars of *Poa pratensis*. Among 16 lines and 14 ecotypes studied by them, considerable variation was observed in the banding pattern of seed EST and PER isozymes.

Quiros (1980) studied the applicability of SGE in the identification of alfalfa mother plants. He observed that the zymograms of PER, EST and ACP from leaf tissues were useful in 'fingerprinting' each of the 21 mother plants tested. He concluded that SGE is a simple, inexpensive and reliable technique for the identification of alfalfa plants.

Quaite and Camlin (1986) produced seed by allowing inter pollination in isolation among the selected genotypes of perennial ryegrass for specific phosphogluco isomerase (PGI) alleles. The experimental seed lots when examined for electrophoretic purity were found to exhibit only the selected PGI genotype. Thus they reported that the use of multilocus techniques could permit electrophoresis to play some future role in the registration of new perennial rye grass cultivars.

2.2.14 Nicotiana species

Smith et al. (1970) observed unique banding patterns of seedling root PER and seed EST in 61 and 55 species of Nicotiana respectively. They reported that no single specific band was common to all Nicotiana species. Comparsion of PER and EST zymograms of 17 artificially synthesised amphidiploids revealed that the bands had mobility as in one or both parents only 25% being new (possible hybrid) bands. They concluded that this method of band assessment is a reliable measure of genetic similarity.

Wilkinson et al. (1985) performed isozyme separations using PAGE for EST, CAT, PER and MDH from leaf samples of 10 tobacco cultivars. Leaf material from the earliest maturity dates exhibited the highest number of isozymes. No cultivar differences were observed for EST. Peroxidases and catalases exhibited the greatest potential for use in cultivar identification.

2.2.15 Cassava

Hussain et al. (1987) developed a methodology for determining the EST isozyme pattern by PAGE in the viable root tissue extracts of 20 cassava cultivars. Each of the 20 cultivars was characterised by a unique band pattern. The patterns were observed to be highly reproducible in band mobility and intensity. Further they reported that this method can be used to identify unknown cassava cultivars by comparing their EST isozyme pattern with those of known cultivars.

Ramirez et al. (1987) developed a methodology based on SGE and PAGE for determining isozyme electrophoregarms of 16 enzymes of cassava varieties as potential genotypic markers. Extracts of 5 different tissues (root, stem, leaf, petiole and bud) were examined. They observed that the nodal portion of the shoots gave isozyme patterns with the largest number of bands. The limited number of varieties that were examined could be distinguished by sequential classification on the basis of the isozyme patterns of ACP, EST, GOT and PGI. Esterases which are a complex group of isozymes showed a large variation in the fast migrating zone with little variation among different tissues.

2.2.16 Pear

Santamour and Demuth (1980) reported that the PER isozyme banding patterns of leaves and cambial tissues on starch gels were sufficient to distinguish among six cultivars of callery pears (*Pyrus calleryana*). Examination of seedlings derived from natural cross pollination among the three cultivars indicated that similar techniques could be used to identify many cultivars that might be developed in future.

2.2.17 Strawberry

Bringhurst *et al*. (1981) have characterised 22 commercially important California strawberry cultivars using three enzyme systems namley, Glucosephosphate isomerase (GPI), Phosphogluco mutase (PGM) and LAP. The 22 cultivars were separated into 14 groups when banding intensities were not considered. Further they opined that the consistent GPI banding intensity if considered, some of the multiple banded types can be separated.

2.2.18 Apple

Weeden and Lamb (1985) characterised 54 apple cultivars using 6 isozyme systems on SGE. Intracultivar variation in isozyme phenotype was not observed, whereas intercultivar polymorphism was sufficient to permit reliable and unambiguous identification of every cultivar. The 6-phospho-gluconate dehydrogenase (6-PGD) and aspartate aminotransferase isozyme systems were most useful for distinguishing the cultivars. Further they reported that the genetic basis of several polymorphisms was known which enables the comparison of the isozyme phenotype observed in a hybrid with that predicted on the basis of parental genotypes.

Weller and Constante (1986) analysed shoot bark and root extracts of 16 different apple root stocks for PER isozymes using PAGE. They observed 13 bands in shoot bark and 8 bands in root extracts. Root PER bands corresponded to ones in the shoot bark PER zymograms. Two of the bands were common to all 16 root stock patterns. The 16 root stocks were grouped according to banding characteristics.

2.2.19 Peaches

Arulsekar et al. (1986) observed three distinct multiple banded zymogram patterns for MDH isozymes among the 290 peach cultivars surveyed by them. Further analysis for segregating progenies revealed only one locus that could be used as a genetic marker in breeding programmes.

2.2.20 Banana

Jarret and Litz (1986) analysed 24 clones of banana and plantain representing various levels of ploidy for isozyme variants of 5 enzymes. Polymorphism was detected in all 5 enzyme systems. Isozymes of GOT were the most useful for discriminating among clones of a particular genomic group.

Studies of Jayaraman and Ramanuja (1987) revealed varietal differences in polyphenol oxidase (PPO) and protein patterns in the pulp tissue of 3 banana cultivars.

2.3 Use in Plant Breeders' Rights (PBR)

Electrophoretic characters have been accepted as a special test of distinctness in at least three cases of PBR. The one International Union for Proection of New Plant Varieties (UPOV) member State to officially grant protection to the three varieties is Sweden.

'Satin' a red fescue variety, is morphologically indistinguishable from two other varieties, 'Dawson' and 'Polar'. Using the technique of IEF, seed EST isozymes were separated and the zymograms of the three varieties compared. 'Satin' zymograms lacked a prominent high-pH band present in the zymograms of both 'Dawson' and 'Polar'. This isozymic difference was accepted as an evidence of varietal novelty and a grant of PBR was issued for 'Satin' in 1978 (Clapham and Almgard, 1978).

'Pernilla', a barley variety was distinguished from morphologically similar 'Gunilla' by differences in gliadin banding patterns and rights were then granted for this cultivar in 1979. 'Kora' a red clover variety could not be clearly distingüished from its parent, 'Disa' and the cultivar 'Reko'. However, general seed protein patterns of the three cultivars were qualitatively dissimilar, allowing PBR to be granted for 'Kora' in 1980 (UPOV, 1980).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Experimental Materials

The material for the present investigation consisted of three *Gossypium arboreum* cultivars, three *Gossypium hirsutum* cultivars, four intra-*hirsutum* hybrids and two *hirsutum* x *barbadense* hybrids which are being cultivated on large area in Maharashtra and adjoining states. Details of the experimental material are presented in Table 1.

Seeds of the parental lines of hybrids and other varieties obtained from the concerned breeders were sown in the experimental block of the Department of Genetics and Plant Breeding, Marathwada Agricultural University, Parbhani during the *kharif* season of 1985 following the standard package of practices. Fertilizers were applied at the rate of 80 Kg N + 40 Kg P_2O_5 + 40 Kg K_2O per hectare. Crosses among the respective parents were effected by hand emasculation and pollination to obtain the hybrid seed. The parental lines and other varieties were selfed to obtain pure seed. Seed obtained from this season was used for the experimental studies.

Table 1. List of cotton cultivars included for the study

Sl No	Variety/Hybrid	Species
1.	Eknath	*G. arboreum*
2.	Sanjay	*G. arboreum*
3.	Rohini	*G. arboreum*
4.	Buri 1007	*G. hirsutum*
5.	SRT 1	*G. hirsutum*
6.	Purnima	*G. hirsutum*
7.	Bikaneri Nerma (B. Nerma)	*G. hirsutum*
8.	AC 738	*G. hirsutum*
9.	NHH 44 (B. Nerma x AC738)	*G. hirsutum* x *G. hirsutum*
10.	AK 32	*G. hirsutum*
11.	DHY 286	*G. hirsutum*
12.	AHH 468 (AK32 x DHY286)	*G. hirsutum* x *G. hirsutum*
13.	G 67	*G. hirsutum*
14.	American Nectariless (AN less)	*G. hirsutum*
15.	H 4 (G67 x AN less)	*G. hirsutum* x *G. hirsutum*
16.	Burinectariless (BN less)	*G. hirsutum*
17.	MCU 5	*G. hirsutum*
18.	Godavari (BN less x MCU5)	*G. hirsutum* x *G. hirsutum*
19.	Laxmi	*G. hirsutum*
20.	SB 289E	*G. barbadense*
21.	Varalaxmi (Laxmi x SB289E)	*G. hirsutum* x *G. barbadense*
22.	DS 28	*G. hirsutum*
23.	SB 425YF	*G. barbadense*
24.	DCH 32 (DS 28 x SB 425YF)	*G. hirsutum* x *G. barbadense*

3.2 Experimental Methods

The present investigation involved following studies :

i. Study of total soluble seed proteins by gel electrophoresis

ii. Study of salt soluble seed proteins by gel electophoresis

The above two studies were made on vertical slab gels following the Sodium dodecyl sulfate - Polyacrylamide gel elctrophoresis (SDS-PAGE) method suggested by Weber and Osborn (1969).

iii. Study of isozymes viz., esterase (EST), malate dehydrogenase (MDH), alcohol dehydrogenase (ADH), peroxidase (PER), catalase (CAT) and acid phosphatase (ACP).

This study was made by polyacrylamide disc gel electrophoresis (PAGE) following the method described by Ornstein (1964) and Davis (1964).

Electrophoretic studies of proteins and isozymes were made by collecting the seed from bolls of two healthy plants separately in each cultivar. Extract from single seed in each cultivar was used in duplicate for electrophoresis, thus making four independent analyses for each cultivar.

3.2.1 Sodium dodecyl sulfate - polyacrylamide gel electrophoresis of proteins

3.2.1.1 Vertical slab gel electrophoresis apparatus

The apparatus (designed by Studier, 1973) 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 the gel. Sample wells are formed in the gel during polymerisation using a perspex sample comb. The details of slab gel preparation are described later in this chapter. The apparatus along with the power supply unit (0 - 500 V and 0 - 100 mA) were supplied by M/s Scientific and Electronics Limited, Bombay.

3.2.1.2 Preparation of the sample

Mature and well filled seeds were selected and decorticated. Decorticated material of single seed was ground in a small pestle and mortar and defatted in a Soxhlet's apparatus using solvent ether.

3.2.1.3 Extraction of water soluble seed proteins (albumins)

Albumins were extracted from the defatted powder using 0.1 M Tris-HCl buffer (pH 7.5) containing 10 mM B-mercaptoethanol. One ml of the buffer was added to the defatted powder in a test tube and the contents were shaken on a test tube shaker for five minutes. The contents were then centrifuged at 10,000 rpm in a refrigerated centrifuge for 20 minutes. The supernatant was filtered and used for electrophoresis after estimating the protein content.

3.2.1.4 Extraction of salt soluble seed proteins (globulins)

Globulins were extracted from the defatted powder by using 0.5 M NaCl in 0.01 M phosphate buffer (pH 7.0).

Homogenization and centrifugation were carried out in the same way as for albumins. The supernatant was filtered and used for electophoresis after protein estimation.

3.2.1.5 Estimation of proteins in the seed extracts

Proteins in the extracts were estimated by the method suggested by Lowry et al. (1951) using alkaline copper and Folin phenol reagent. The Folin phenol reagent was obtained from the V.P. Chest Institute, University of Delhi, New Delhi.

Reagents

A : 2 g NaOH, 10 g Na_2CO_3 , 0.1 g Sodium-Potassium tartrate per 500 ml water

B : 0.5 g $CuSO_4.5H_2O$ per 100 ml water

C : 10 ml solution A + 0.2 ml solution B mixed prior to use

D : 1 part of Folin phenol (2 N) mixed with 1 part of water prior to use

Preparation of standard curve

Standard curve was prepared by taking different concentrations of the standard protein Bovine serum albumin

(Sigma Chemicals Ltd). A stock solution was prepared by adding 40 mg of bovine serum albumin (BSA) in 100 ml of distilled water which gave 400 ug of BSA/ml. From this stock solution, different dilutions were made in test tubes as indicated in Table 2.

Table 2 - Concentrations of Bovine serum albumin and the optical density recorded

Test Tube No	Stock solution (ml)	Distilled water added (ml)	Total volume (ml)	BSA ug/ml	Optical* density (O.D.)
0	-	1.0	1.0	0	0.00
1	0.1	0.9	1.0	40	0.15
2	0.2	0.8	1.0	80	0.25
3	0.3	0.7	1.0	120	0.34
4	0.4	0.6	1.0	160	0.42
5	0.5	0.5	1.0	200	0.50
6	0.6	0.4	1.0	240	0.60
7	0.7	0.3	1.0	280	0.70
8	0.8	0.2	1.0	320	0.80
9	0.9	0.1	1.0	360	0.85
10	1.0	-	1.0	400	0.90

* Optical density was recorded after adding the required reagents

To the contents of each test tube, 5 ml of reagent C were added, shaken well and incubated for 20 minutes at room temperature, then one ml of reagent 'D' was added, shaken and kept for one hour. A bluish colour was developed, the optical density was measured at 700 nm on 'Spectronic-20'. The standard curve was prepared by plotting O.D. against concentrations (Fig. 1).

Determination of proteins in the seed sample

From the protein extracts of seed, 20 ul aliquot was taken and O.D. was measured following the procedure described above. The protein content in the sample extract was determined by comparing the reading on the standard curve.

An appropriate volume of the protein extract was diluted with the sample buffer (containing SDS, Mercaptoethanol, glycerol and tracking dye) in such a way that 50 ul of the diluted sample contained about 200 ug of protein. The diluted sample was incubated in a hot water bath (95 C) for 3 to 4 minutes, followed by cooling to room temperature. The sample was either used immediately for electrophoresis or stored in a refrigerator. However, stored samples were warmed before electrophoresis.

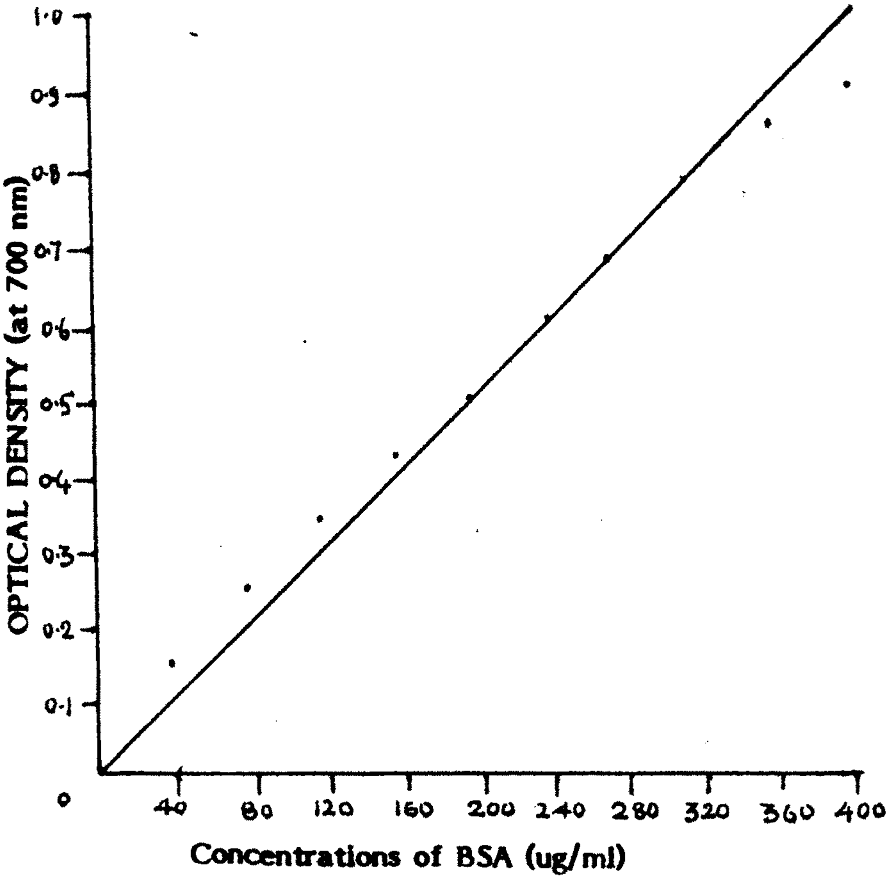

Fig. 1. Standard curve for protein determination

3.2.1.6 Stock solutions

i. **Extraction Buffer** (0.1 M 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 B-Mercaptoethanol was added and then the volume was made up to 100 ml by maintaining the pH at 7.5. The solution was stored in a dark bottle at 4° C.

ii. **Sample buffer** - The sample buffer used to dilute and incubate the protein extract was prepared by mixing the following :

Distilled Water	4.00 ml
0.5 M Tris-HCl, pH 6.8	1.00 ml
Glycerol	0.80 ml
10% SDS (w/v)	1.60 ml
B-Mercaptoethanol	0.40 ml
0.05% Bromophenol blue (w/v)	0.20 ml
	8.00 ml

iii. **Acrylamide-bisacrylamide** (22.2 : 0.6) was prepared by dissolving 22.2 g of acrylamide (Sigma) and 0.6 g bisacrylamide in a total volume of 100 ml water. The solution was filtered through Whatman No.1 filter paper and stored at 4 ° C in a dark bottle.

iv. **Gel buffer** : Sodium phosphate buffer (0.15 M, pH 7.2) was prepared by dissolving 25.637 g of Na_2HPO_4 and 8.82 g of NaH_2PO_4 in one litre of water.

v. 10% SDS (w/v) was prepared by dissolving 10 g Sodium Lauryl Sulfate (SISCO Lab, Bombay) in water to make 100 ml volume. Slight heating was necessary to obtain clear solution, which was stable at room temperature but precipitated in cold.

vi. 1.5% APS (w/v) was prepared by dissolving 150 mg of ammonium persulphate (Glaxo) in 10 ml of water. This was made fresh weekly or just before use.

vii. **TEMED** (Tetramethyl ethelene diamine) was used as such (Fluka Chemicals, Germany). It was stored at 4 °C in a dark bottle.

viii. **Staining solution** : The staining solution was prepared by dissolving 1.25 g Coomassie brilliant blue R250 (SISCO Lab, Bombay) in 454 ml of 50% methanol and 46 ml of glacial acetic acid. Ths solution was filtered through Whatman No. 1 filter paper.

ix. **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.

x. **Reservoir buffer** : It was prepared by mixing 200 ml of the gel buffer, 10 ml of 10% SDS and 790 ml of distilled water.

3.2.1.7 Gel Preparation

The gel of 7.5% acrylamide concentration for the SDS-Phosphate (continuous) buffer system consisted of :

Phosphate buffer (0.15 M, pH 7.2)	30.00 ml
Acrylamide:bisacrylamide (22.2:0.6)	20.30 ml
10% SDS (w/v)	0.60 ml
Distilled water	7.56 ml
1.5% APS (w/v)	1.50 ml
TEMED *	0.04 ml

	60.00 ml
	--------

* 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 :

The cleaned glass plates were held apart at the correct distance by thin (1.5 mm) perspex spacers. The sealing of 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 (Fig. 2).

The gel mixture was prepared by adding the correct volumes of all components. The contents were gently mixed and the gel solution 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 fit snugly against the glass plates. Special care was take to ensure that air bubbles were not trapped beneath the comb. The assembly was left undisturbed for the gel to polymerise (30 to 40 minutes) (Fig. 2).

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.

3.2.1.8 Sample loading

The sample buffer containing about 200 ug of protein in 50 ul 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.

3.2.1.9 Electrophoresis

The electrophoresis apparatus (Fig. 3) 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 bottom of the gel which took about 6 to 7 hours. After the run, the power supply was switched off.

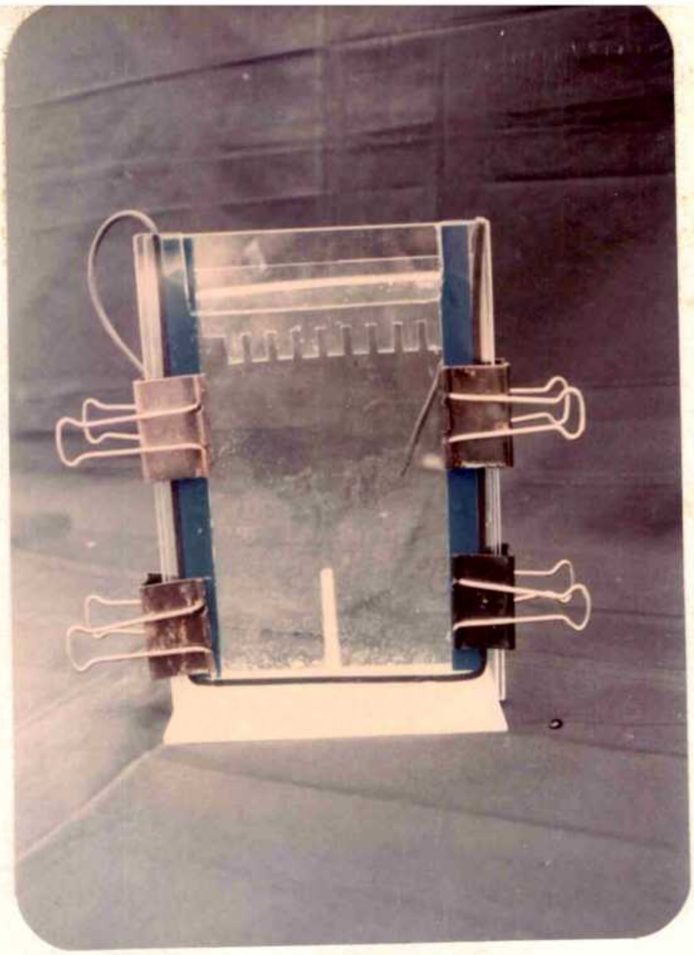

Fig. 2. Polymerisation of the vertical slab gel

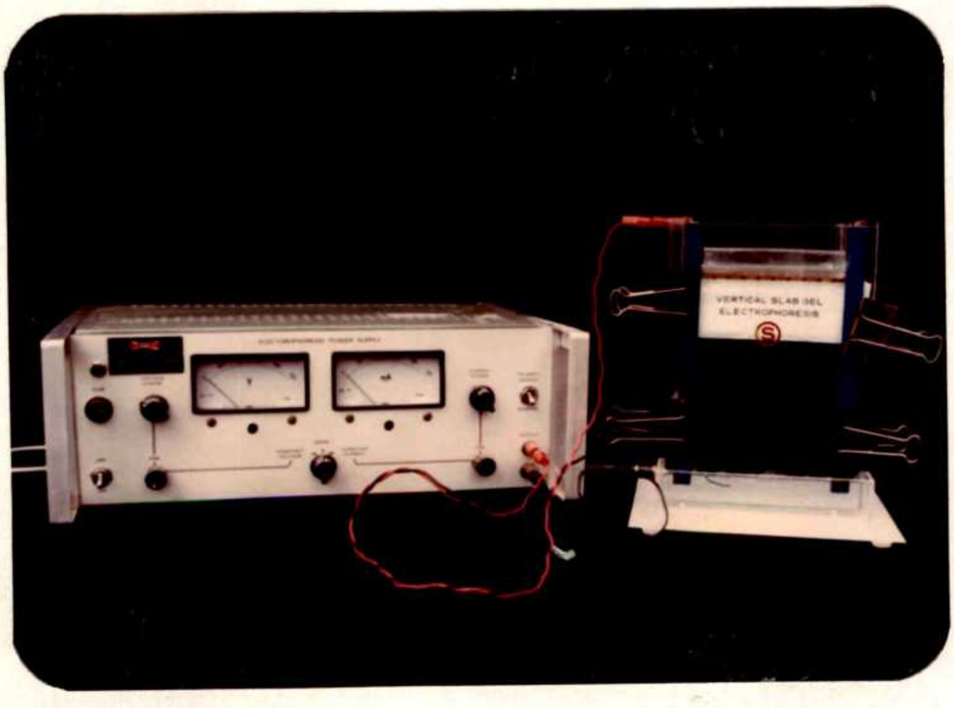

Fig. 3. Vertical slab gel electrophoresis apparatus

3.2.1.10 Recovery of Gels

Slab gels were recovered by gently levering the glass plates apart at the end away from the notch (to avoid damage to the fragile notched end). The gel resting on the unnotched glass plate was carefully slided 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 the position of tracking dye.

3.2.1.11 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 every 2 to 3 hours, until the protein bands were visible on clear background which took about 10 to 12 hours. The gels were stored in trays containing a solution of 7.5% glacial acetic acid.

3.2.2 Disc electrophoresis of isozymes

The method followed was to stain for specific enzyme activity in situ. In contrast to dissociating (SDS) buffer system, the zone electrophoresis of enzymes under non-dissociating buffer conditions was designed to fractionate the mixture in such a way that subunit interaction, native protein conformation and biological activity were preserved. The method suggested by Ornstein (1964) and Davis (1964) was followed using a disc gel electrophoresis apparatus.

Unless specified, all the steps and procedures were performed at low temperature (0 to 4° C) both to reduce loss of enzyme activity through denaturation and to minimise attack by any proteases in the sample.

3.2.2.1 Disc gel electrophoresis apparatus

The cylindrical gel tubes were made of glass with an inner diameter of 0.6 cm, wall thickness of 0.1 cm and length of 7.0 cm. During gel preparation, rod gel holders were kept exactly vertical in the racks made of perspex. For electrophoresis, the rod gels were transferred to an

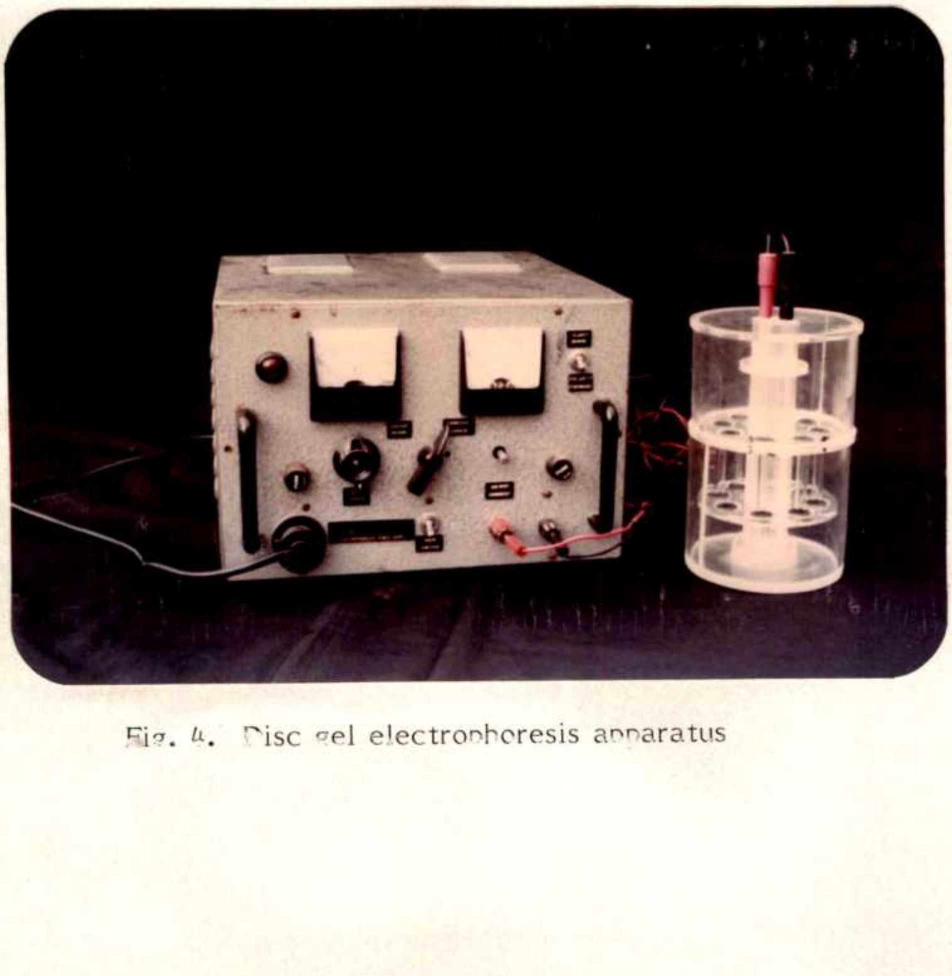

Fig. 4. Disc gel electrophoresis apparatus

apparatus as shown in Fig. 4. This apparatus was made mainly of perspex. The gel tubes were suspended by means of silicone rubber grommets held in holes drilled in the base of the upper buffer reservoir. In the apparatus most of the gel length was immersed in the buffer held in the lower buffer reservoir, which aided in the dissipiation of heat during electrophoresis. Platinum electrodes were at the same distance from each gel tube and hence the electrodes were circular in shape and located centrally.

3.2.2.2 Stock solutions

i. **Extraction buffer** : The enzymes from single seeds were extracted by 0.1 M Tris-HCl buffer (pH 7.5) containing 10 mM B-Mercaptoethanol. This buffer was prepared by dissolving 1.21 g of Tris in 50 ml of water, to which 0.08 ml of B-Mercaptoethanol was added and the pH was adjusted to 7.5 by adding 1 N HCl. The final volume was made up to 100 ml by adding distilled water. The buffer was stored at 4° C in a dark bottle.

ii. **Resolving gel buffer** (1.50 M pH 8.8) : 18.15 g of Tris was dissolved in about 50 ml of water and by adding 1 N HCl, the pH was adjusted to 8.8. The volume was made up to 100 ml by adding distilled water. The buffer was stored at 4° C in a dark bottle.

iii. **Reservoir buffer** (Tris-glycine pH 8.3) : 3.0 g Tris and 14.4 g glycine were dissolved in 1 litre of water and the pH was adjusted to 8.3. The buffer was stored at 4° C.

iv. Acrylamide-bisacrylamide (30.0 : 0.8) : was prepared by dissolving 30 g of acrylamide and 0.8 g of bisacrylamide in a total volume of 100 ml water. The solution was filtered through Whatman No. 1 filter paper and stored at 4° C in a dark bottle.

v. **Ammonium persulfate** (1.5% w/v) : 0.15 g of ammonium persulfate was dissolved in 10 ml of water. This was made just before use.

vi. **TEMED** : Tetramethyl ethelene diamine was used as such. This undiluted solution was stored at 4° C in a dark bottle.

3.2.2.3 Extraction of native proteins (enzymes)

Seeds were surface sterilised and soaked in water for 72 hours on a filter paper in a sterilised petri dish. The enzymes were extracted from these seeds by macerating decorticated single seed with 1 ml of extraction buffer

(0.1 M Tris-HCl, pH 7.5) in a pre-chilled pestle and mortar in an ice bucket. The paste was transferred to a pre-chilled centrifugation tube.

The contents were centrifuged in a refrigerated centrifuge at 15,000 rpm for 30 minutes at 0 - 4 °C. The supernatant was transferred to pre-chilled glass vials which were strored in a deep freeze at - 10 °C.

In the seed extract, proteins were estimated by following the method of Lowry et al. (1951) described earlier in this chapter.

3.2.2.4 Gel mixture preparation

A gel of 10% acrylamide concentration was prepared by mixing the following reagents :

Resolving gel buffer	7.50 ml
Acrylamide : bisacrylamide	10.00 ml
Distilled water	11.00 ml
1.5% APS (w/v)	1.50 ml
TEMED	0.01 ml
	30.00 ml

3.2.2.5 Preparation of rod gels

i. Glass tubes were cleaned by soaking them in chromic acid overnight, rinsed with distilled water and finally with ethanol before drying.

ii. Gel tubes were placed in racks which hold tubes snugly in vertical position. The lower end was sealed by slipping them into rubber bungs.

iii. The gel mixture was prepared by adding the reagents mentioned above. TEMED was added just before pouring the gel mixture into the gel tubes.

iv. Each of the 8 tubes was filled (leaving about 1.0 cm space at the top) with gel mixture carefully avoiding trapping any air bubbles.

v. Once the gel tube was filled, the solution was overlayed with water to ensure flat gel miniscus. Care was taken to prevent mixing of overlay with gel solution.

vi. The gels were left undisturbed to polymerise for about 30 minutes. An interface was formed between water and gel.

The water layer was removed by a tissue paper wick and gel surface rinsed with reservoir buffer.

vii. Rod gels were placed in the rubber grommets of the upper buffer reservoir of the electrophoresis apparatus.

viii. Reservoir buffer was added to the lower reservoir of the apparatus. The upper reservoir having rod gels was fitted in the correct position. Air bubbles, if any, were removed from the bottom of the gel by flicking the top of the gel tube.

ix. Reservoir buffer was added to the upper reservoir.

3.2.2.6 Loading of samples and electrophoresis

The sample aliquot (50 ul) containing 200 ug of protein, glycerol and tracking dye (0.05% bromophenol blue) was carefully loaded onto the gel surface using a micropipette. The dense sample flowed on to the gel surface and formed a sharply-defined layer.

The disc electrophoresis apparatus was connected to the power pack with anode (+) to the lower reservoir and cathode (-) to the upper reservoir. An electric current of 1.5 mA was applied till the sample entered the gel and a constant current of 2.5 mA per rod gel was applied until the tracking dye reached bottom of the gel. After the run, the current was switched off and the rod tubes were removed from the apparatus.

3.2.2.7 Recovery of gels

Rod gels were taken out of the tubes by rimming. The glass tube having rod gel was held in one hand whilst a syringe fitted with a long fine blunt needle was used to squirt water in between the gel surface and the tube wall, slowly rotating the glass tube. The intact gel usually slided out fairly easily. The position of the tracking dye was marked for later use in 'Rm' determinations.

3.2.2.8 Enzyme activity staining

After electrophoresis, the rod gels were processed immediately for the isozyme band determination. Different gels were incubated for different enzyme activity staining systems (Vallejos, 1983).

i. **Esterases** (E.C. 3.1.1.2)

The two reaction solutions were prepared as follows for EST activity staining :

A.	1% α- naphthyl acetate in 50% acetone (33 mg in 3.0 ml)	3.0 ml
	Phosphate buffer (0.2 M, pH 4.3)	50.0 ml
B.	Fast blue RR salt (100 mg in 25 ml of phosphate buffer 0.2 M, pH 9.2)	25.0 ml
	Distilled water	25.0 ml

Solution A and solution B were mixed together just before incubation. Each gel was immersed in 12 ml of the rigorously stirred reaction mixture. The gels were incubated in dark at 30° C for 2 to 3 hours until greyish to black colour bands appeared. The staining solution was discarded, the gels were washed in tap water and fixed in 7% acetic acid. Zymograms were traced immediately as bands faded away slowly.

ii. **Malate dehydrogenase** (E.C. 1.1.1.37)

The staining solution for the MDH activity was prepared just before incubation as follows :

Tris-HCl (0.1 M, pH 7.5)	100 ml
L-Malic acid (1.0 M, pH 7.5)	3 ml
Nicotinamide Adenine Dinucleotide (NAD)	30 mg
Methyl Thiazolyl Tetrazolium (MTT)	20 mg
Phenazine Methosulphate (PMS)	4 mg

The gels were incubated in the above reaction mixture (12 ml per gel) in the dark at 30° C for 20 to 30 minutes. Purple colour bands were observed. The staining solution was discarded to stop the reaction. The gels were washed with distilled water and fixed in 7% acetic acid. Zymograms were traced immediately as the bands faded away slowly.

iii. **Alcohol dehydrogenase** (E.C. 1.1.1.1)

The staining solution for the ADH activity was prepared just before incubation as follows :

Tris-HCl (0.1 M, pH 7.5)	100 ml
NAD	30 mg
MTT	20 mg
PMS	4 mg
Ethanol*	6 ml

* Added just before incubation

The gels were incubated in the above reaction solution. Each gel was immersed in about 12 ml of the solution and incubated in dark at 30 °C for about 20 to 30 minutes. Light to dark purple coloured bands were observed. The gels were then fixed in 7% acetic acid. Zymograms were traced.

iv. Peroxidase (E.C. 1.11.1.7)

The gels were rinsed in phosphate buffer (0.05 M, pH 5.1) followed by immersion in the reaction mixture prepared freshly as follows :

A.	25% acetic acid (v/v)	50.0 ml
	Benzidine hydrochloride	1.2 g
B.	6% H_2O_2 (w/v)	20.0 ml
	Distilled water	30.0 ml

Solution A and solution B were mixed just before pouring onto the gels. Sky blue coloured bands appeared within 2 minutes and were recorded immediately since the bands faded away rapidly.

v. **Catalase** (E.C. 1.11.1.6)

The gels (prepared with 0.5% starch) were incubated in a solution prepared as follows for the CAT enzyme activity :

A.	Na-thiosulphate (60 mM)	30.0 ml
	3% H_2O_2 (w/v)	70.0 ml
B.	Potassium Iodide (90 mM)	100.0 ml
	Glacial acetic acid	0.5 ml

Solution A was prepared just before pouring onto the gels. Each gel was immersed in about 12 ml of solution A for 30 seconds. The solution A was poured off, followed by addition of solution B. The chromophores formed slowly. The zymogram was recorded as soon as the achromatic bands became evident.

vi. **Acid phosphatase** (E.C. 3.1.3.2)

The staining solution for ACP activity was prepared just before incubation as follows :

Na acetate (50 mM, pH 5.5)	100 ml
Mg $Cl_2.6H_2O$ (1 M)	1 ml
Fast Garnet GBC salt	100 mg
1% α- Naphthyl acid phosphate in 50% acetone (33 mg in 3 ml)	3 ml

Each gel was immersed in 12 ml of the rigorously stirred reaction mixture. The gels were incubated in dark at 30° C for 2 to 3 hours until orange or red colour bands appeared. The staining solution was discarded, the gels were rinsed with tap water and fixed in 7% glacial acetic acid. Zymograms were traced immediately as bands tend to fade away slowly.

3.2.3 Photography of the stained protein/isozyme bands

Photographs of the wet gels were taken by illuminating them with diffused flourescent light from beneath. Photographs were taken by using 'Canon' camera and 35 mm Kodak colour film roll.

3.2.4 Measurement of 'Rm' values

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

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

3.2.5 Preparation of the banding patterns and detection of differences among the cultivars

By visual screening of the gels, protein/isozyme bands were traced onto the graph and mean 'Rm' value calculated for four independent electrophoretic runs made for each cultivar and the banding patterns were drawn. The banding patterns thus obtained were observed to detect the differences among the cultivars. These differences were measured as qualitative and quantitative variations.

Qualitative Variation

When a particular protein/isozyme band (as designated by its 'Rm' value) was present in the electrophoregram of one cultivar but absent in that of another, the variation was referred as qualitative variation.

Quantitative Variation

When a particular protein/isozyme band was observed in the electrophoregrams of two or more different cultivars, but differed in band size or staining intensity, the variation was referred as quantitative variation. This variation was categorised as dense, medium, light, weak and faint bands depending on the intensity of staining (Fig.5).

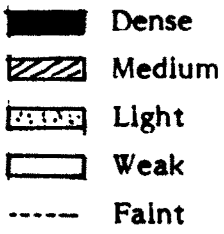

Fig. 5. Notation followed for categorising staining intensity of protein/isozyme band

RESULTS

4. RESULTS

The experimental findings of the present investigation are presented under three sections.

1. Water soluble seed proteins (albumins)
2. Salt soluble seed proteins (globulins)
3. Isozymes
    - i) Esterase (EST)
    - ii) Malate dehydrogenase (MDH)
    - iii) Alcohol dehydrogenase (ADH)
    - iv) Peroxidase (PER)
    - v) Catalase (CAT)
    - vi) Acid phosphatase (ACP)

Qualitative and quantitative differences in the banding patterns of the different cotton genotypes were analysed. Qualitative differences were based on presence or absence of the specific bands in the profiles. Quantitative differences were determined on the basis of the staining intensities of the bands in question. The staining intensities were defined as : dense, medium, light, weak and faint (Fig. 5).

4.1 Water soluble seed proteins (albumins)

Electrophoretic patterns of water soluble seed proteins (albumins) of the material under study were obtained by SDS-PAGE technique using phosphate (continuous) buffer system. The banding patterns represented by electrophoregrams are presented in Fig. 6b to Fig. 13b, corresponding drawings are depited in Fig. 6a to Fig. 13a. Some bands which were faint on the gels are not visible in the photographs. However, these have been drawn in the diagram by visual screening of the gels. Consistent results were obtained in four sample runs from the single seed extracts of two different plants in each cultivar.

4.1.1 *Gossypium arboreum* varieties

Three varieties of *G. arboreum* viz., Eknath, Sanjay and Rohini were studied. Electrophoregrams of the seed albumins of these three varieties are presented in Fig. 6b and the depiction is done in Fig. 6a. From these figures it is evident that all the three varieties had 14 protein bands each. However, qualitative and quantitative differences were observed among the cultivars for their banding pattern. In all 19 bands were recognised in this group. Nine bands were found to be common to all the 3 varieties. Band Nos. 6, 13 and 15 (Rm 0.32, 0.65 and 0.68) were specific to Rohini. Band No. 12 (Rm 0.56) was specific to Sanjay. Band No. 14, 16 and 17 (Rm 0.66 0.72 and 0.82) were observed in both Eknath and Sanjay. Quantitative difference was observed in the Band No. 2 (Rm 0.18) which was present in all the 3 entries. Intensity of this band was observed to be medium in Rohini, light in Sanjay and faint in Eknath. Band No. 11 (Rm 0.55) was observed in Eknath and Rohini.

4.1.2 *Gossypium hirsutum* varieties

The three varieties of *G. hirsutum* under study were Buri 1007, SRT1 and Purnima. From the Fig. 7a it is evident that there existed both qualitative and quantitative differences in the protein banding patterns among the 3 cultivars. In all 18 bands were recognised in this group. Buri 1007 exhibited 14 bands while albumins of SRT1 and Purnima were resolved into 10 and 15 bands respectively. Eight bands were found to be common in all the 3 entries. Band Nos. 13 and 15 (Rm 0.66 and 0.70) were specific to

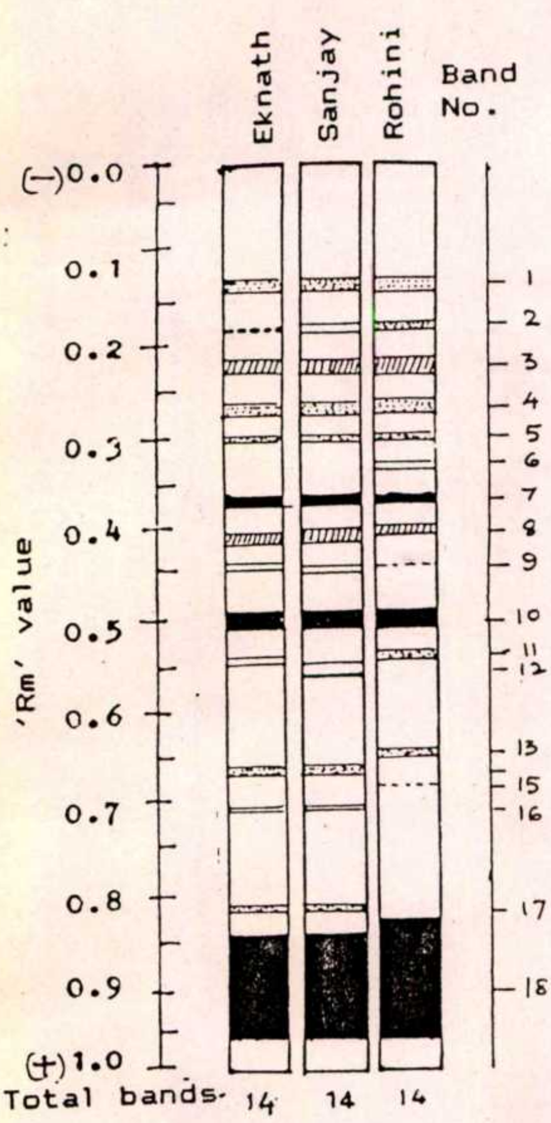

Fig. 6a

Fig. 6a SDS-PAGE profiles of seed albumins in G. arboreum varieties

Fig. 6b Electrophoregrams

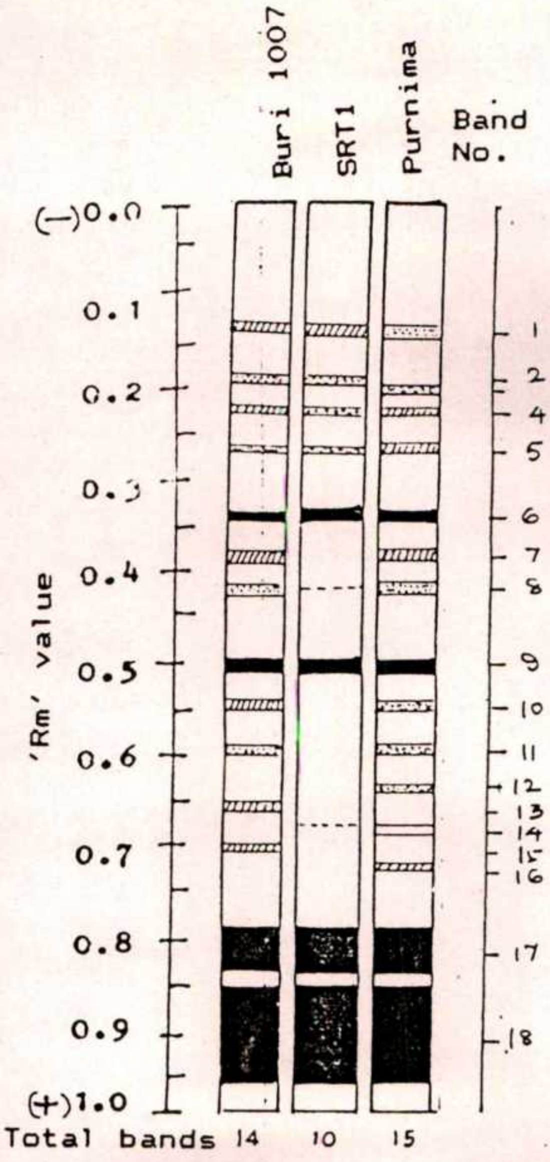

Fig. 7a

Fig. 7a SDS-PAGE profiles of seed albumins in G. hirsutum varieties

Fig. 7b Electrophoregrams

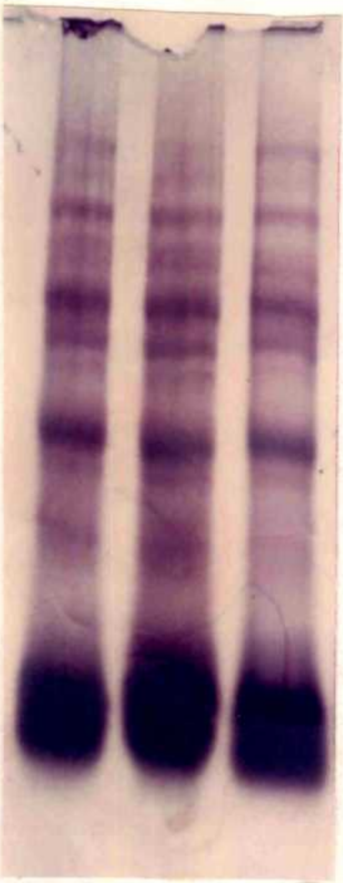

Fig. 6b

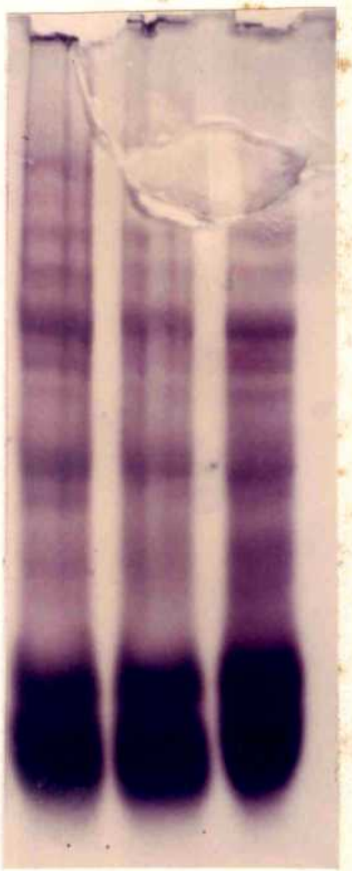

Fig. 7b

Buri 1007. Band Nos. 3, 12 and 16 (Rm 0.20, 0.64 and 0.73) were specific to Purnima. Band No. 2 (Rm 0.18) was present in both Buri 1007 and SRT1 but absent in Purnima. Band Nos. 7, 10 and 11 (Rm 0.38, 0.55 and 0.60) were present in Buri 1007 and Purnima but absent in SRT1. Band No. 14 (Rm 0.68) was present in SRT1 and Purnima but absent in Buri 1007.

Quantitative differences were also observed in the protein bands. Intensity of Band No. 8 (Rm 0.42) was medium in Buri 1007 and Purnima, while it was faint in SRT1. Band No. 4 (Rm 0.24) was of medium intensity in Buri 1007 and Purnima but light in SRT1. Band No. 14 (Rm 0.68) was weak in Purnima and faint in SRT1.

4.1.3 Intra-*hirsutum* hybrids

4.1.3.1 NHH44 and its parents

From the Fig. 8a and Fig. 8b showing the banding patterns of seed albumins of NHH44 and its parents, it is evident that there existed qualitative and quantitative differences among the cultivars. The hybrid NHH44 recorded 13 bands while its female (B. Nerma) and male (AC738) parents exhibited 12 bands each. In all, 18 bands were recognised in this group, out of which 7 bands were common in all the 3 genotypes. A faint band No. 14 (Rm 0.62) was specific to the hybrid. Five bands viz., Nos. 3, 6, 10, 13 and 16 (Rm 0.17, 0.29, 0.44, 0.58 and 0.67) were specific to the female parent. Five bands viz., Nos. 2, 5, 9, 12 and 15 (Rm 0.16, 0.27, 0.41, 0.55 and 0.64) were present in the male parent as well as the hybrid but absent in the female parent.

Quantitative differences existed in Band Nos. 1, 9 and 12 which were lighly stained in the hybrid and medium in male parent.

4.1.3.2 AHH468 and its parents

From the Fig. 9a it is evident that there existed both qualitative and quantitative differences in the seed albumin banding patterns among the 3 genotypes. AK 32, the female parent had 12 bands while the hybrid and male parent (DHY286) had 15 bands each. In all 17 bands were recognised in this group, 10 bands were found to be common in all the three. Band Nos. 6, 11 and 12 (Rm 0.31, 0.48 and 0.52) were

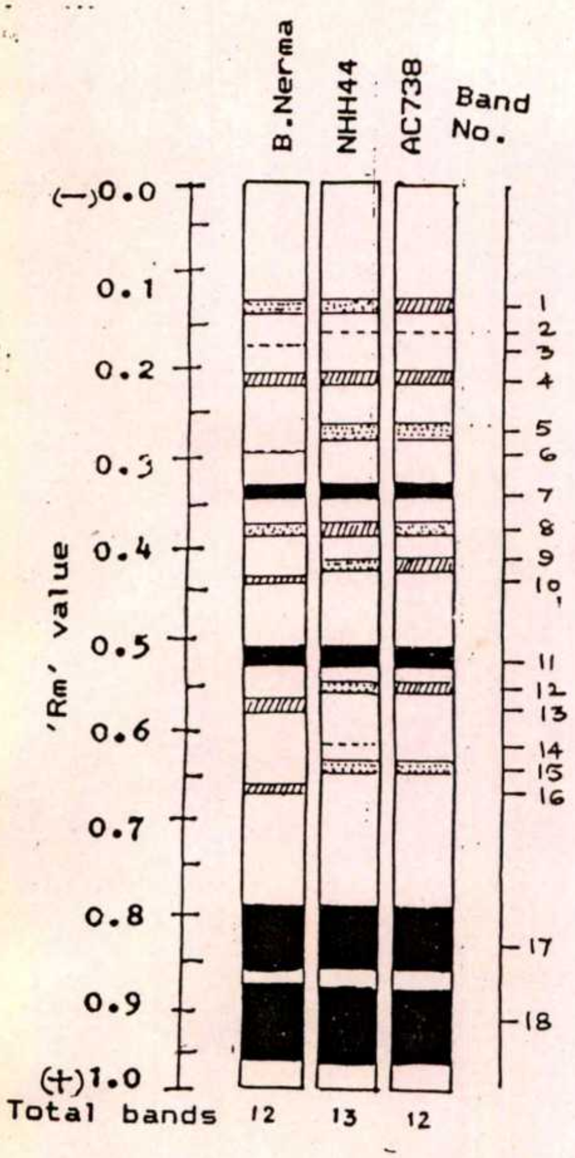

Fig. 8a

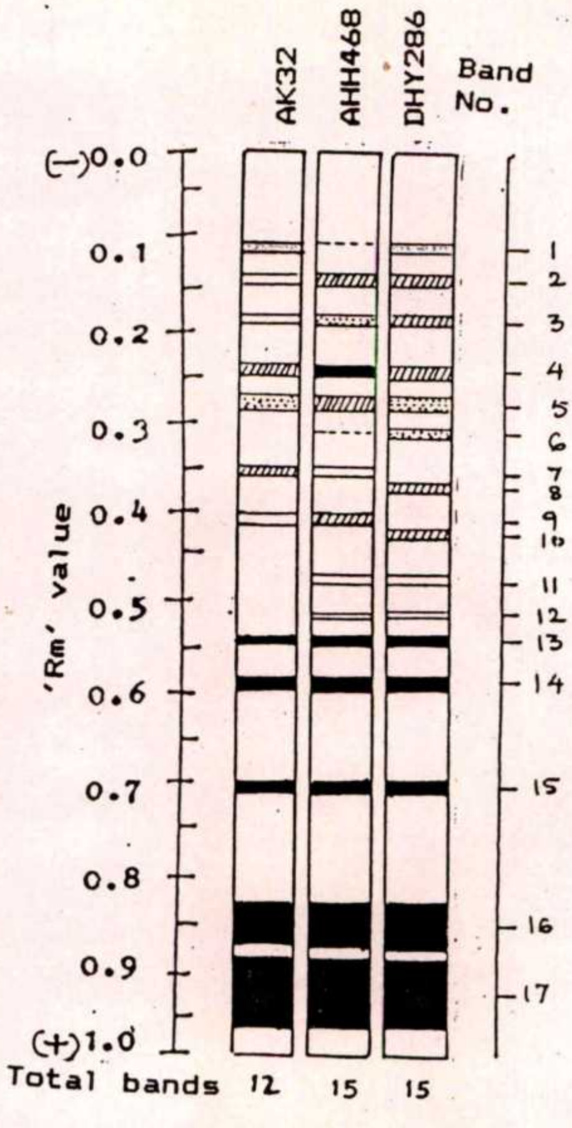

Fig. 9a

Fig. 8a SDS-PAGE profiles of seed albumins in NHH44 and its parents

Fig. 8b Electrophoregrams

Fig. 9a SDS-PAGE profiles of seed albumins in AHH468 and its parents

Fig. 9b Electrophoregrams

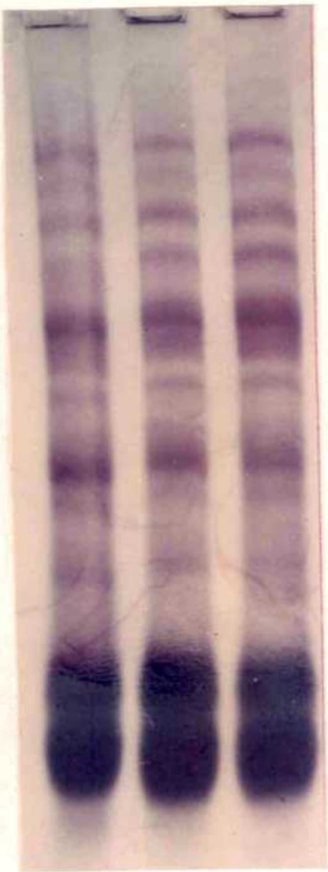

Fig. 8b

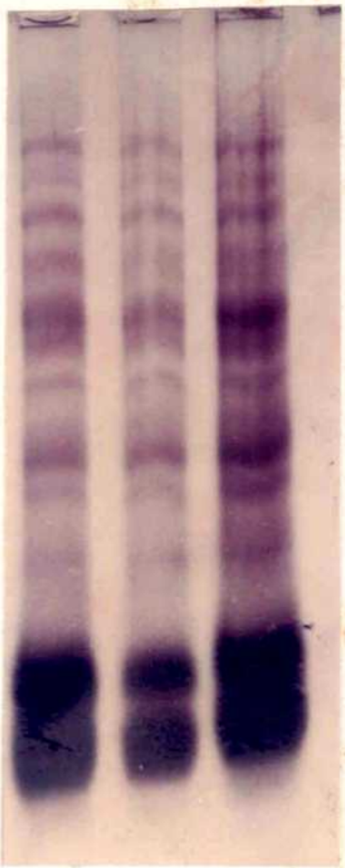

Fig. 9b

observed in the hybrid and the male present but absent in the female parent. Band Nos. 7 and 9 (Rm 0.35 and 0.41) were found in the female parent and hybrid but absent in the male parent.

Quantitative differences were observed in Band Nos. 1, 2 and 3. Band No. 1 was faint in the hybrid and light in both the parents. Band No. 2 (Rm 0.14) was medium in the hybrid and the male parent but lightly stained in the female parent. Band No. 3 (Rm 0.19) was of medium intensity in female, weak in male parent and light in the hybrid. Band No. 4 (Rm 0.24) was dense in hybrid, medium in both the parents.

4.1.3.3 H4 and its parents

The electrophoregrams of the water soluble seed proteins of H4 and its parents are presented in Fig.10a and Fig. 10b. From the figures it is evident that there existed qualtitative and quantitative differences in the banding pattern of the cultivars. The hybrid H4 and its female parent G67 had 13 bands while the male parent American Nectariless had 12 bands. However, in all 18 bands were recognised in this group, out of which 8 bands were common to all the 3 genotypes. Band Nos. 8, 11, 13 and 16 (Rm

0.54, 0.63, 0.68 and 0.75) were specific to G67, while Band No. 14 (Rm 0.73) was specific to the male parent. Band No. 15 (Rm 0.74) was specific to the hybrid H4. Band No. 6 (Rm 0.38) was found in the female parent and the hybrid but not in the male parent. Band Nos. 9, 10 and 12 (Rm 0.56, 0.62 and 0.66) were observed in the hybrid and the male parent but were absent in the female parent.

Quantitative differences were observed in Band Nos. 7 ~~and 9~~ (Rm 0.49 ~~and 0.56~~) which was were dense in the male parent, medium in the hybrid and weakly stained in the female parent. Band No. 2 (Rm 0.17) was dense in the hybrid and slight in both the parents.

4.1.3.4 Godavari and its parents

From the Fig. 11a and Fig. 11b it is evident that there existed qualitative and quantitative differences in the the seed albumin banding pattern of the 3 cultivars. The hybrid and the male parent exhibited more or less similar banding patterns, both producing 14 bands each while the female parent had only 11 bands. Three bands viz., Band Nos. 2, 5 and 8 (Rm 0.13, 0.25 and 0.38) were observed in the hybrid as well as the male parent but not

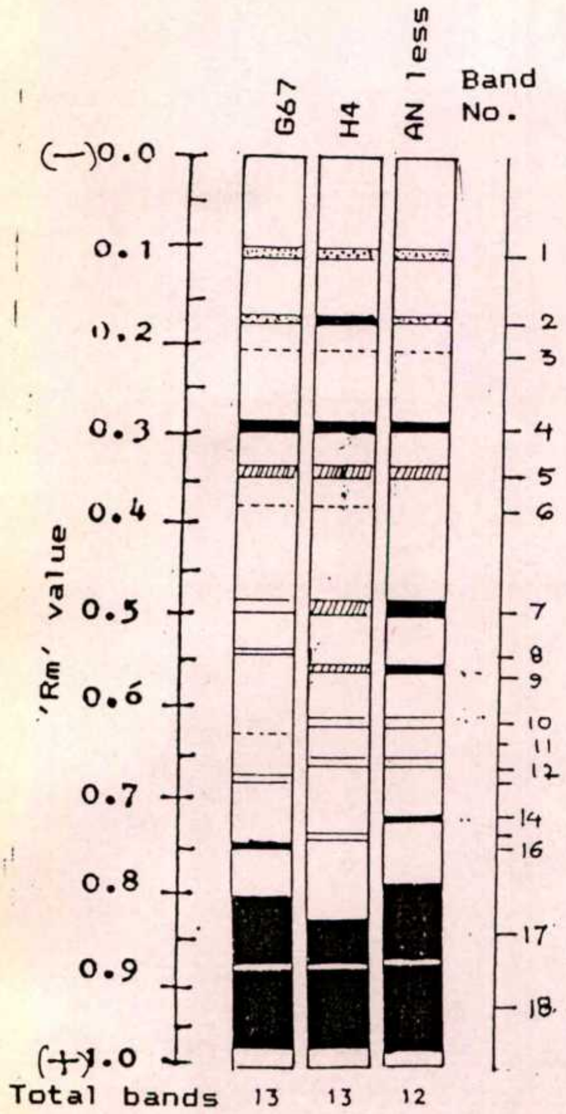

Fig. 10a

Fig. 10a SDS-PAGE profiles of seed albumins in H4 and its parents

Fig. 10b Electrophoregrams

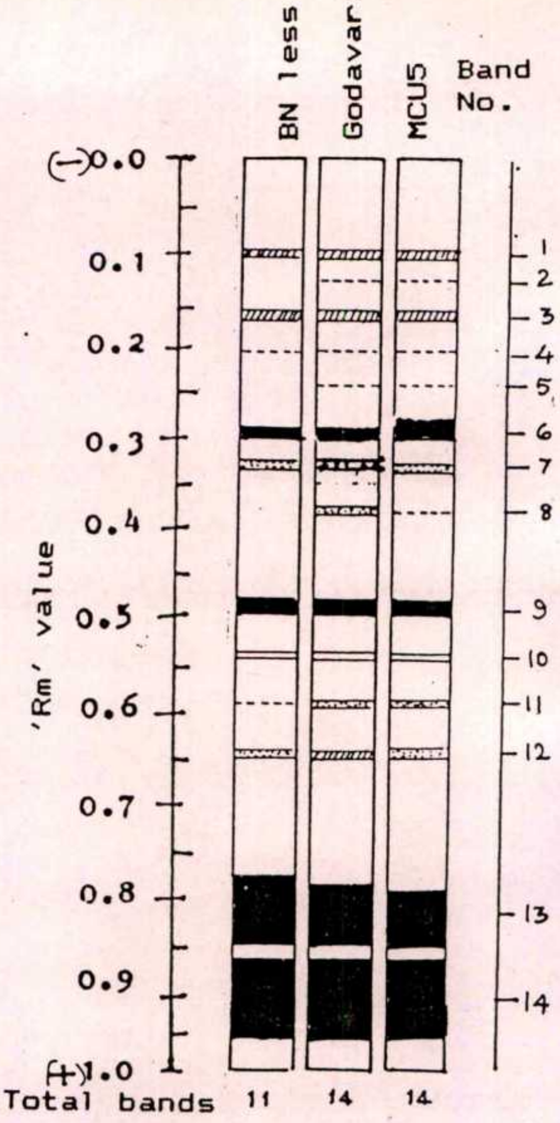

Fig. 11a

Fig. 11a SDS-PAGE profiles of seed albumins in Godavari and its parents

Fig. 11b Electrophoregrams

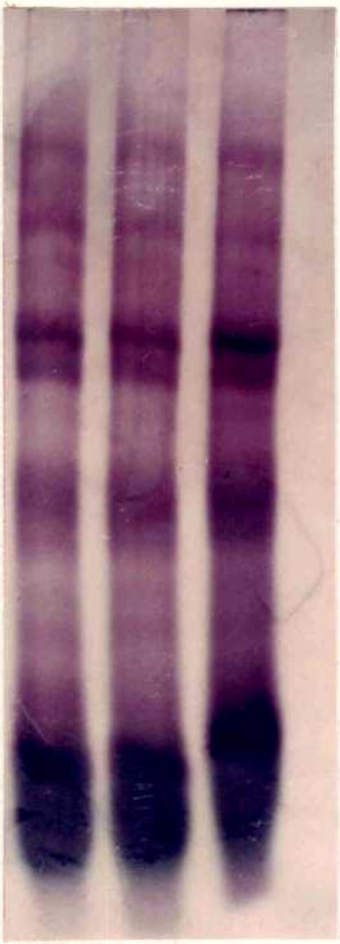

Fig. 10b

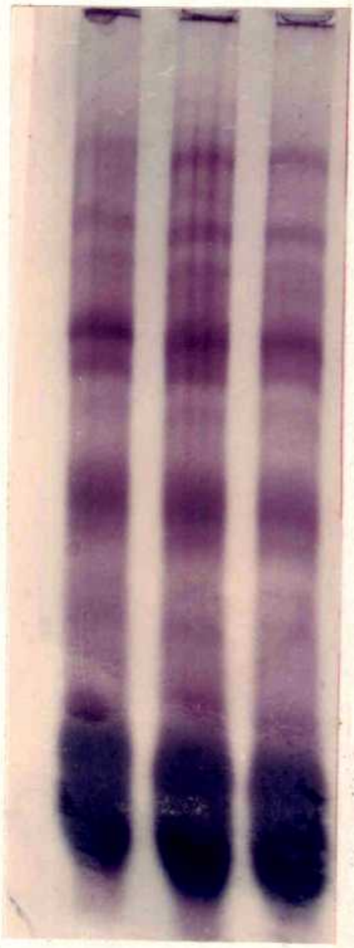

Fig. 11b

in the female parent. Band No. 8 (Rm 0.37) was of medium intensity in the hybrid and faint in the male parent. Band No. 12 (Rm 0.65) was medium in the hybrid and light in both the parents.

4.1.4 *Hirsutum* x *barbadense* hybrids

4.1.4.1 Varalaxmi and its parents

From the Fig. 12a and Fig. 12b it is evident that there existed both qualitative and quantitative differences in the seed albumin banding patterns of the interspecific hybrid Varalaxmi and its parents (Laxmi and SB289E). The hybrid produced 14 bands while both the parents exhibited 12 bands each. In all 15 bands were recognised in this group, out of which 9 bands were common to all the 3 genotypes. Band Nos. 5, 8 and 11 (Rm 0.32, 0.45 and 0.55) were observed in the hybrid and the male parent only, while band Nos. 12 and 13 (Rm 0.58 and 0.63) were observed in the hybrid and the female parent only. Band No. 1 and 2 (Rm 0.13 and 0.17) were weak in both the parents and medium in the hybrid. Band No. 10 (Rm 0.53) was specific to the female parent.

4.1.4.2 DCH32 and its parents

From the Fig. 13a and Fig. 13b it is evident that there existed qualitative and quantitative differences in the seed albumin banding patterns of the hybrid DCH32 and its parents. In the female parent (DS28) 13 bands were observed while the male parent (SB425YF) and the hybrid had 16 bands each. A total of 17 bands were recognised in this group. Band No. 7 (Rm 0.32) was specific to the female parent. Band Nos. 1, 2, 4 and 10 (Rm 0.10, 0.12, 0.17 and 0.45) were found in the hybrid and the male parent only. Band No. 4 (Rm 0.17) was of medium intensity in the male parent and weak in the hybrid. Band Nos. 5, 6, 14 and 15 (Rm 0.24, 0.27 and 0.63 and 0.66) were of medium intensity in the male parent, light in the hybrid and weak in the female parent.

4.2 Salt soluble seed proteins (globulins)

The electrophoretic patterns of the salt soluble seed proteins (globulins) in the varieties, hybrids and their respective parents studied by SDS-PAGE technique are presented in Figs. 14b to Fig. 21b. The results were

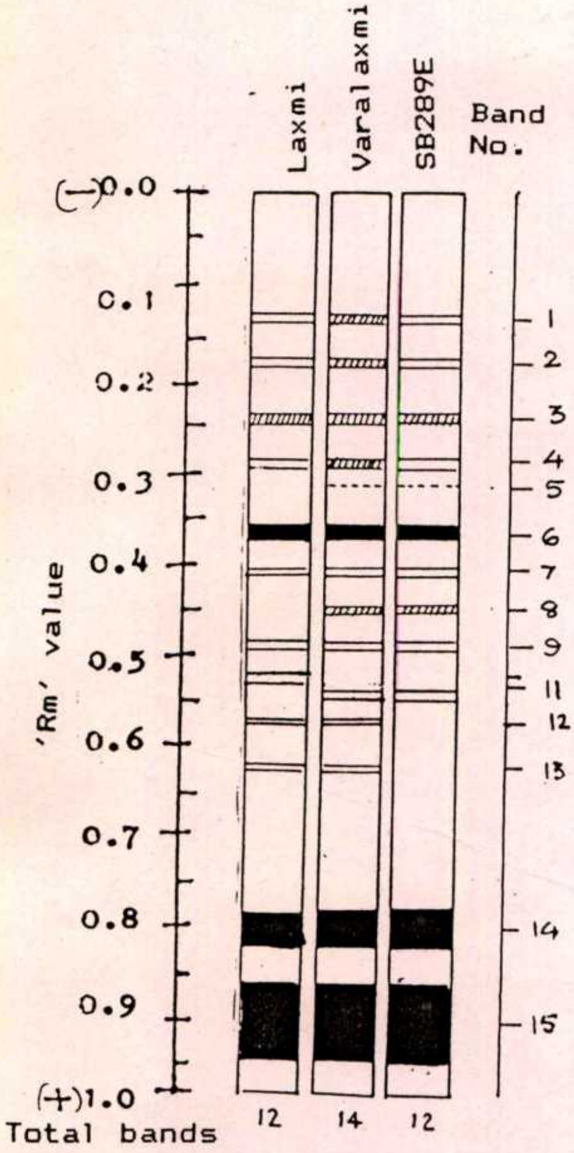

Fig. 12a

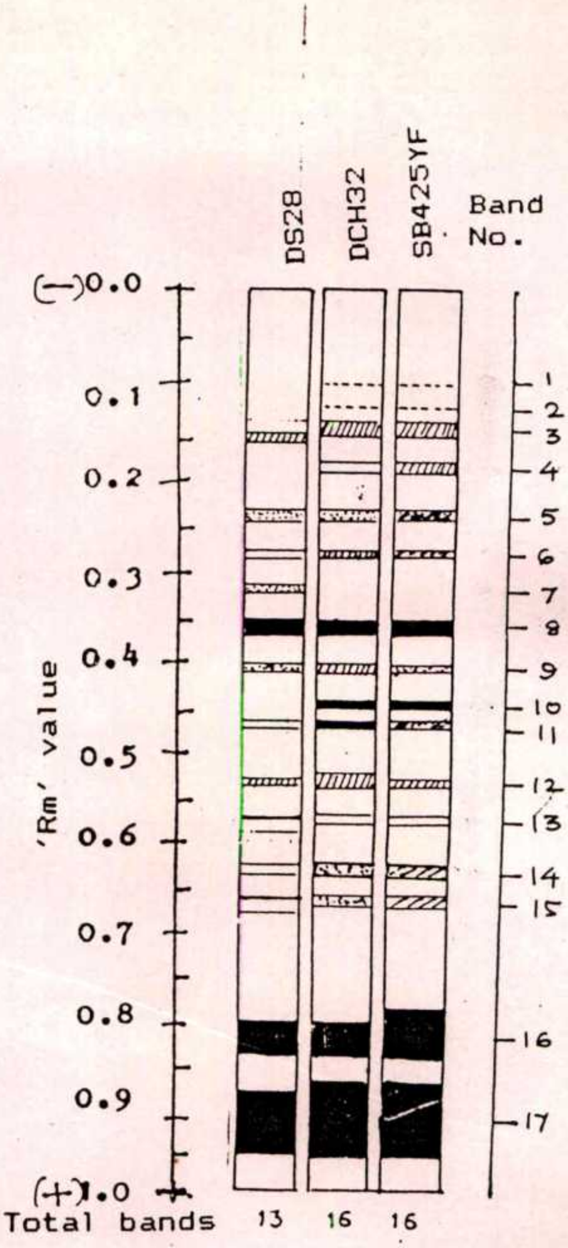

Fig. 13a

Fig. 12a SDS-PAGE profiles of seed albumins in Varalaxmi and its parents

Fig. 12b Electrophoregrams

Fig. 13a SDS-PAGE profiles of seed albumins in DCH32 and its parents

Fig. 13b Electrophoregrams

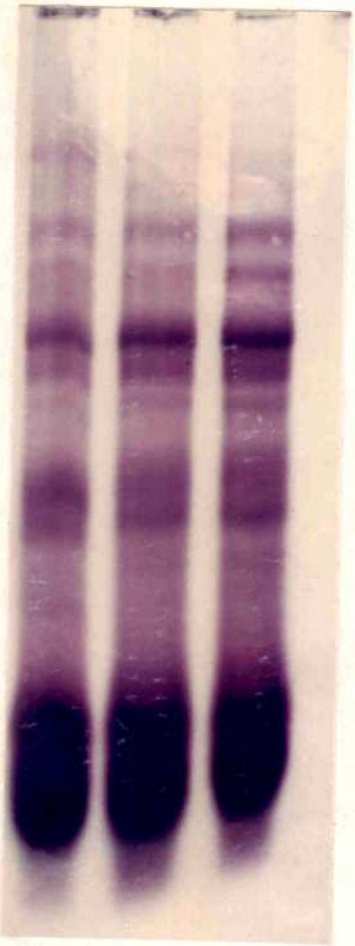

Fig. 12b

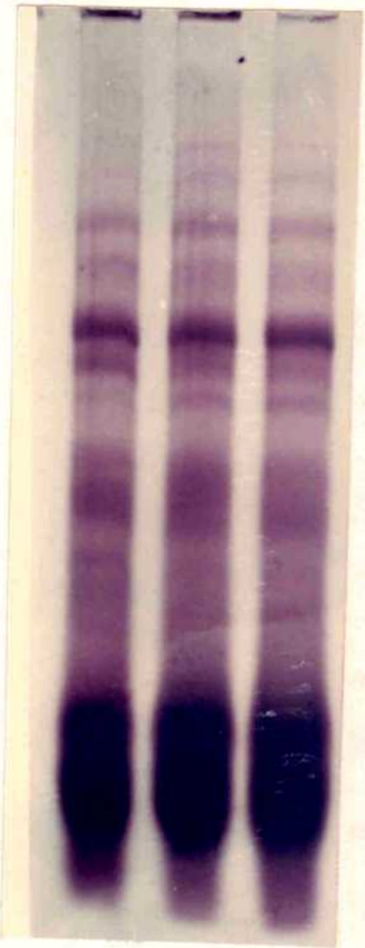

Fig. 13b

observed to be consistent in both the sample extracts obtained from each seed of two different plants per cutlivar. The protein profiles are drawn by visual screening of the gels and presented in Fig.14a to Fig. 21a.

4.2.1 *Gossypium arboreum* varieties

The three *arboreum* varieties viz., Eknath, Sanjay and Rohini were studied for their salt soluble seed protein banding patterns. The resulting protein profile is presented in Fig. 14b and its sketch is presented in Fig. 14a. From this it is evident that, the three varieties exhibited contrasting qualitative differences in the banding pattern. Eknath had 9, Sanjay 5 and Rohini 7 bands. A total of 12 bands were recognised in this group. In these 3 varieties, four Band Nos. 1, 2, and 9 (Rm 0.36, 0.41, 0.66 and 0.78) were common. Band Nos. 3, 5, 8 and 11 (Rm 0.54, 0.58, 0.72 and 0.94) were specific to Eknath. Band No. 4 and 6 (Rm 0.55 and 0.60) were specific to Rohini. Quantitative differences among the bands were not conspicuous.

4.2.2 *Gossypium hirsutum* varieties

The three varieties studied in this group were Buri 1007, SRT1 and Purnima. The seed globulin banding patterns and their depictions are presented in Fig. 15b and Fig. 15a. Qualitative differences were observed among the varieties for banding pattern. Buri 1007 and Purnima exhibited 7 bands each while in SRT1, 8 bands were observed. A total of 10 bands were recognised in this group. Five bands (Nos. 1, 2, 3, 4 and 7) were common in all the 3 varieties. Band No. 5 (Rm 0.60) was observed in Buri 1007 and Purnima but was absent in SRT1. Band No. 8 (Rm 0.87) was present in Buri 1007 and SRT1 but absent in Purnima. Band Nos. 6 and 10 (Rm 0.65 and 0.93) were specific to SRT1. Band No. 9 (Rm 0.91) was specific to Purnima.

4.2.3 Intra-*hirsutum* hybrids

4.2.3.1 NHH44 and its parents

From the banding pattern (Fig.16b) and its corresponding depiction (Fig. 16a) it is evident that there existed both qualitative and quantitative differences in

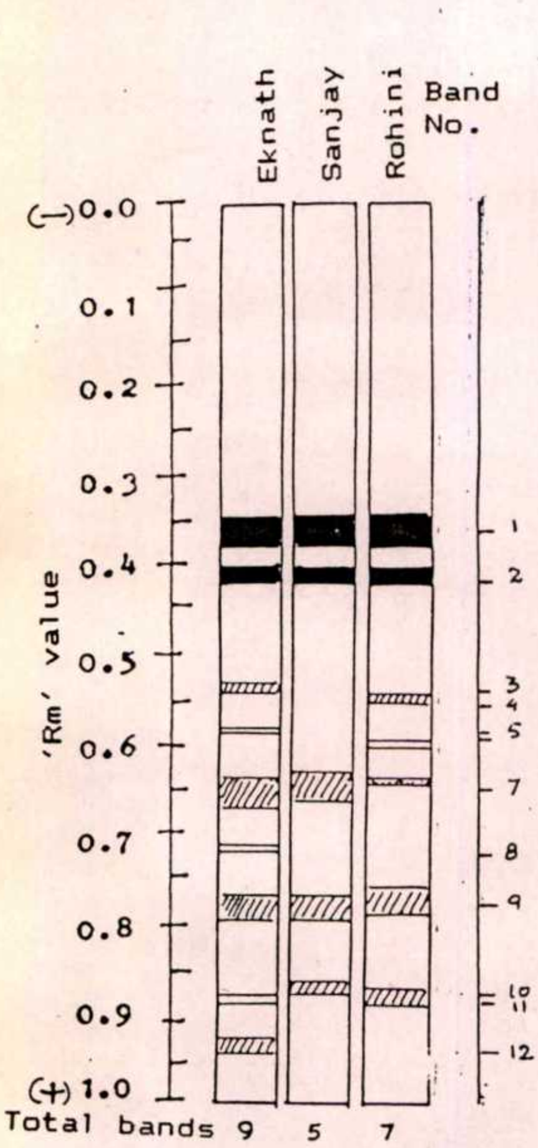

Fig. 14a

Fig. 14a SDS-PAGE profiles of seed globulins in *G. arboreum* varieties

Fig. 14b Electrophoregrams

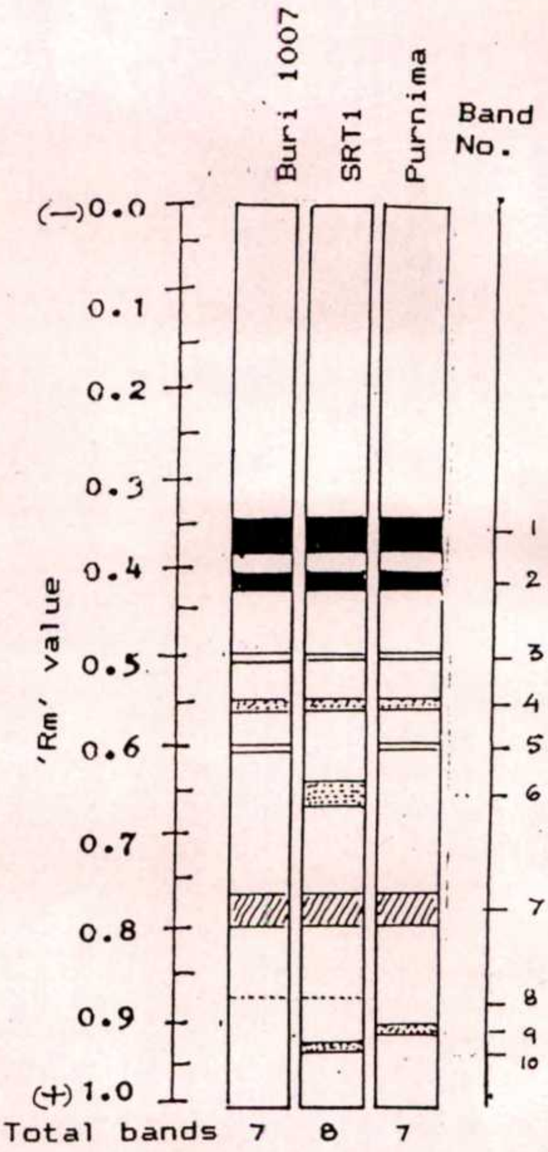

Fig. 15a

Fig. 15a SDS-PAGE profiles of seed globulins in *G. hirsutum* varieties

Fig. 15b Electrophoregrams

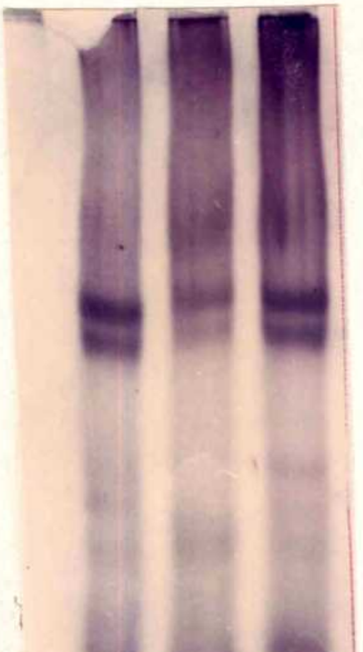

Fig. 14b

Fig. 15b

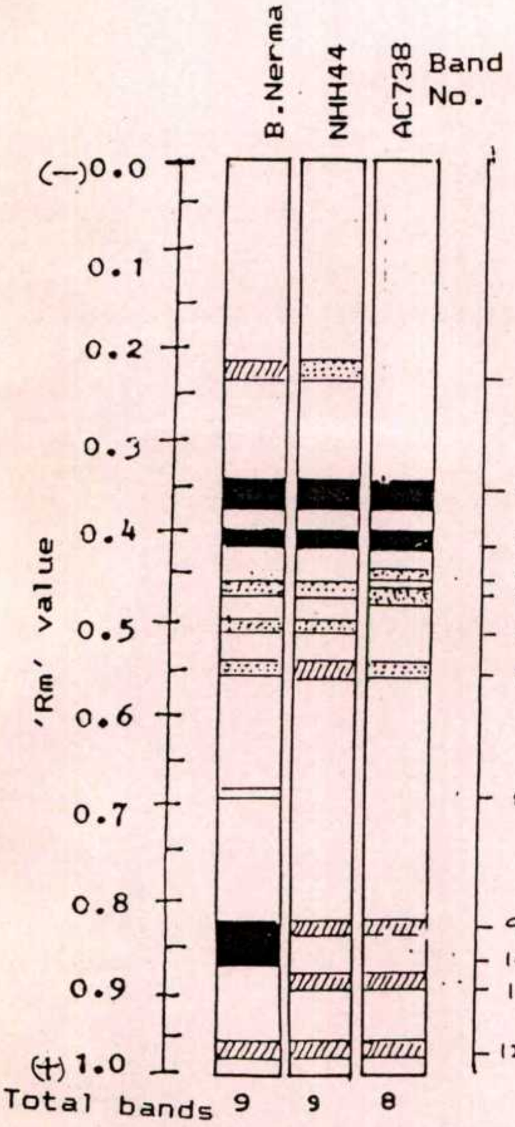

Fig. 16a

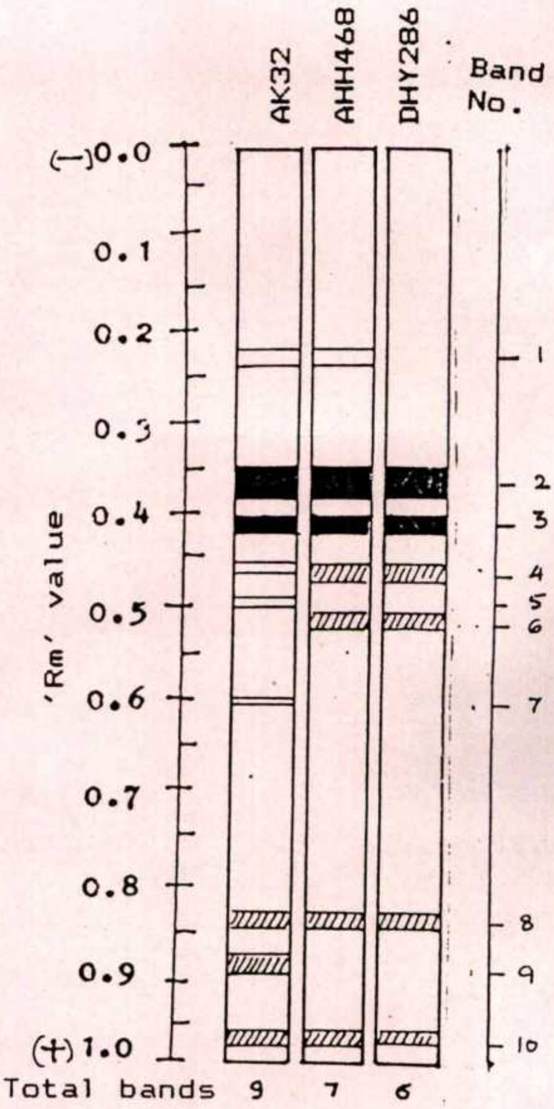

Fig. 17a

Fig. 16a SDS-PAGE profiles of seed globulins in NHH44 and its parents

Fig. 16b Electrophoregrams

Fig. 17a SDS-PAGE profiles of seed globulins in AHH468 and its parents

Fig. 17b Electrophoregrams

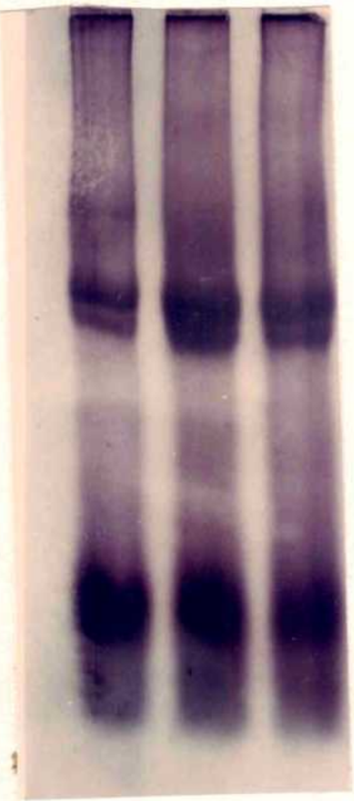

Fig. 16b

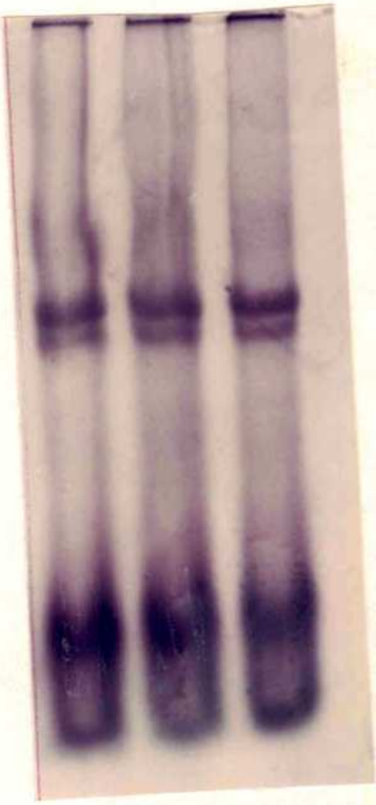

Fig. 17b

the seed globulin banding pattern of the hybrid NHH44 and its parents. A total of 12 bands were recognised in this group, out of which 5 bands were common to all the 3 genotypes. The female parent (Bikaneri Nerma) and the hybrid exhibited 9 bands each while the male parent (AC738) exhibited 8 bands. Band No. 8 (Rm 0.68) was specific to the female parent and Band No. 4 (Rm 0.45) was specific to the male parent. Band Nos. 1 and 6 (Rm 0.24 and 0.50) were present in the female parent and the hybrid but not in the male parent. Band No. 11 (Rm 0.89) was observed in the male parent and the hybrid but was absent in the female parent.

4.2.3.2 AHH468 and its parents

The globulin banding patterns of the hybrid AHH468 and its parents are presented in Fig. 17b and Fig. 17a. The Fig. 17a reveals qualitative and quantitative variation in the globulin profiles of the 3 genotypes. In the female parent, 9 bands were observed, in the hybrid 7 bands and in the male parent 6 bands. A total of 10 bands were recognised in this group, out of which 4 bands were common to all the 3 genotypes. The male parent as well as the hybrid exhibited similar pattern for 6 bands. Band No. 1 (Rm 0.23) was present in the hybrid as well as the female parent. Band Nos. 5, 7 and 9 (Rm 0.50, 0.60 and 0.88) were specific to the female parent. Band No. 4 (Rm 0.46) was weakly stained in the female parent and medium in the male parent and the hybrid.

4.2.3.3 H4 and its parents

Globulin profiles of the hybrid H4 and its female G67 and male parent (American Nectariless) are presented in Fig. 18b and depicted in Fig. 18a. The zymogram reveals that both the parents had 8 bands each while the hybrid had only 7 bands. A total of 11 bands were recognised in this group, out of which 4 bands were common in all the 3 genotypes. Band Nos. 3 and 4 (Rm 0.46 and 0.55) were present in both the parents but absent in the hybrid. However, an unique densely stained band (Band No. 5, Rm 0.58) was observed in hybrid. Band No. 10 (Rm 0.92) was specific to the female parent. Band No. 11 (Rm 0.96) was present in the hybrid as well as the male parent. Quantitative differences were observed in band Nos. 6 and 7 (Rm 0.66 and 0.72), these bands were stained with medium intensity in the hybrid and faintly stained in both the parents. Band No. 8 (Rm 0.80) was specific to the hybrid while band No. 9 (Rm 0.82) was observed in both the parents.

4.2.3.4 Godavari and its parents

The zymogram and its depiction in the hybrid Godavari along with its female parent (Buri Nectariless) and male parent (MCU5) are presented in Fig. 19b and Fig. 19a. From Fig. 19a it is evident that both the parents exhibited 8 bands while the hybrid exhibited 7 bands. A total of 10 bands were recognised in this group, out of which 5 bands were common to all the 3 genotypes. Band Nos. 3 and 4 (Rm 0.46 and 0.52) which were present in both the parents were not observed in the hybrid, instead a densely stained band (Band No. 5, Rm 0.55) was specifically observed in the hybrid. Band No. 10 (Rm 0.96) was specific to the female parent. Band No. 9 (Rm 0.88) was observed in the hybrid as well as the male parent but was absent in the female parent. Quantitative differences were observed in band Nos. 6 (Rm 0.66 and 0.70) which were faint in both the parents, but of medium intensity in the hybrid. Band No. 7 (Rm 0.70) was medium in hybrid, weak in ♀ and faint in ♂ parent.

4.2.4 <u>Hirsutum</u> x <u>barbadense</u> hybrids

4.2.4.1 Varalaxmi and its parents

From the Fig. 20a which show the banding patterns of globulins of the interspecific hybrid Varalaxmi along with its female parent (Laxmi) and male parent (SB289E), it is evident that the female parent had 7 bands while the

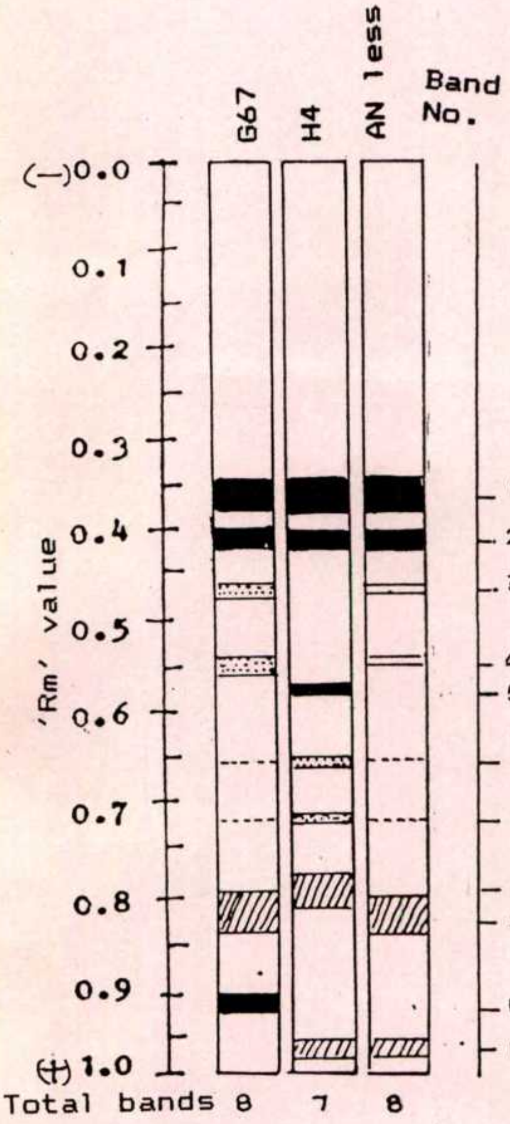

Fig. 18a

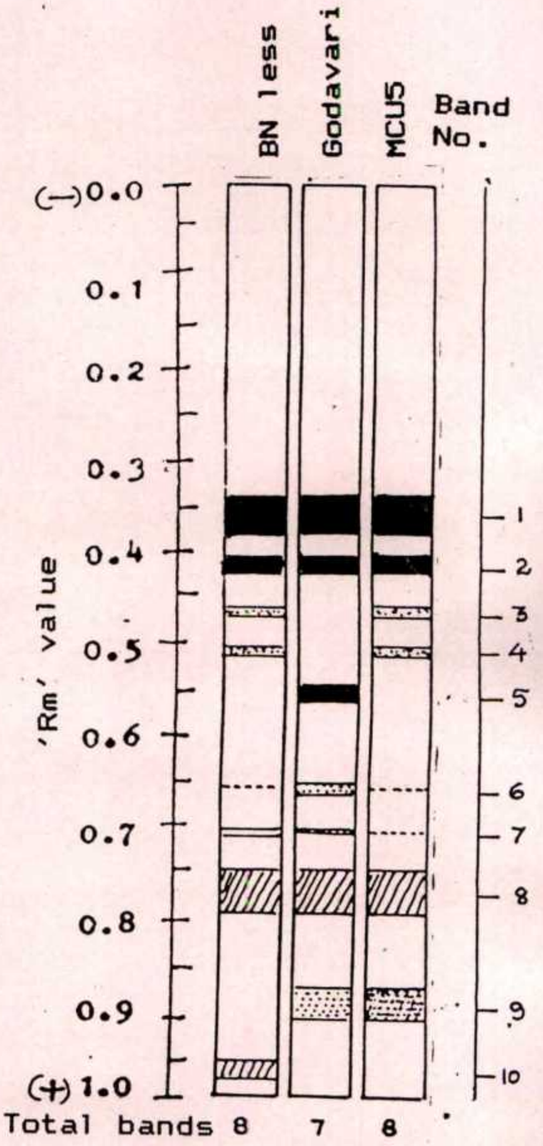

Fig. 19a

Fig. 18a SDS-PAGE profiles of seed globulins in H4 and its parents

Fig. 18b Electrophoregrams

Fig. 19a SDS-PAGE profiles of seed globulins in Godavari and its parents

Fig. 19b Electrophoregrams

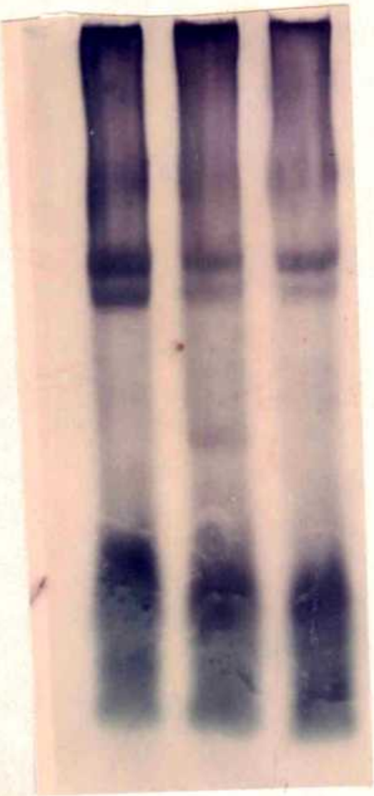

Fig. 18b

Fig. 19b

Fig. 20b

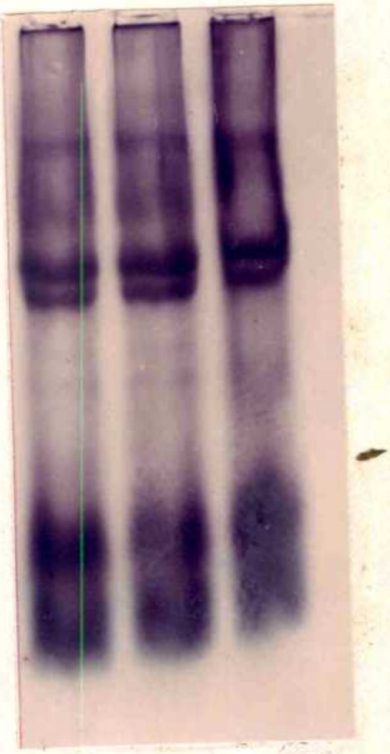

Fig. 21b

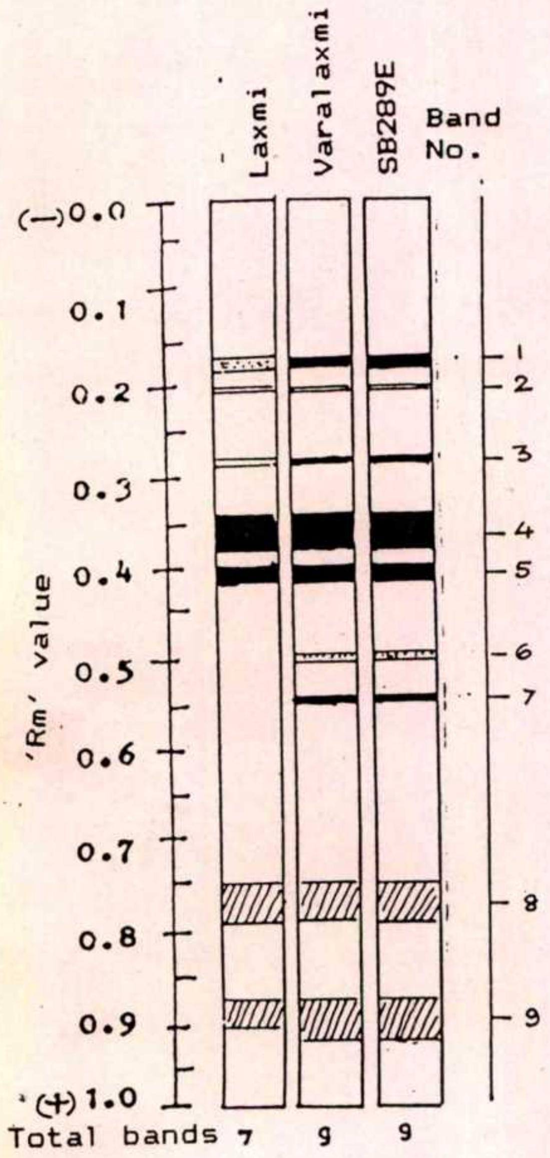

Fig. 20a

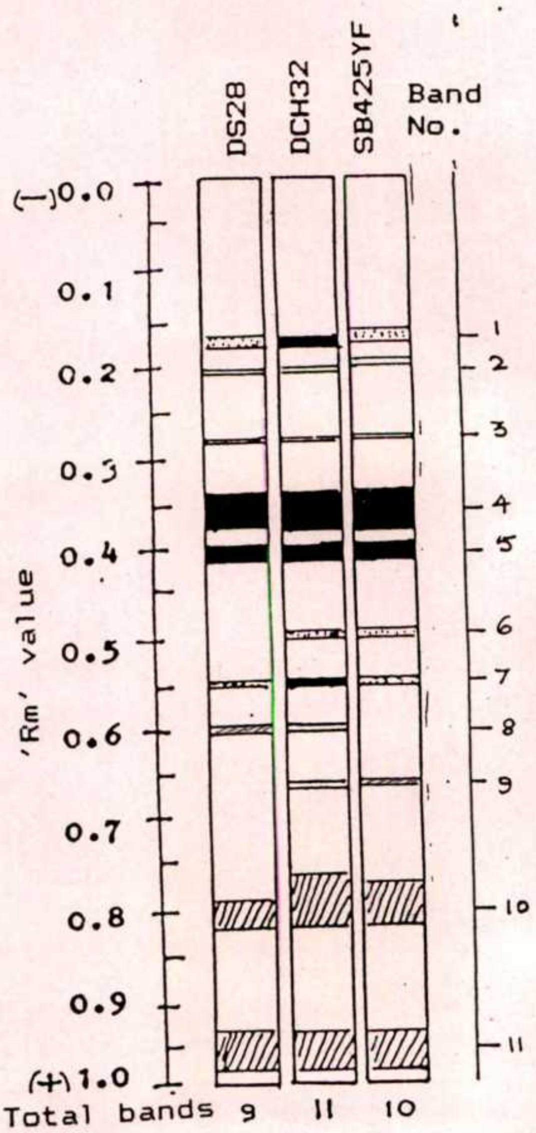

Fig. 21a

Fig. 20a SDS-PAGE profiles of seed globulins in Varalaxmi and its parents

Fig. 21a SDS-PAGE profiles of seed globulins in DCH32 and its parents

Fig. 20b Electrophoregrams

Fig. 21b Electrophoregrams

male parent and the hybrid exhibited 9 bands each. A total of 9 bands were recognised in this group, out of which 7 bands were common to all the 3 genotypes. Band No. 6 and 7 (Rm 0.50 and 0.55) were present in the hybrid and the male parent but were absent in the female parent. The hybrid and the male parent exhibited similar banding pattern both qualitatively and quantitatively.

4.2.4.2 DCH32 and its parents

The globulin banding patterns of the interspecific hybrid DCH32 along with its female (DS28) and male parent (SB425YF) are presented in Fig. 21b and depicted in Fig. 21a. From the Fig. 21a it is evident that the hybrid exhibited 11 bands while its female parent and male parent exhibited 9 and 10 bands respectively. Eight bands were common to all the three genotypes. Band Nos. 6 and 9 (Rm 0.50 and 0.66) were present in the male and the hybrid but absent in the female parent. Band No. 8 (Rm 0.60) was faintly stained in the hybrid and of medium intensity in the female parent. It was absent in the male parent. Band No. 9 was of medium intensity in the male parent while it was faintly stained in the hybrid. Band No's 1 and 7 (Rm 0.17 and 0.55) were dense in the hybrid and light in both the parents.

4.3 Isozymes

4.3.1 Esterases (EST)

Electrophoretic patterns of the EST isozymes in the 24 cotton cultivars are depicted in Fig. 22 to Fig.29.

4.3.1.1 *G. arboreum* varieties

From the Fig. 22 it is evident that each of the three varieties exhibited different type of zymogram. Eknath exhibited 6 bands, Sanjay 7 bands and Rohini 5 bands. However, band Nos. 1, 8 and 10 were common in all the three varieties. Band No. 2 and 4 (Rm 0.28 and 0.36) were specific to Eknath, band Nos. 5 and 9 (Rm 0.41 and 0.71) were specific to Sanjay and band No. 6 (Rm 0.45) was specific to Rohini.

4.3.1.2 *G. hirsutum* varieties

From the Fig. 23 representing the EST zymograms of the three *G. hirsutum* varieties, viz., Buri 1007, SRT1 and Purnima it is evident that 3 varieties exhibited conspicuous qualitative differences. In Buri 1007 and

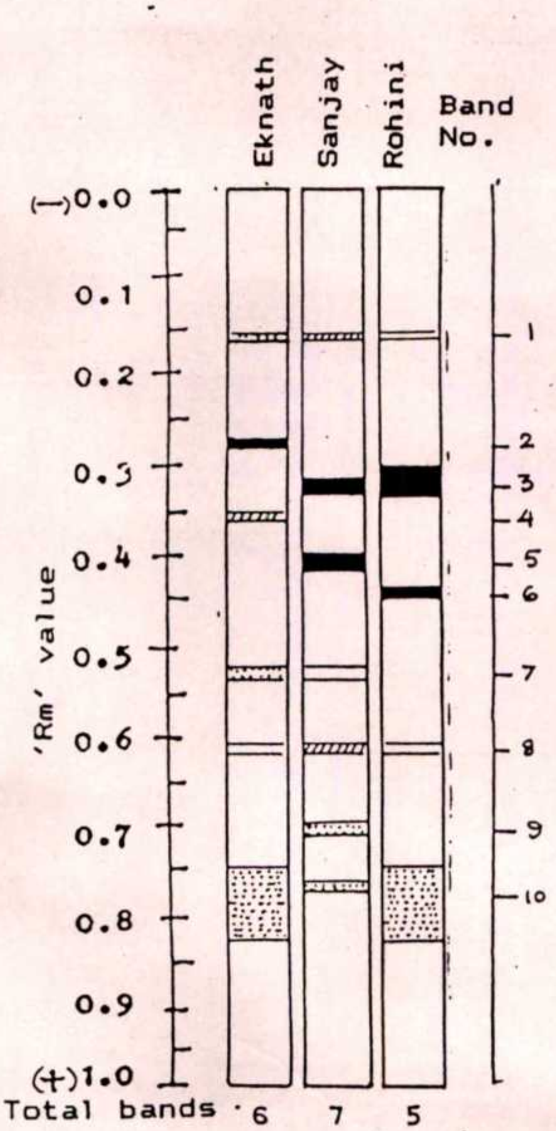

Fig. 22

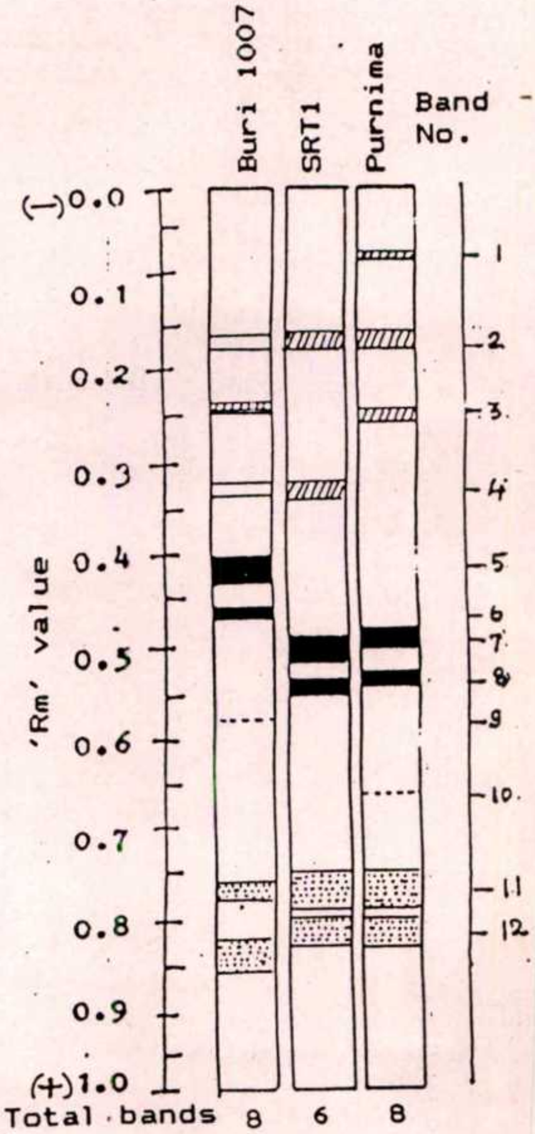

Fig. 23

Fig. 22 PAGE patterns of seed esterases in G. arboreum varieties

Fig. 23 PAGE patterns of seed esterases in G. hirsutum varieties

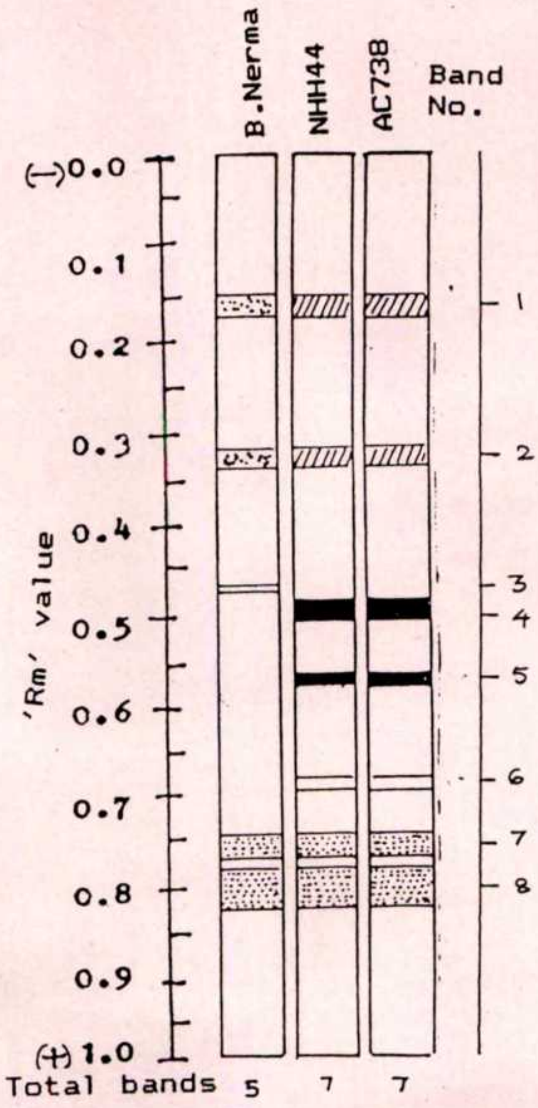

Fig. 24

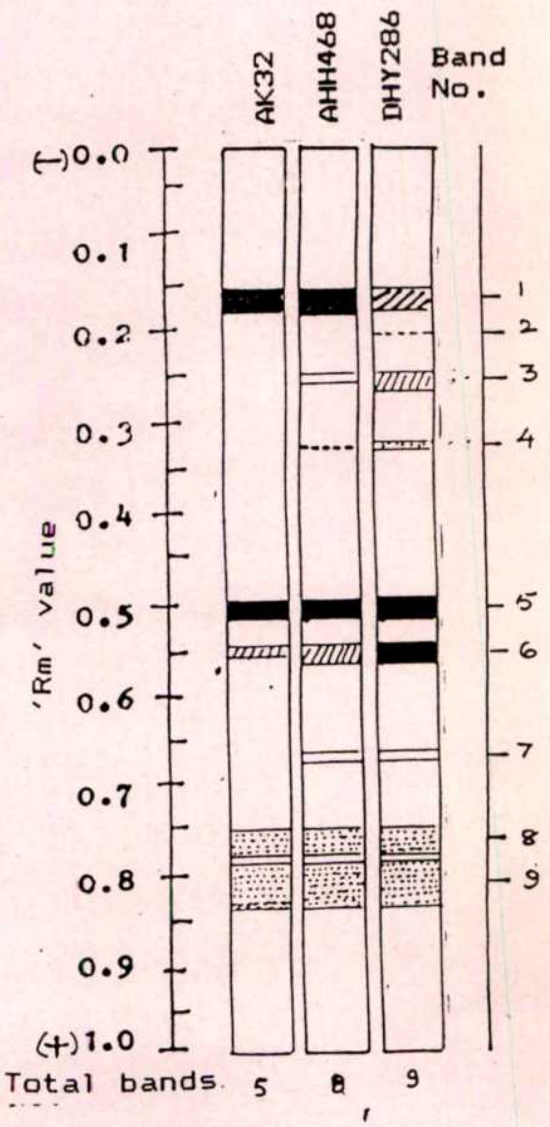

Fig. 25

Fig. 24 PAGE patterns of seed esterases in NHH44 and its parents

Fig. 25 PAGE patterns of seed esterases in AHH468 and its parents

Purnima, 8 bands were resolved while in SRT1 6 bands were observed. Only 3 bands (Nos. 2, 11 and 12) were common to all the three varieties. Band No. 1 and 10 (Rm 0.08 and 0.66) were specific to Purnima, while band Nos. 5, 6 and 9 (Rm 0.42, 0.47 and 0.57) were specific to Buri 1007. Band No. 3 (Rm 0.24) was present in both Buri 1007 and Purnima. Band No. 4 (Rm 0.33) was observed in Buri 1007 and SRT1 but absent in Purnima. Band Nos. 7 and 8 (Rm 0.50 and 0.54) were observed in SRT1 and Purnima but absent in Buri 1007.

4.3.1.3 Intra-*hirsutum* hybrids

A. NHH44 and its parents

From the Fig.24 representing the esterase zymogram of the hybrid NHH44 and its parents (Bikaneri Nerma and AC738), it is evident that there existed both qualitative and quantitative differences among the genotypes. The female parent had 5 esterase bands while the male parent and the hybrid had 7 bands each. Band Nos. 7 and 8 were common in all the 3 entries. Band No. 3 (Rm 0.47) was specific to the female parent. Band Nos. 4, 5 and 6 (Rm 0.50, 0.57 and 0.68) were observed in the hybrid and the male parent only. Band Nos. 1 and 2 (Rm 0.17 and 0.34) were of medium intensity in the hybrid and the male parent while it was lightly stained in the female parent.

B. AHH468 and its parents

From the Fig. 25 representing the esterase zymogram of the hybrid AHH468 and its parents (AK32 and DHY286), it is evident that there existed both qualitative and quanititative differences among the genotypes. The female parent exhibited 5 bands, the male parent 9 bands and the hybrid 8 bands. Five bands (Nos. 1, 5, 6, 8 and 9) were common in all the 3 genotypes. Band No. 2 (Rm 0.21) was specific to the male parent. Band Nos. 3, 4 and 7 (Rm 0.26, 0.33 and 0.67) were observed in the hybrid and the male parent only. Band No. 1 (Rm 0.16) was densely stained in the hybrid and female parent while it was of medium intensity in the male parent. Band No. 4 (Rm 0.32) was faint in the hybrid and light in the male parent. Band No. 6 (Rm 0.55) was of medium intensity in the hybrid and the female parent but densely stained in the male parent.

C. H4 and its parents

From the Fig. 26 representing the esterase zymogram of the hybrid H4 and its parents (G67 and American Nectariless), it is evident that there existed both qualitative and quantitative differences among the genotypes in respect of their banding pattern. The hybrid H4 had 9 EST isozyme bands, while both parents exhibited 7 bands each. Band No. 4 (Rm 0.33) was specific to the female parent. Band No. 5 (Rm 0.42) was specific to the hybrid. Band Nos. 2, 6, 7, 9 and 10 were common to all the 3 genotypes. Band No. 1 (Rm 0.08) was present in the hybrid and the female parent, while Band Nos. 3 and 8 (Rm 0.26 and 0.67) was present in the hybrid and the male parent. Band No. 2 was densely stained in the female parent, medium in the hybrid and light in the male parent.

D. Godavari and its parents

The esterase zymograms of the hybrid Godavari and its parents (Buri × Nectariless and MCU5) are presented in Fig. 27. From this it is evident that there existed both qualitative and quantitative differences among the genotypes for esterases. Eight isozymes of esterase were detected in the hybrid Godavari, 7 in the female and 9 in the male parent. Six bands (Nos. 1, 2, 6, 8, 9 and 10) were common to all the three genotypes. Band No. 4 (Rm 0.32) was specific to the hybrid, while band No. 5 (Rm 0.35) was specific to the male parent. Band No. 3 (Rm 0.26) which was observed in both the parents was absent in the hybrid. Band

No. 7 (Rm 0.56) was observed in the hybrid as well as the male parent but absent in the female parent. Band No. 6 (Rm 0.50) was densely stained in the female parent and of medium intensity in the male parent and the hybrid.

4.3.1.4 *Hirsutum* x *barbadense* hybrids

A. Varalaxmi and its parents

The esterase zymograms of the hybrid Varalaxmi and its parents are presented in Fig. 28. From this it is evident that there existed both qualitative and quantitative differences among the three genotypes in their EST banding patterns. The female parent and the hybrid had 7 bands each while the male parent exhibited 6 bands. Band No. 3 (Rm 0.25) was specific to the hybrid. Band No. 1 (Rm 0.08) was specific to the female parent. Band No. 7 (Rm 0.60) was lightly stained in the female parent and faint in the hybrid. Band No. 5 (Rm 0.42) was densely stained in the hybrid and of medium intensity in the male parent. Band No. 4 (Rm 0.27) was present in both the parents but absent in the hybrid. Band No. 2 (Rm 0.16) was dense in the hybrid and the male parent but medium in intensity in the female parent. Band No. 6 (Rm 0.50) was observed to be dense in the hybrid and the female parent but light in the male parent.

B. DCH32 and its parents

The zymograms of the esterase isozyme of the interspecific hybrid DCH32 and its parents are presented in Fig. 29. From the figure it is evident that there existed both qualitative and quantitative differences among the 3 genotypes for their esterase banding pattern. The hybrid and the female parent exhibited 7 bands each while the male parent had 6 bands. Three bands (Nos. 5, 9 and 10) were common in all the 3 genotypes. Band Nos. 1 and 8 (Rm 0.09 and 0.60) were specific to the female parent. Band No. 2 (Rm 0.17) was dense in the male parent and light in the hybrid. Band No. 3 (Rm 0.25) was observed in the hybrid and the female parent only. Band No. 4 (Rm 0.27) was specific to the male parent. Band No. 5 (Rm 0.42) was dense in the female, medium in the hybrid and faint in the male parent. Band No. 6 (Rm 0.48) was observed in the hybrid as well as the female parent, but was absent in the male parent. Band No. 7 (Rm 0.58) was densely stained in the hybrid and of medium intensity in the male parent.

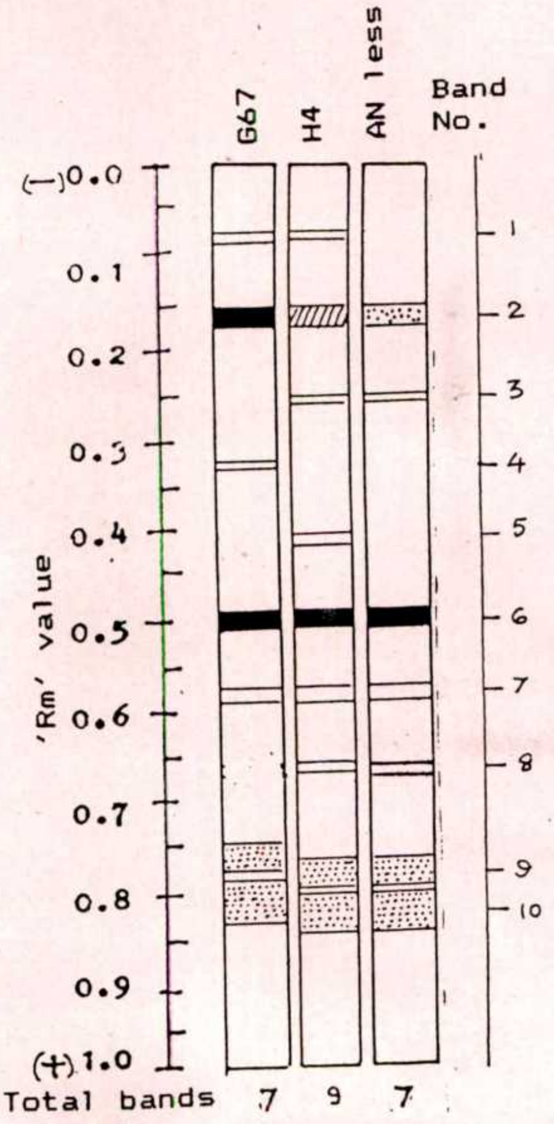

Fig. 26

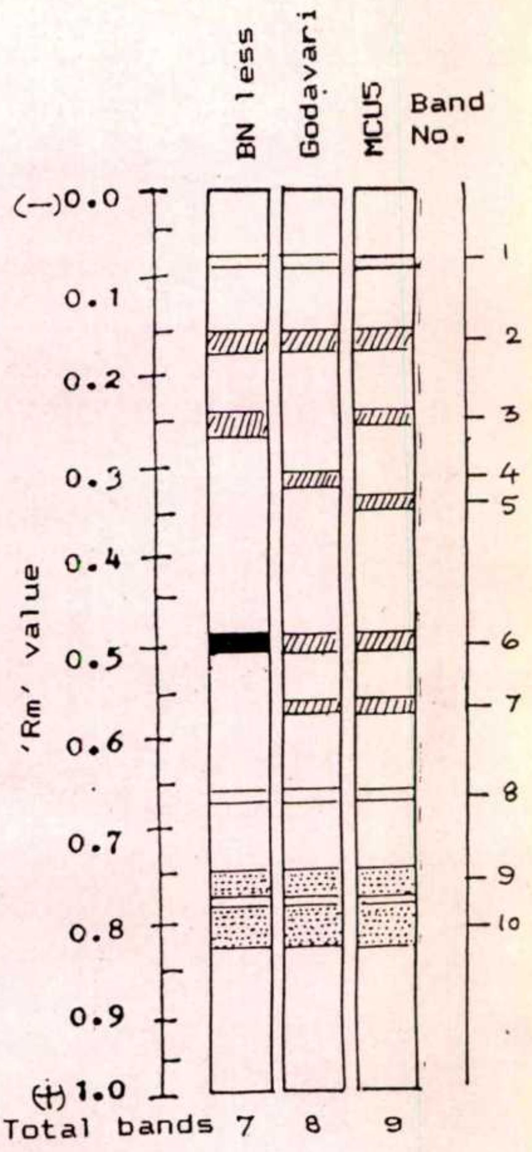

Fig. 27

Fig. 26 PAGE patterns of seed esterases in H4 and its parents

Fig. 27 PAGE patterns of seed esterases in Godavari and its parents

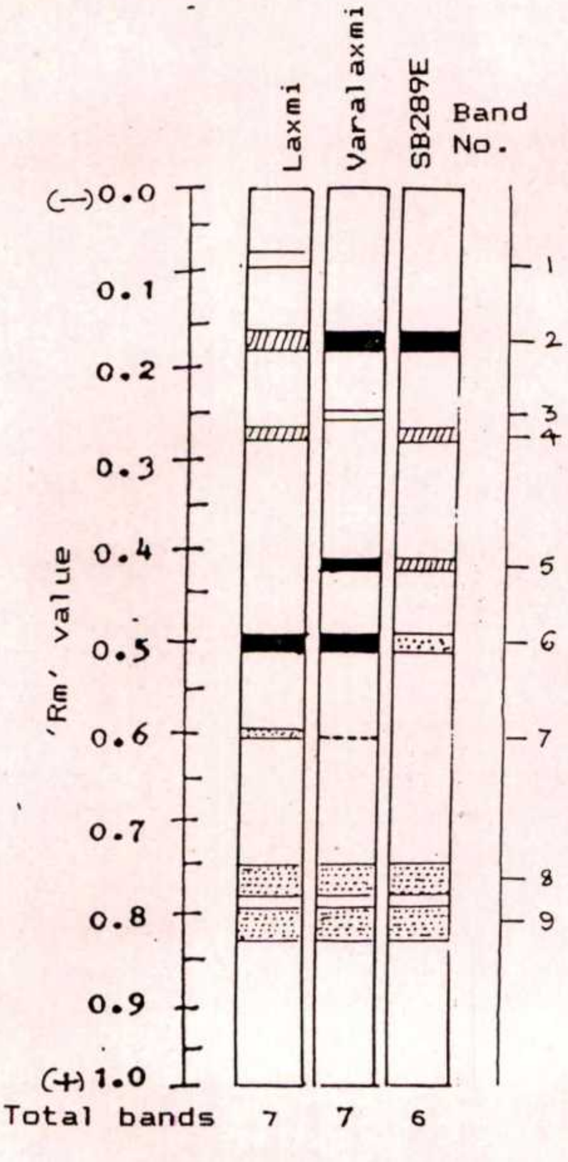

Fig. 28

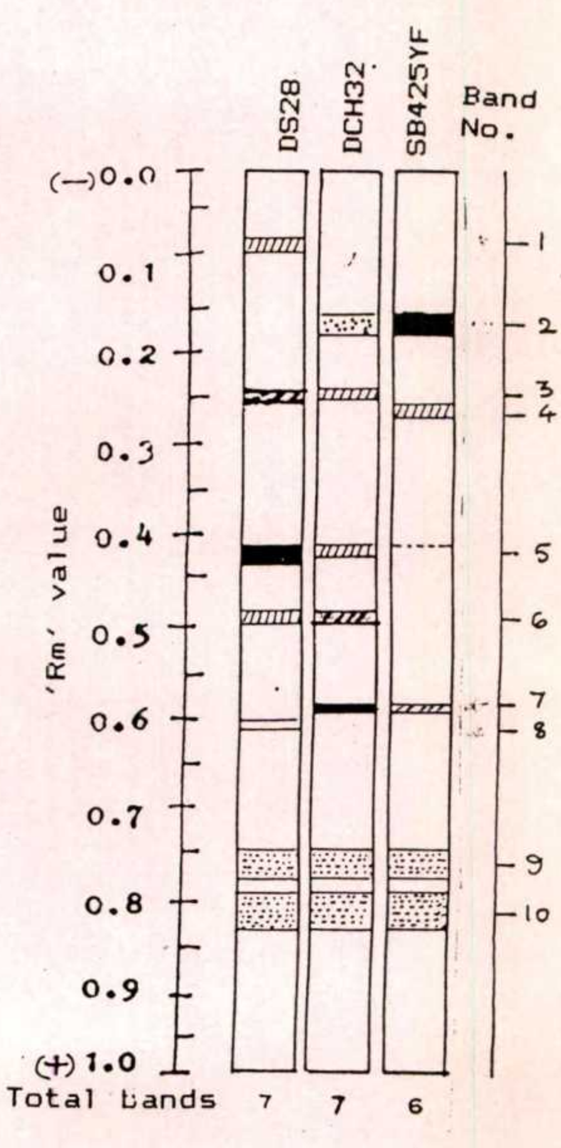

Fig. 29

Fig. 28 PAGE patterns of seed esterases in Varalaxmi and its parents

Fig. 29 PAGE patterns of seed esterases in DCH32 and its parents

4.3.2 Malate dehyrdogenase (MDH)

Banding patterns of MDH isozymes drawn by visual screening of the gels are presented in Fig. 30 to Fig. 37.

4.3.2.1 G. arboreum varieties

From the Fig. 30 it is evident that each of the three varieties exhibited different type of zymogram. Qualitative and quantitative differences were observed among the varieties in their banding pattern. Qualitative variations were found to be predominating. In Eknath, 8 bands were recorded, while in Sanjay and Rohini 5 and 6 bands respectively were recorded. In all 13 bands were recognised in this group of varieties. Band Nos. 1 and 7 (Rm 0.04 and 0.16) were common in all the three cultivars. Band Nos. 9 and 12 (Rm 0.25 and 0.33) were present in Sanjay and Rohini. Band No. 9 (Rm 0.25) was broader in Rohini than in Sanjay. Band No. 7 (Rm 0.16) which was found in all the three cultivars was observed to be dense in Sanjay medium in Rohini and light in Eknath.

4.3.2.2 G. hirsutum varieties

In the MDH zymograms of three *hirsutum* varieties, qualitative and quantitative differences were observed

(Fig. 31). Buri 1007 and SRT1 exhibited 4 bands each while 3 bands were resolved in Purnima. In all 8 bands were recognised in these 3 varieties. Band Nos. 2 and 7 (Rm 0.04 and 0.28) were present in Buri 1007 and SRT1. Band No. 3 and 8 (Rm 0.10 and 0.32) were specific to Purnima. Band No. 5 (Rm 0.16) was specific to SRT1. Band No. 6 (Rm 0.20) was observed in Buri 1007 and Purnima. This band was broad in Purnima and narrow in Buri 1007.

4.3.2.3 Intra-*hirsutum* hybrids

A. NHH44 and its parents

From the Fig. 32 representing the MDH activity in the hybrid NHH44 and its parents (Bikaneri Nerma and AC738) it is evident that there existed both qualitative and quantitative differences among the 3 genotypes in their banding patterns. The female parent, Bikaneri Nerma had 3 bands while the hybrid and the male parent (AC738) had 4 bands each. In all 7 bands were observed in the three cultivars. The male parent and the hybrid exhibited more or less similar banding pattern though quantitative variation in band No. 4 (Rm 0.20) was observed. This band was broader in the male parent than in the hybrid. Band No. 3, 5 and 6 (Rm 0.15, 0.23 and 0.30) were specific to the female parent. Band Nos. 1, 2, 4 and 7 (Rm 0.04, 0.10, 0.20 and 0.30) were observed in the hybrid and the male parent but were absent in the female parent.

B. AHH468 and its parents

The MDH zymograms of the hybrid AHH468 along with its parents, AK32 and DHY286 are presented in Fig. 33. From the figure it is evident that there existed both qualitative and quantitative differences in their banding patterns. In all 8 bands were observed in this group. In female parent (AK32) 6 bands were observed. The male parent (DHY286) exhibited 4 bands and the hybrid (AHH468) 5 bands. Band Nos. 1 and 4 (Rm 0.05 and 0.16) were common in all the 3 genotypes. Band Nos. 2, 6 and 8 (Rm 0.07, 0.23 and 0.33) were specific to the female parent. All the 4 bands present in the male parent were also found in the hybrid. Band No. 3 (Rm 0.12) was observed in the female parent as well as the hybrid, but was absent in the male parent. Band Nos. 5 and 7 (Rm 0.22 and 0.30) were observed in the male parent and the hybrid but were absent in the female parent.

C. H4 and its parents

From the Fig. 34 representing the MDH zymogram of the hybrid H4 and its parents (G67 and American Nectariless) it is evident that there existed both qualitative and quantitative differences among the genotypes. In all 9 bands were observed out of which 2 bands were common in all the 3 genotypes. The hybrid H4 exhibited 6 bands while both of its parents exhibited 5 bands each. Band Nos. 4 and 7 (Rm 0.20 and 0.28) were specific to G67. Band No. 8 (Rm 0.30) was specific to the male parent, while band No. 9 (Rm 0.34) was specific to the hybrid H4. Band No. 1 (Rm 0.04) was present in both G67 and H4 but absent in the male parent. Band Nos. 5 and 6 (Rm 0.22 and 0.25) were observed in the hybrid and the male parent, but were absent in the female parent. Quantitative differences were observed in band Nos. 2 and 3 which were faint in the hybrid and light in both the parents. Band No. 5 (Rm 0.22) was broad in the hybrid while narrow in the male parent.

D. Godavari and its parents

The MDH activity in the hybrid Godavari and its parents (Buri Nectariless and MCU5) is represented in Fig. 35. Qualitative variation was observed among the 3 genotypes. The female parent had 3 bands while the hybrid and the male parent had 5 bands each. The hybrid and the male parent exhibited similar banding patterns. A total of 7 MDH bands were observed in the 3 genotypes. Only one band (No. 7, Rm 0.31) was common to all the 3 cultivars. Band

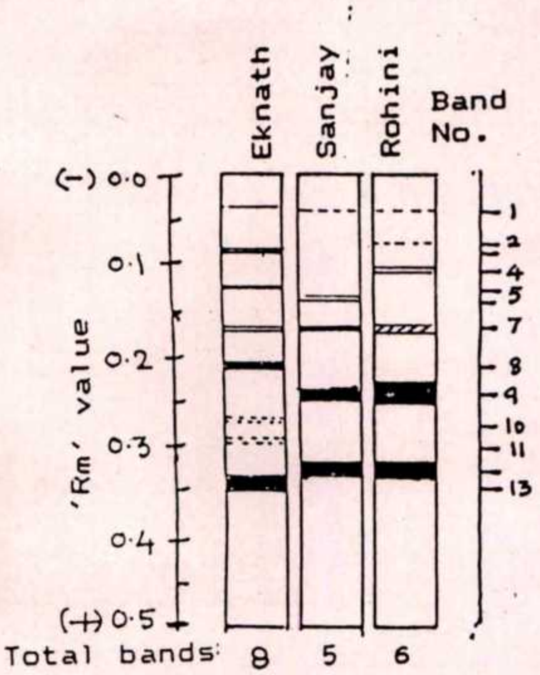

Fig. 30

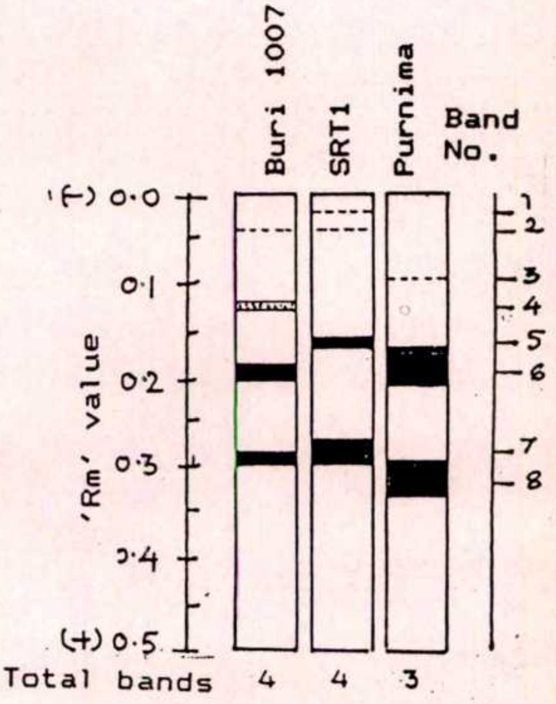

Fig. 31

Fig. 30 PAGE patterns of MDH in seed of G. arboreum varieties

Fig. 31 PAGE patterns of MDH in seed of G. hirsutum varieties

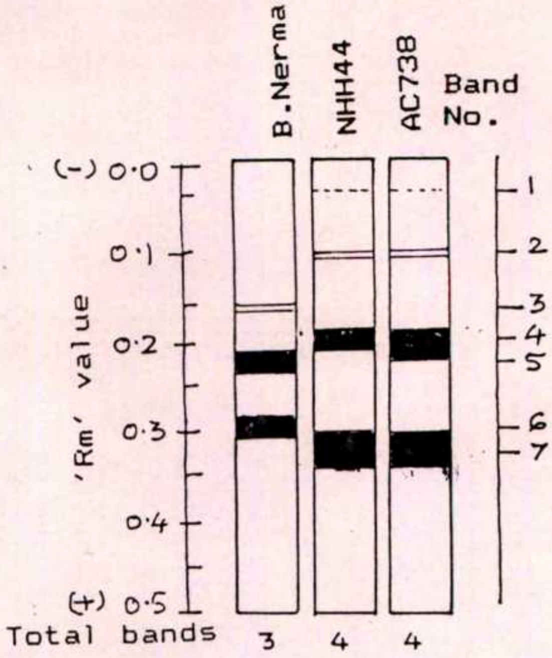

Fig. 32

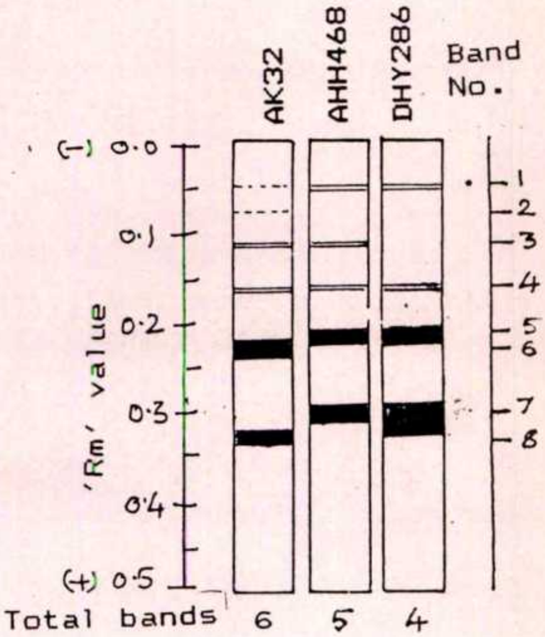

Fig. 33

Fig. 32 PAGE patterns of MDH in seed of NHH44 and its parents

Fig. 33 PAGE patterns of MDH in seed of AHH468 and its parents

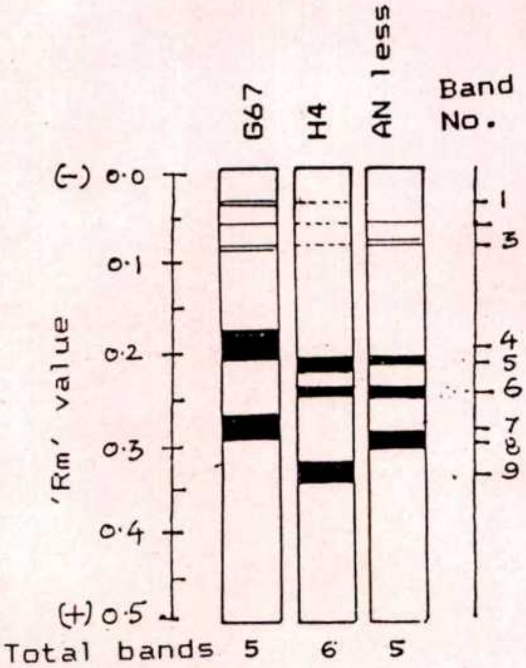

Fig. 34

Fig. 34 PAGE patterns of MDH in seed of H4 and its parents

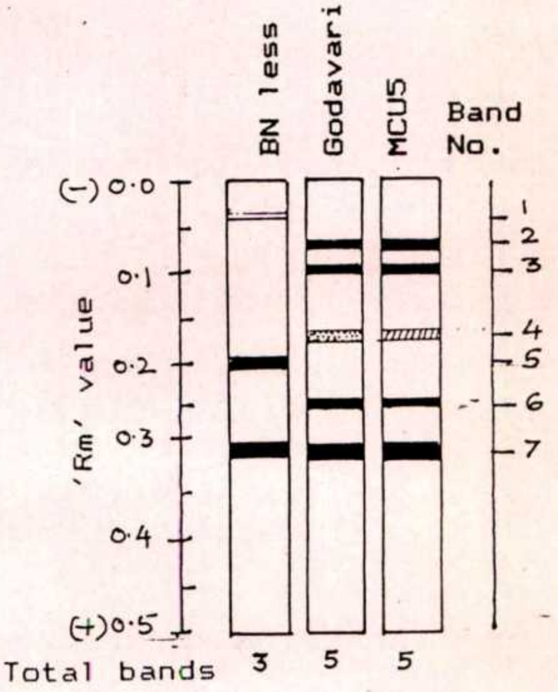

Fig. 35

Fig. 35 PAGE patterns of MDH in seed of Godavari and its parents

No. 1 and 5 (Rm 0.04 and 0.20) were specific to the female parent. Band Nos. 2, 3, 4 and 6 (Rm 0.07, 0.10, 0.16 and 0.25) were present in the hybrid as well as the male parent but absent in the female parent.

4.3.2.4 Hirsutum x barbadense hybrids

A. Varalaxmi and its parents

The MDH zymograms of the interspecific hybrid Varalaxmi and its parents are presented in Fig. 36. There existed both qualitative and quantitative differences in the MDH zymograms. In all 9 bands were resolved. The female parent (Laxmi) had 7 bands, the male parent (SB289E) 5 bands and the hybrid (Varalaxmi) 6 bands. Four bands (Nos. 2, 5, 6 and 7; Rm 0.05, 0.16, 0.22 and 0.30) were common to all the 3 genotypes. Band Nos. 1 and 8 (Rm 0.03 and 0.33) were specific to Laxmi. Band No. 3 (Rm 0.10) was specific to SB289E while band No. 4 (Rm 0.12) was specific to the hybrid Varalaxmi. Band No. 9 (Rm 0.45) was present in the female parent and hybrid only. This band was dense in the female parent and faint in the hybrid.

B. DCH32 and its parents

From the Fig. 37 representing the zymogram of the MDH activity in the interspecific hybrid DCH32 and its parents, it is evident that there existed qualitative and quantitative variation in the banding pattern of the cultivars. The hybrid and the male parent exhibited similar banding patterns, both recording 3 bands each while the female parent had 5 bands. A total of 6 bands were recognised in this group with 3 bands common in them. Band Nos. 2, 3 and 5 (Rm 0.06, 0.10 and 0.30) were specific to the female parent. Band No. 6 (Rm 0.32) was narrow in the hybrid and broad in the male parent.

4.3.3 Alcohol Dehydrogenase (ADH)

Drawings of the ADH zymograms were made by visual screening of the gels and are presented in Fig.38 to Fig.45

4.3.3.1 *G. arboreum* varieties

Fig. 38 shows that each of the three varieties of *arboreum* species exhibited a different type of zymogram. The differences among them were qualitative. Each variety exhibited 2 bands. A total of 3 bands were recognised in this group. In Eknath and Sanjay, band No. 1 (Rm 0.25) was observed. Band No. 2 (Rm 0.30) was observed in Sanjay and Rohini while Band No. 3 (Rm 0.35) was recorded in Eknath and Rohini.

4.3.3.2 G. hirsutum varieties

As shown in Fig. 39 qualitative and quantitative differences existed among the varieties in respect of their ADH isozymes. A total of 3 bands were observed in this group. Each variety had 2 bands. Band No. 1 (Rm 0.18) was specific to Buri 1007. Band No. 2 (Rm 0.24) was observed in SRT1 and Purnima. Band No. 3 (Rm 0.33) was found in all the three entries with differential intensity of staining: dense in Purnima, medium in SRT1 and light in Buri 1007.

4.3.3.3 Intra-hirsutum hybrids

A. NHH44 and its parents

Fig.40 representing the ADH isozyme activity in the seed of the hybrid NHH44 and its parents (B. Nerma and AC738) reveals the occurence of qualitative and quantitative variation among the genotypes. The hybrid NHH44 exhibited 3 bands while both of its parents resolved only 2 bands. In all, 5 bands were recognised in this group. Band No. 1 (Rm 0.05) was specific to the hybrid. Band No. 2 (Rm 0.17) was found in the female as well as the hybrid. Band No. 3 (Rm 0.29) was present in the male parent and the hybrid. Band No. 4 (Rm 0.34) was specific to the female parent.

B. AHH468 and its parents

As shown in Fig. 41 qualitative and quantitative variations existed among the hybrid AHH468 and its parents for the ADH isozyme. A total of 3 bands were recognised in this group. In the hybrid and the male parent, 3 bands were observed while the female parent exhibited only 2 bands. Band No. 1 (Rm 0.04) was observed in the male parent and the hybrid, but was absent in the female parent. Band Nos. 2 and 3 (Rm 0.17 and 0.29) were found in all the 3 entries, however, they differed in the intensity of staining. These bands were resolved to be dense in the hybrid, while medium in both the parents.

C. H4 and its parents

Fig. 42 reveals the occurence of qualitative and quantitative variations among the hybrid H4 and its parents for the ADH isozymes. A total of 3 bands were recognised in this group. Each genotype exhibited 2 bands. Band No. 1 (Rm 0.20) was specific to the female parent. Band No. 2 (Rm 0.24) was present in the hybrid as well as the male parent. Band No. 3 (Rm 0.29) was common in all the 3 genotypes. This band was dense in female parent and light in the hybrid and the male parent.

D. Godavari and its parents

Qualitative and quantitative variations were observed in the hybrid Godavari and its parents for the ADH isozyme banding pattern (Fig. 43). A total of 4 bands were recognised in this group with three bands common in all the three genotypes. Band No. 1 (Rm 0.04) was observed in the male parent as well as the hybrid but was absent in the female parent. Band No. 2 (Rm 0.20) though present in all the genotypes, could be differentiated quantitatively. This band was dense and wide in the male parent and the hybrid, while in the female it was thin and medium. Band Nos. 3 and 4 (Rm 0.27 and 0.38) found in all the 3 genotypes differed quantitatively. These bands were dense in the hybrid, medium in the male parent and light in the female parent.

4.3.3.4 Hirsutum x barbadense hybrids

A. Varalaxmi and its parents

The Fig. 44 represents the zymograms of the ADH in the seeds of the interspecific hybrid Varalaxmi and its parents (Laxmi and SB289E). Qualitative and quantitative variation was observed among the genotypes. A total of 4

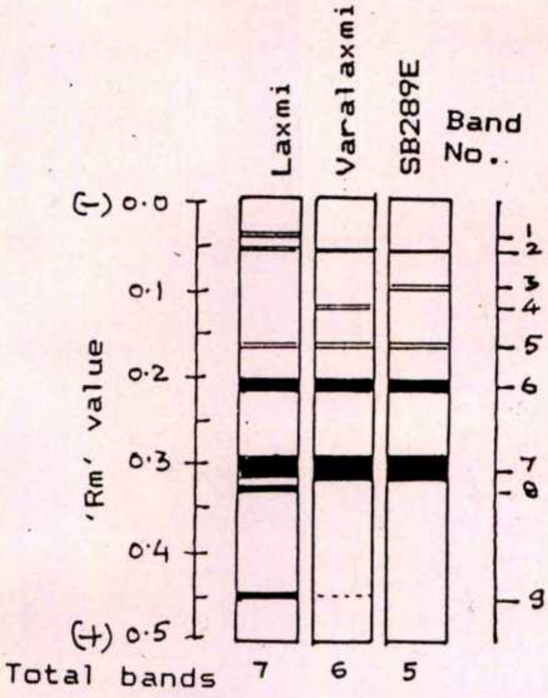

Fig. 36

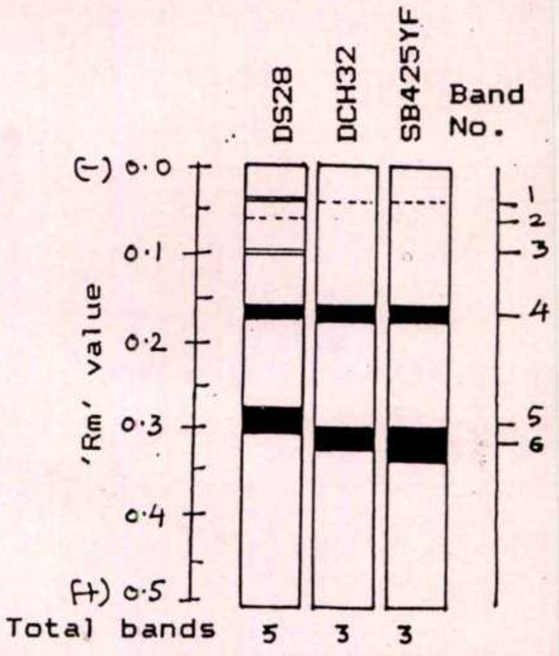

Fig. 37

Fig. 36 PAGE patterns of MDH in seed of Varalaxmi and its parents

Fig. 37 PAGE patterns of MDH in seed of DCH32 and its parents

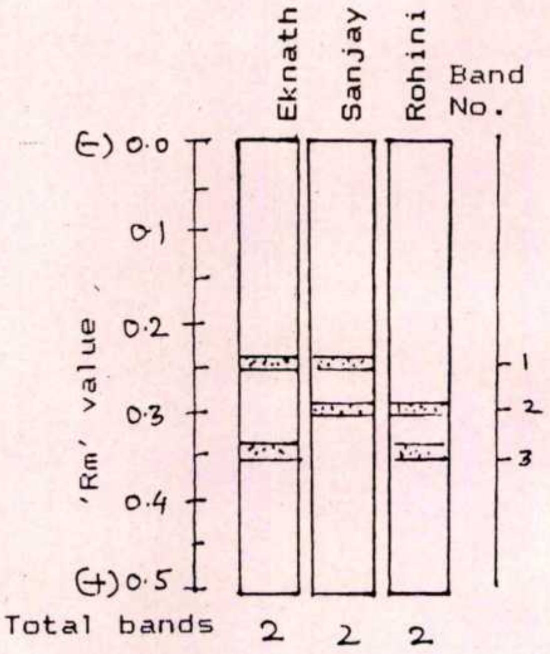

Fig. 38

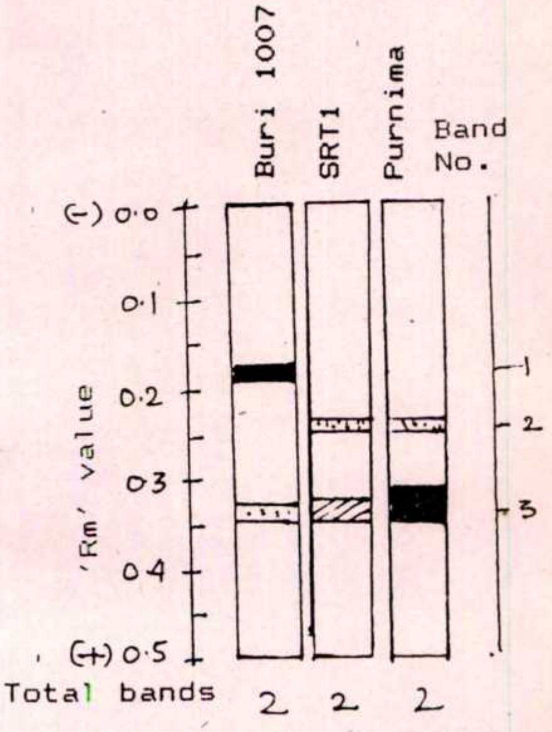

Fig. 39

Fig. 38 PAGE patterns of ADH in seed of G. arboreum varieties

Fig. 39 PAGE patterns of ADH in seed of G. hirsutum varieties

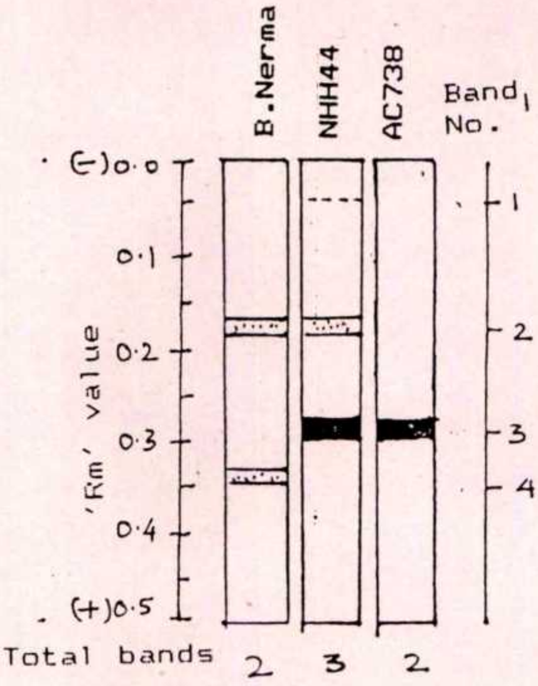

Fig. 40

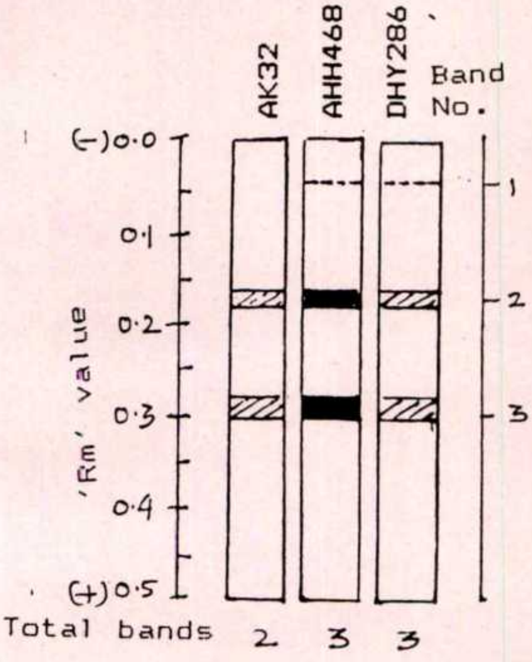

Fig. 41

Fig. 40 PAGE patterns of ADH in seed of NHH44 and its parents

Fig. 41 PAGE patterns of ADH in seed of AHH468 and its parents

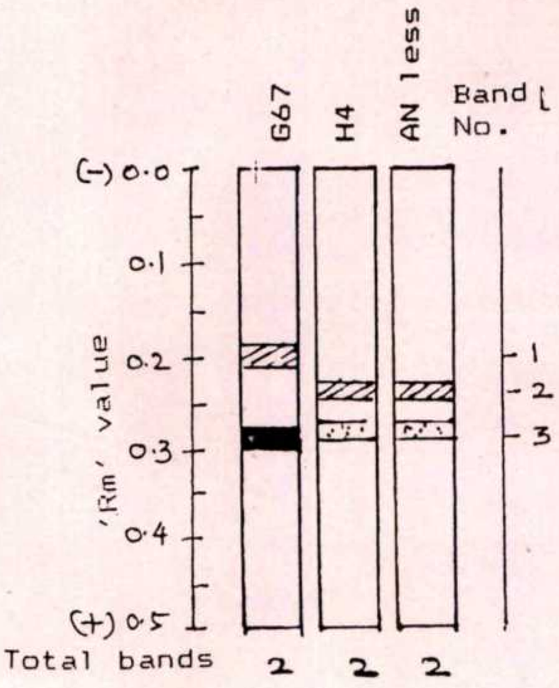

Fig. 42

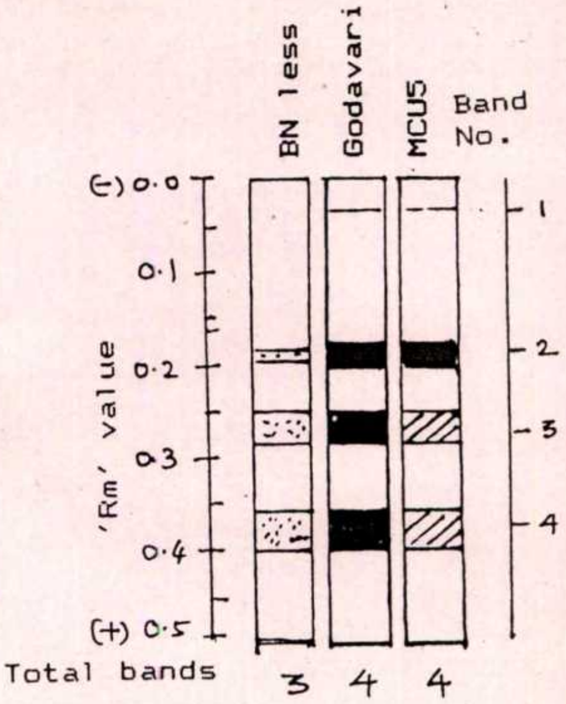

Fig. 43

Fig. 42 PAGE patterns of ADH in seed of H4 and its parents

Fig. 43 PAGE patterns of ADH in seed of Godavari and its parents

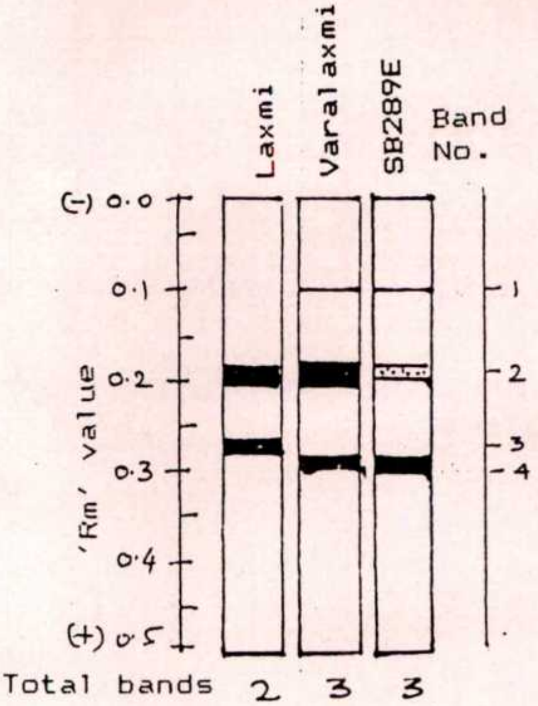

Fig. 44

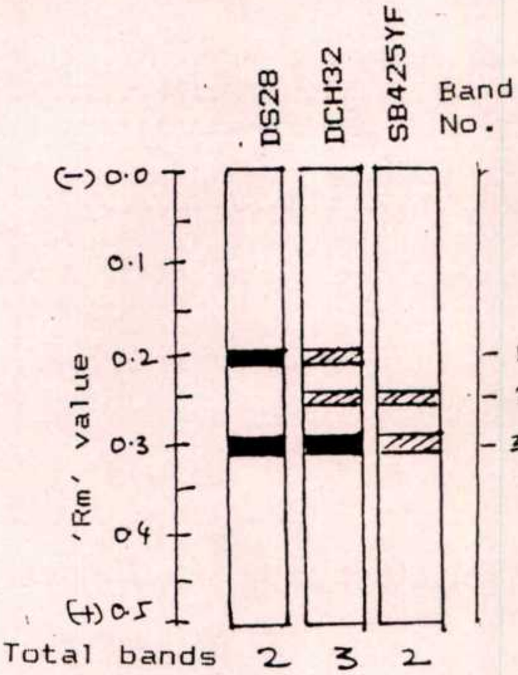

Fig. 45

Fig. 44 PAGE patterns of ADH in seed of Varalaxmi and its parents

Fig. 45 PAGE patterns of ADH in seed of DCH32 and its parents bands were recognised in this group. The female parent exhibited 2 bands while the hybrid and the male parent exhibited 3 bands each. Band No. 1 (Rm 0.10) was found in the hybrid and the male parent. Band No. 2 (Rm 0.20) was observed in all the genotypes, but differed quantitatively. This band was observed to be lightly stained and thin in the male parent. It was dense and wide in the female parent and the hybrid. Band No. 3 (Rm 0.28) was specific to the female parent. Band No. 4 (Rm 0.30) was present in the male parent and the hybrid only.

B. DCH32 and its parents

The ADH zymograms of the interspecific hybrid DCH32 alongwith its parents (DS28 and SB425YF) are presented in Fig. 45. There existed both qualitative and quantitative variations among the genotypes. A total of 3 bands were recognised in this group. Both the parents had 2 bands each while the hybrid exhibited 3 bands. Band No. 1 (Rm 0.20) was observed in the female parent and the hybrid. Band No. 2 (Rm 0.25) was observed in the hybrid and the male parent. Band No. 3 (Rm 0.30) which was observed in all the 3 genotypes differed quantitatively. This band was dense in the female parent and the hybrid, but medium in the male parent.

4.3.4 Peroxidase (PER)

The PER zymograms obtained are depicted in Fig. 51 after visual screening of the gels.

4.3.4.1 *G. arboreum* varieties

The banding pattern of PER isozymes was observed to be similar in all the three cultivars of *arboreum*. Two bands, one at Rm 0.10 and the other at Rm 0.30 were observed with no variation among the cultivars (Fig. 51)

4.3.4.2 *G. hirsutum* varieties

The PER isozyme patterns of the three *hirsutum* varieties were observed to be similar (Fig. 51). Each variety exhibited two bands one at Rm 0.15 and the other at 0.28.

4.3.4.3 Intra-*hirsutum* varieties

All the four intra-*hirsutum* hybrids alongwith their respective parents exhibited similar PER isozyme patterns, each resolving into two bands at Rm 0.15 and 0.28.

4.3.4.4 Hirsutum x barbadense hybrids

The PER isozyme pattern of the two inter-specific hybrids (Varalaxmi and DCH32) had one band intermediate between their respective parents. Band No. 3 (Rm 0.18) was present in both the hybrids only (Fig. 51).

4.3.5 Catalase (CAT)

The CAT zymograms obtained are depicted in Fig. 52 after visual screening of the gels.

4.3.5.1 G. arboreum varieties

The banding pattern of CAT isozymes was observed to be similar in all the 3 cultivars of arboreum. Two bands, one at Rm 0.20 and the other at Rm 0.28 were observed with no variation among the cultivars (Fig. 52).

4.3.5.2 G. hirsutum varieties

The CAT isozyme banding patterns of the three hirsutum varieties was observed to be similar (Fig. 52). Each variety exhibited 3 bands, No. 1, 2 and 3 at Rm 0.20, 0.25 and 0.30 respectively.

4.3.5.3 Intra-*hirsutum* hybrids

All the four intra-*hirsutum* hybrids along with their respective parents exhibited similar CAT isozyme banding patterns, each resolving into 3 bands at Rm 0.20, 0.25 and 0.30 (Fig. 52).

4.3.5.4 *Hirsutum* x *barbadense* hybrids

The CAT isozyme banding pattern of the 2 inter-specific hybrids (Varalaxmi and DCH32) was observed to be similar to that of their male parents, each exhibiting 3 bands at Rm 0.20, 0.25 and 0.34. The female parents of these 2 hybrids also exhibited 3 bands, at Rm 0.20, 0.25 and 0.30. The band No. 3 in the hybrids and the male parents was observed to be wider than in the female parents (Fig. 52).

4.3.6 Acid phosphatase (ACP)

The ACP isozyme banding pattern revealed that all the 24 genotype under study exhibited similar banding pattern, each producing only 2 bands No. 1 and 2 at Rm 0.08 and 0.18 respectively (Fig. 53).

DISCUSSION

5. DISCUSSION

Seed certification schemes, controlled pedigree systems and rules and regulations for seed growing and distribution are all aimed at maintaining cultivar trueness and purity of the seed. However, inspite of this possibilities always exist for unwanted seed of other cultivars or other types to contaminate the originally clean seed lots. To discover and control such contaminations, pre- and post-control tests are conducted by means of cultivar identification and purity determination. All have one general aim to find effective, reliable and scientifically based methods which can be used for cultivar identification of some of the agricultural crops. Both field and laboratory methods have been formulated. As the field plot techniques are tedious and cumbersome, the biochemical tests are developed for the varietal identification and genetic purity determination, by which a large number of samples can be handled within a short span of time. Moreover, the tests can be carried all the year round (Olsen, 1975). The tests could be complementary to field tests in samples involving disputes. Several investigators have emphasised that seed protein and isozyme electrophoresis is a reliable method for the identification of individual and cultivars of cotton (Cherry, 1970; Cherry et al., 1972; Choi and Chang, 1973; Ibragimov, 1973; Hancock, 1982 and Kapse and Nerkar, 1985).

The present investigation was undertaken with the object to identify and determine genetic purity of cotton varieties and hybrids using the electrophoretic patterns of protiens and isozymes in single seeds. The results are discussed in the following :

5.1 *G. arborueum* varieties

The three *arboreum* varieties viz., Eknath, Sanjay and Rohini could be distinguished from one another on the basis of banding pattern of water and salt soluble seed proteins, and EST, MDH and ADH isozyme patterns. However, PER, CAT and ACP isozymes could not provide the discriminative patterns. For water soluble seed proteins, all the three varieties produced same number (14) of bands but certain bands differed in their 'Rm' values which were helpful in identifying them individually. One band (No. 12, Rm 0.56) was specific to Sanjay while 3 bands (Nos. 6, 13 and 15; Rm 0.32, 0.65 and 0.68) were specific to Rohini. Eknath could be differentiated from others by the quantitative difference in one of the bands (Band No. 2; Rm 0.18) (Fig.6a).

Salt soluble seed proteins (globulins) depicted contrasting differences. The varieties could be easily identified by the qualitative variations exhibited by them (Fig.14a). Eknath produced 9 bands, Sanjay 5 bands while

Rohini resolved 7 bands though only 4 bands were common in all the three entries.

Regarding esterases (EST) banding pattern,varieties could be identified by the presence of certain specific bands. Eknath, Sanjay and Rohini produced 6, 7 and 5 bands respectively. One band (No. 6; Rm 0.45) was specific to Rohini while 2 bands (Nos. 2 and 4; Rm 0.28 and 0.36) were specific to Eknath as well as Sanjay (Band Nos. 5 and 9; Rm 0.41 and 0.71) (Fig.22).

The Malate dehydrogenase (MDH) isozyme banding pattern varied in the three varieties, aiding in their identification. Eknath, Sanjay and Rohini resolved into 8, 5 and 6 MDH isozyme bands respectively. The varieties could be identified by the presence of 4 specific bands in Eknath, one specific band in Sanjay and 2 specific bands in Rohini apart from quantitative differences in two of their bands (Fig.30). The ADH isozyme banding pattern was also helpful in identifying the varieties by the presence of certain specific bands even though all of them produced two bands each (Fig.38).

The PER, CAT and ACP isozyme banding patterns were not helpful in identifying the varieties as they were similar in all the 3 varieties (Fig.51, Fig.52 and Fig.53).

Similar differences in the protein banding patterns were reported in 4 different varieties of _G. arboreum_ by Cherry _et al_. (1970) and in 2 varieties studied by Kapse and Nerkar (1985). In the isozyme pattern studied, Cherry _et al_. (1972) also reported differences among the varieties within different species of _Gossypium_ for EST. This polymorphism was not reported for LAP and CAT.

5.2 _G. hirsutum_ varieties

The three _hirsutum_ varieties viz., Buri 1007, SRT1 and Purnima could be distinguished from one another on the basis of banding pattern of albumins, globulins, EST, MDH and ADH isozyme patterns. PER, CAT and ACP could not provide the discriminative patterns. For seed albumins, Purnima was having more number of bands (15) followed by Buri 1007 (14 bands) and SRT1 (10 bands). Buri 1007 could be identified by the presence of 2 specific bands (Nos. 13 and 15; Rm 0.66 and 0.70) and Purnima by 3 specific bands (Nos. 3, 12 and 16; Rm 0.20, 0.61 and 0.73). SRT1 could be also differentiated from others by the quantitative difference in 3 of the bands (Nos. 4, 8 and 14; Rm 0.24, 0.42 and 0.68) (Fig.7a).

By the seed globulins, the 3 genotypes were easily identifiable. SRT1 produced 8 bands while 2 others resolved into 7 bands each. SRT1 was having 2 specific bands (Nos. 6 and 10; Rm 0.65 and 0.93) while Purnima had one specific band (No. 9; Rm 0.91) to aid in identification. Buri 1007 could be identified by the absence of these bands (Fig.15a).

With the esterases (EST) banding patterns, the varieties could be identified by the presence or absence of certain specific bands. SRT1 resolved 6 bands while Buri 1007 and Purnima produced 8 bands each. Buri 1007 could be identified by the presence of 3 specific bands (Nos. 5, 6 and 9; Rm 0.42, 0.47 and 0.57) while Purnima had two specific bands (Nos. 1 and 10; Rm 0.08 and 0.66) to isolate it from the others. SRT1 lacked certain specific bands which were observed in the other 2 varieties (Fig.23).

With regard to the MDH isozyme patterns, Purnima produced 3 bands while Buri 1007 and SRT1 produced 4 bands each. However, no band was common for all the 3 genotypes and each genotype exhibited unique type of MDH isozyme pattern (Fig.31) aiding in identifying them. The ADH isozyme pattern exhibited one specific band (No. 1; Rm 0.18) in Buri 1007. The varieties could also be distinguished from one another by the quantitative differences in one of the bands (No. 3; Rm 0.33) (Fig.39).

The PER (2 bands), CAT (3 bands) and ACP (2 bands) isozyme patterns were not helpful in identifying the varieties as they were similar in all the three varieties (Fig. 51, Fig. 52 and Fig. 53).

Similar results were reported by Cherry *et al*. (1971) and Kapse and Nerkar (1985) who observed seed protein banding differences among the different varieties of *G. hirsutum*. In the isozyme analysis, Cherry *et al*. (1972) also observed qualititative and quantitative differences among the varieties of *hirsutum* for EST while such polymorphism was not observed for LAP and CAT isozyme patterns. Cherry and Katterman (1971) also reported high polymorphism for non-specific seed esterases in natural population of *G. thurberi*.

5.3 Intra-*hirsutum* hybrids

5.3.1 NHH44 and its parents

The hybrid NHH44 could be distinguished from its parents on the basis of the banding pattern of seed albumins, globulins, EST, MDH and ADH. However, PER, CAT and ACP could not provide the discriminative patterns. For the seed albumins, the hybrid NHH44 exhibited 13 bands while both of its parents produced 12 bands each. The hybrid could be identified from its parents by the presence of a specific band (No. 14; Rm 0.62) lacking in its parents. The hybrid NHH44 could also distinguished from its female parent by the presence of 5 bands (Nos. 2, 5, 9, 12 and 15; Rm 0.16, 0.27, 0.41, 0.55 and 0.64) which are also present in the male parent. The female parent could be identified by the presence of 5 specific bands (Nos. 3, 6, 10, 13 and 16; Rm 0.17, 0.29, 0.44, 0.58 and 0.67) in it (Fig. 8a).

By the seed globulin banding patterns the hybrid could be distinguished from its female parent by the absence of 2 bands (Nos. 8 and 11; Rm 0.68 and 0.89). It could also be distinguished from its male parent by the absence of one band (No. 4; Rm 0.45) and presence of band Nos. 1 and 6 (Rm 0.24 and 0.50) in it (Fig.16a).

In the seed EST banding pattern, the female parent had 5 bands while the hybrid and male parent produced 7 bands each. Further, the female parent could be identified by the presence of a specific band (No. 3; Rm 0.47). Both male parent and the hybrid were more or less similar. However, hybrid could be distinguished by its mediumly stained band (No. 2; Rm 0.34) compared to light staining in the male parent (Fig. 24).

By the MDH isozyme banding pattern, the female parent could be distinguished by the presence of 3 bands while both the hybrid and the male parent had 4 bands each. Female parent had two specific bands (No. 3 and 5; Rm 0.15 and 0.30) as its uniqueness. The hybrid and the male parent were more or less similar for MDH pattern. However, the hybrid could be distinguished by the quantitative difference in the band No. 4 (Rm 0.20) (Fig. 32). This band was broader in the male parent than in the hybrid.

In the ADH isozyme banding pattern, the hybrid could be discriminated by the presence of 3 bands while both of its parents exhibited 2 bands each. Band No. 1 (Rm 0.05) was specific to the hybrid as its uniqueness. The female parent could be distinguished from the hybrid as well as male parent by the presence of a specific band (No. 4; Rm 0.35) in the female parent. The female parent could also be discriminated by the absence of band No. 3 (Rm 0.29) found in the hybrid and the male parent. Further, male parent could also be isolated by the absence of a band (No. 2; Rm 0.17) (Fig. 40).

The PER, CAT and ACP isozyme patterns were not helpful in discriminating the hybrid from its parents. As they were observed to be similar (Fig. 51, Fig. 52 and Fig. 53) in all the 3 genotypes.

Similar results were reported by Kapse and Nerkar (1985) who observed one or two extra bands in the hybrid that were inherited from the male parent. Cherry *et al.* (1971) also reported that the F1 plants as well as the parental seed mixture had additive banding patterns from their parents.

5.3.2 AHH468 and its parents

By the seed albumin banding pattern, the hybrid and the male parent were distinguishable by the presence of 15 bands in each, while the female parent had only 12 bands. The hybrid could be discriminated from its female parent by the presence of 3 extra bands (Nos. 6, 11 and 12; Rm 0.31, 0.48 and 0.52) inherited from the male parent (Fig. 9a). Further, the hybrid could be differentiated from its male parent by presene of band Nos. 7 and 9 (Rm 0.35 and 0.41) lacking in the latter. The quantitative differences in band Nos. 1, 3, 4 and 6 were also useful in differentiating the male parent and the hybrid (Fig. 9a).

In the salt soluble seed proteins banding pattern, the hybrid resolved into 7 bands while female and male parent produced 9 and 6 bands respectively. The hybrid could be distinguished from its female parent by the absence of 3 bands (Nos. 5, 7 and 9; Rm 0.50, 0.60 and 0.88) in the hybrid. Further, band No. 4 (Rm 0.46) was light in the female parent and mediumly stained in the hybrid. The hybrid could be discriminated from its male parent by the presence of one extra band (No. 1, Rm 0.23) in the hybrid (Fig. 17a).

By the seed EST isozyme patterns, the hybrid could be identified by the presence of 8 bands from its female parent having 5 bands and male parent having 9 bands. The hybrid could be distinguished from its female parent by having 3 bands (Nos. 3, 4 and 7; Rm 0.26, 0.33 and 0.67) extra inherited from its male parent. The hybrid lacked one band (No. 2, Rm 0.21) which was present as faint band in the male parent (Fig. 25).

In the MDH isozyme pattern, the hybrid exhibited 5 bands while its female and male parents resolved into 6 and 4 bands respectively. The hybrid could be discriminated from its female parent by the presence of two bands (Nos. 5 and 7; Rm 0.22 and 0.30), inherited from its male parent. Further, the hybrid could also be distinguished from its male parent by the presence of one band (No. 3, Rm 0.12), also found in the female parent (Fig. 33).

By the ADH isozyme pattern, the hybrid could be identified from its female parent by the presence of one band (No. 1; Rm 0.05), inherited from its male parent. Both the hybrid and male parent resolved into 3 bands while its female parent exhibited 2 bands. Further, the hybrid could be discriminated by 2 dense bands (No. 2 and 3; Rm 0.17 and 0.29) compared to mediumly stained ones in both the parents (Fig. 41).

The PER, CAT and ACP were not helpful in distinguishing the hybrid from its parents, as they were observed to exhibit similar pattern in all the 3 genotypes (Fig. 51, Fig. 52 and Fig. 53).

The results obtained, also agree with the earlier reports of Kapse and Nerkar (1985) and Cherry et al. (1972) for the seed albumins, EST and CAT patterns.

5.3.3 H4 and its parents

In the seed albumin profiles, the hybrid H4 and its female parent produced 13 bands each, while the male parent exhibited 12 bands. The hybrid could be distinguished by the presence of a specific band (No. 15; Rm 0.74 which was absent in both the parents. It could be differentiated from its female parent by the presene of 4 bands (8, 11, 13 and

16; Rm 0.56, 0.62, 0.66 and 0.75) which were absent in the latter. Further, the hybrid could be discriminated from its male parent by the presence of band No. 6 (Rm 0.38) which was not observed in the latter. In addition, the hybrid differed in the intensity of two bands (Nos. 7 and 9; Rm 0.49 and 0.56) compared to its parents (Fig. 10a) to aid in its identification.

In the seed globulin banding pattern, the hybrid had 7 bands while both of its parents exhibited 8 bands each. The hybrid could be identified by the presence of a specific densely stained band (No. 5; Rm 0.58) which was lacking in either of the parents. Further, the hybrid could be differentiated from its female parent by band Nos. 3, 4 and 9 (Rm 0.46, 0.55 and 0.82) which lacked in the former. The hybrid could also be discriminated from its male parent by the absence of two bands (Nos. 3 and 4; Rm 0.46 and 0.55) which were observed in the latter. In addition, the hybrid differed in the staining intensity of two bands (Nos. 6 and 7; Rm 0.66 and 0.72) compared to both of its parents (Fig. 18a) aiding in its identification.

By the seed EST banding pattern, the hybrid could be identified by the presence of 9 bands while both of its parents had 7 bands each. The hybrid H4 could be distinguished from both of its parents by the presence of a specific band (No. 5; Rm 0.42) that was absent in the latter. The hybrid could be differentiated from its female parent by the presence of two bands (Nos. 3 and 8; Rm 0.26 and 0.67) which were absent in the latter. The hybrid could be discriminated from its male parent by the absence of band No. 1 (Rm 0.08) in the latter. The difference in staining intensity of band No. 2 (Rm 0.17) could also aid in identifying the hybrid from its parents (Fig. 26).

In the MDH isozyme patterns, the hybrid exhibited 6 bands while both of its parents produced 5 bands each. The hybrid could be separated from both of its parents by the presence of a specific band (No. 9; Rm 0.34) in it. It could be distinguished from its female parent by the presence of two bands (Nos. 5 and 6; Rm 0.22 and 0.25) that were absent in the latter. Further, the hybrid could also be discriminated from its male parent by the presence of band No. 1 (Rm 0.04) which is lacking in the latter. Quantitative differences in 3 bands (Nos. 2, 3, and 5) could also aid in identifying the hybrid from its parents (Fig. 34).

By the ADH isozyme patterns, the hybrid could be distinguished from its female parent by the presence of band No. 2 (Rm 0.24) that was absent in the latter. However, the hybrid and the male parent had similar patterns with quantitative differences in band No.3 (Rm 0.29) (Fig. 42). The PER, CAT and ACP isozyme patterns were similar in all the 3 genotypes and were not helpful in discriminating them (Fig.51, Fig.52 and Fig.53).

Thus, the seed albumin, globulin, EST and MDH patterns were most useful in differentiating the hybrid from its parents and aided in identifying them individually and in determining genetic purity of these cultivars.

Similarly, the protein banding pattern differences were reported in Gossypium species by Kapse (1983) and Kapse and Nerkar (1985). Cherry et al. (1971) reported that F1 individuals produced banding pattern that was additive of seed protein of their parents. Further, Cherry et al. (1972) also reported differences and additive banding pattern for synthetic mixture of seed extracts when analysed for EST, however, such polymorphism was not observed in CAT and LAP isozymes in several different Gossypium species studied by them.

5.3.4 Godavari and its parents

In the seed albumin profiles, the hybrid Godavari and its male parent exhibited 14 bands each while in the female parent only 11 bands were observed. The hybrid could be distinguished from its female parent by the presence of

3 bands (Nos. 2, 5 and 8; Rm 0.13, 0.25 and 0.38) in the former and absence in the latter. The hybrid and the male parent were more or less similar except quantitative differences in band Nos. 8 and 12 (Rm 0.37 and 0.65) (Fig. 11a).

In the seed globulin profiles, the hybrid resolved into 7 bands while both of its parents exhibited 8 bands each. The hybrid could be separated by the presence of a densely stained band (No. 5; Rm 0.55) which was absent in either of the parents. The hybrid could be differentiated from its female parent by the presence of band No. 9 (Rm 0.88) in the former and the absence of band Nos. 3, 4 and 10 (Rm 0.46, 0.52 and 0.96) in the former (Fig. 19a). Quantitative differences in two bands (Nos. 6 and 7; Rm 0.66 and 0.70) were also helpful in identifying the hybrid from its parents.

By the EST isozyme pattern, the hybrid could be identified by the presence of 8 bands compared to its female parent and male parent having 7 and 9 bands respectively. The hybrid could be distinguished by the presence of a specific band (No. 4; Rm 0.32) that is absent in both the parents. It could be differentiated from its female parent by the presence of band No. 7 (Rm 0.56) which is absent in the latter. The hybrid could also be distinguished from its male parent by the absence of band Nos. 3 and 5 (Rm 0.26 and 0.35) in the former. Further, the hybrid could be discriminated from its parents by the quantitative differences in band No. 6 (Rm 0.50) (Fig. 27).

In the MDH isozyme patterns, the hybrid and the male parent were similar both qualitatively and quantitatively each producing 5 bands. Its female parent exhibited 3 bands. The hybrid could be distinguished from its female parent by the absence of two bands (Nos. 1 and 5; Rm 0.04 and 0.20) and the presence of 4 bands (Nos. 2, 3, 4 and 6; Rm 0.07, 0.10, 0.16 and 0.25) in the former (Fig. 35).

In the ADH isozyme pattern, the hybrid and male parent produced 4 bands each while its female parent exhibited 3 bands. The hybrid could be distinguished from its female parent by the presence of band No. 1 (Rm 0.04) absent in the latter. The hybrid and the male parent could be differentiated by the quantitative differences in band Nos. 2, 3 and 4 (Rm 0.20, 0.27 and 0.38) (Fig. 43). The PER, CAT and ACP isozyme patterns were similar in all the 3 genotypes and were not helpful in discriminating them (Fig. 51, 52 and 53).

Thus, the studies revealed that the seed albumins, globulins, EST and MDH patterns were the most useful in differentiating in the hybrid from its parents and aided in identifying the hybrid and in determining genetic purity of the cultivars.

5.4 *Hirsutum* x *barbadense* hybrids

5.4.1 Varalaxmi and its parents

In the seed albumin profiles, the hybrid Varalaxmi and its male parent exhibited 14 bands each while its female parent produced 12 bands. The hybrid could be distinguished from its female parent by the presence of 3 bands (Nos. 5, 8 and 11; Rm 0.32, 0.45 and 0.55) which were absent in the latter. The hybrid could also be differentiated from its male parent by the presence of two bands (Nos. 12 and 13; Rm 0.58 and 0.63) which were not found in the latter. Further, the hybrid could be discriminated from both of its parents by the quantitative variation in the band Nos. 1 and 2 (Rm 0.13 and 0.17) (Fig. 12a).

In the seed globulin proflies, the hybrid and the male parent exhibited 9 bands each, while the female parent produced 7 bands. The hybrid could be differentiated from its female parent by the presence of two bands (Nos. 6 and 7; Rm 0.50 and 0.55) which were not found in the latter. The hybrid and the male parent had similar banding patterns both qualitatively and quantitatively (Fig. 20a).

In the seed EST isozyme patterns, the hybrid and its female parent produced 7 bands while its male parent recorded 6 bands. The hybrid could be discriminated from its parents by the presence of a specific band (No. 3; Rm 0.25) which was not found in either of the parents. The hybrid could be distinguished from its female parent by the presence of band No. 5 (Rm 0.42) and absence of band Nos. 1 and 4 (Rm 0.08 and 0.27) in the former. From the male parent the hybrid could be differentiated by the absence of band No. 4 (Rm 0.27) and the presence of band No. 7 (Rm 0.60) in the latter (Fig. 28).

In the MDH isozyme patterns, the hybrid recorded 6 bands while its female parent and the male parent produced 7 and 5 bands respectively. The hybrid could be discriminated from both of its parents by the presence of a specific band (No. 4; Rm 0.12) in it and absent in its parents. The hybrid could be distinguished from its female parent by the absence of two bands (Nos. 1 and 8; Rm 0.03 and 0.33) in the former. The hybrid could be differentiated from its male parent by the presence of band No. 9 (Rm 0.45) which was absent in the latter (Fig. 36).

By the ADH isozyme pattern, the hybrid could be distinguished from its female parent by the presence of band Nos. 1 and 4 (Rm 0.10 and 0.30) lacking in the latter. The female could be discriminated by the presence of a specific band (No. 3; Rm 0.28) in it. The hybrid could also be differentiated from its male parent by the quantitative differences in band No. 2 (Rm 0.20) (Fig. 44).

In the PER isozyme pattern, all the 3 genotypes recorded 2 bands each. The hybrid could be discriminated from its parents by the presence of a specific band (No. 3; Rm 0.16) which was not found in either of the parents. The hybrid could be distinguished from its male parent by the presence of band No. 5 (Rm 0.28) in it while absent in the latter (Fig. 51). By the CAT isozyme pattern, the hybrid could be differentiated from its female parent by the quantitative difference in the band No. 3 (Rm 0.30). However, both the hybrid and the male parent exhibited similar banding pattern (Fig. 52). The ACP isozyme pattern was not helpful in discriminating the genotypes as the pattern was similar in all of them (Fig. 53).

Thus, in these different analyses, the seed albumins, globulins, EST and MDH patterns were helpful in differentiating the genotypes and were useful in identifying and determining the genotypic purity of the cultivars. The ADH, PER and CAT were helpful only to certain extent while ACP patterns are not useful for such purpose.

5.4.2 DCH32 and its parents

In the seed albumin patterns, the hybrid and male parent exhibited 16 bands each while its female parent prooduced 13 bands. The hybrid could be differentiated from its female parent by the presence of two faintly stained bands (Nos. 1 and 2; Rm 0.10 and 0.12) and two mediumly stained bands (Nos. 4 and 10; Rm 0.17 and 0.45) in it which were absent in the latter. Band No. 7 (Rm 0.32) was observed in the female parent and not in the hybrid. The hybrid and the male parent had more or less similar banding patterns. However, they could be discriminated by the quantitative differences in band Nos. 4 and 12 (Rm 0.18 and 0.53) (Fig. 13a).

In the seed globulin pattern, the hybrid resolved into 11 bands while its female parent and male parent exhibited 9 and 10 bands respectively. The hybrid could be distinguished from its female parent by the presence of band Nos. 6 and 9 (Rm 0.50 and 0.66) which were absent in the latter. From the male parent the hybrid could be discriminated by the band No. 8 (Rm 0.60) which was observed in the hybrid and not in the male parent. Further, the hybrid could be differentiated from both of its parents by the quantitative difference in band Nos. 1, 7, 8, 9 and 10 (Rm 0.17, 0.55, 0.60, 0.67 and 0.80) (Fig. 21a).

In the EST isozyme patterns, the hybrid and the female parent resolved into 7 bands each while its male parent exhibited 6 bands. The hybrid could be distinguished from its female parent by the presence of two bands (Nos. 2 and 7; Rm 0.17 and 0.58) in it and absente in the latter. The female parent could also be differentiated from the hybrid by the presence of band Nos. 1 and 8 (Rm 0.09 and 0.60) in it and absente in the hybrid. The hybrid could be discriminated from its male parent by the presence of band No. 6 (Rm 0.48) and absence of band No. 4 (Rm 0.27) in it in contrast to the male parent. Further, the hybrid could be distinguished from both of its parents by the quantitative differences in band Nos. 2, 5 and 7 (Rm 0.17, 0.42 and 0.58) (Fig. 29).

In the MDH patterns, the hybrid and the male parent were similar both producing 3 bands while the female parent recorded 5 bands. The hybrid could be differentiated from its female parent by the absence of one faint and one mediumly stained band (Nos. 2 and 3; Rm 0.06 and 0.10) in the former. It could also be distinguished from the female parent by the absence of band No. 5 (Rm 0.30) in the former. The hybrid could be discriminated from its male parent by the quantitative differences in band No. 6 (Rm 0.32) (Fig. 37).

By the ADH isozyme pattern, the hybrid could be distinguished from its parents by the presence of 3 bands in it while its both parents recorded two bands each. The hybrid could be differentiated from its female parent by the presence of band No. 2 (Rm 0.26) in it and absence in the latter. Similarly the hybrid could be discriminated from its male parent by the presence of band No. 1 (Rm 0.22) in it and absence in the latter. Further, the hybrid could be distinguished from both of its parents by the quantitative difference in band Nos. 1 and 3 (Rm 0.20 and 0.30) (Fig. 45).

By the banding pattern of PER isozyme, the hybrid could be distinguished from its female parent by the presence of two bands Nos. 3 and 6 (Rm 0.16 and 0.30) in it while absent in the latter. The hybrid could be distinguished from its male parent by the absence of band No. 4 (Rm 0.20) in the former. (Fig. 51). In the CAT isozyme pattern, the hybrid could be discriminated from its female parent by the quantitative difference in band No. 3 (Rm 0.30). The hybrid and the male parent were similar for this character (Fig. 52). In the ACP isozyme pattern, the hybrid and its parents recorded similar phenotype and were not useful in discriminating the genotypes (Fig. 53).

Thus, in different analyses, the seed albumins, globulins, EST and MDH patterns were most useful in differentiating the genotypes and are useful in identifying and determining genetic purity of the cultivars. The ADH, PER and CAT were helpful in differentiating the inter-specific hybrids while ACP was not useful for such purpose.

Similar results were reported for the seed albumins by Cherry et al. (1971), Kapse (1983) and Kapse and Nerkar (1985). For the isozyme analyses Cherry et al. (1972) reported the differences in EST patterns and similarities for CAT patterns in different cultivars of Gossypium species.

5.5 Seed albumin banding patterns

From Fig. 46, the overall differential banding pattern of seed albumins reveals great variation in the number and intensity of bands among the different species and cultivars. The number of bands in each genotype ranged from 10 to 16. A total of 31 bands were recognised for the material (24 cultivars) under study. No two cultivars were exactly alike, when qualitative and quantitative variations were studied in them. Thus, each genotype exhibited unique type of banding pattern. This method of study is like fingerprinting of the cultivars and can as well be considered for determination of genetic purity of cultivars.

Similar observations are reported in cotton by Cherry _et al_. (1970), Ibragimov _et al_. (1973), Zapruder _et al_. (1980), Kapse (1983) and Kapse and Nerkar (1985). In other crop plants also similar results are reported such as wheat (Shewry _et al_., 1978b and Zillman and Bushuk, 1979), rice (Siddiq _et al_., 1972), sorghum (Tripathi _et al_., 1983), maize (Scandalios, 1969) and beans (Hussain _et al_., 1986).

5.6 Seed globulin banding patterns

From Fig. 47, the overall differential banding pattern of salt soluble seed proteins (globulins) reveals qualitative and quantitative variations among the different cultivars of Gossypium species. The number of bands in each cultivar ranged from 5 to 1[illegible]. A total of 20 bands were recognised in the 24 cultivars studied, out of which two bands were common in all the genotypes. Critical study of the banding patterns revealed that no two genotypes are exactly alike in their globulins banding pattern. Thus, this study aided in establishing the trueness of the cotton cultivars.

Similar results for seed globulins are also reported in cotton (Castleberry and Coleman, 1972 and Ibragimov *et al*., 1973) and other crops such as in wheat (Dhaliwal, 1977), rice (Sarkar and Bose, 1984 and Chauhan and Nanda, 1984), beans (Ziegenfus and Clarkson, 1971 and Barrat, 1980) and forages (Dalling *et al*., 1979).

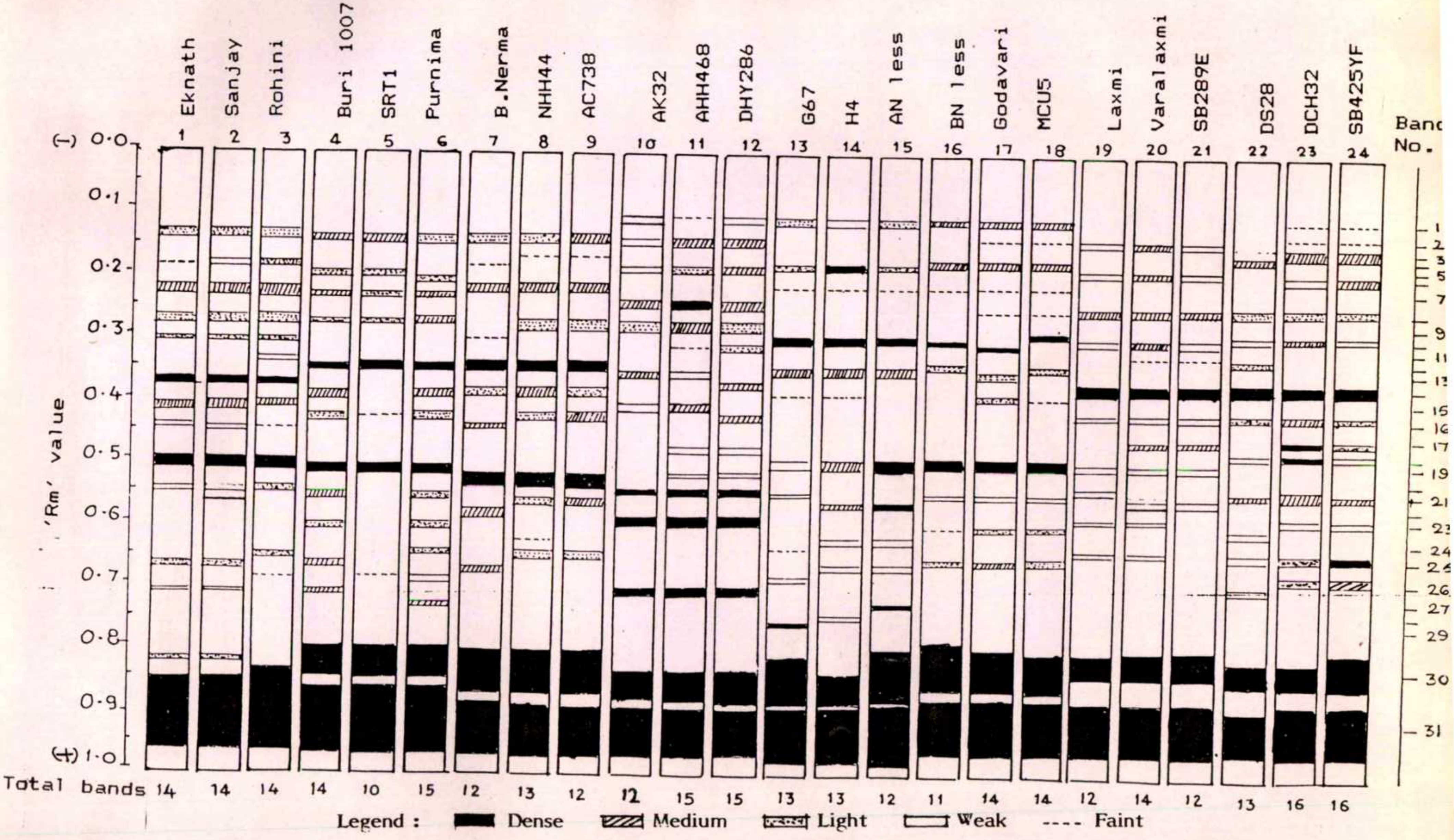

Fig. 46 Schematic diagram of the SDS-PAGE profiles of seed albumins in cotton varieties, hybrids and their parents

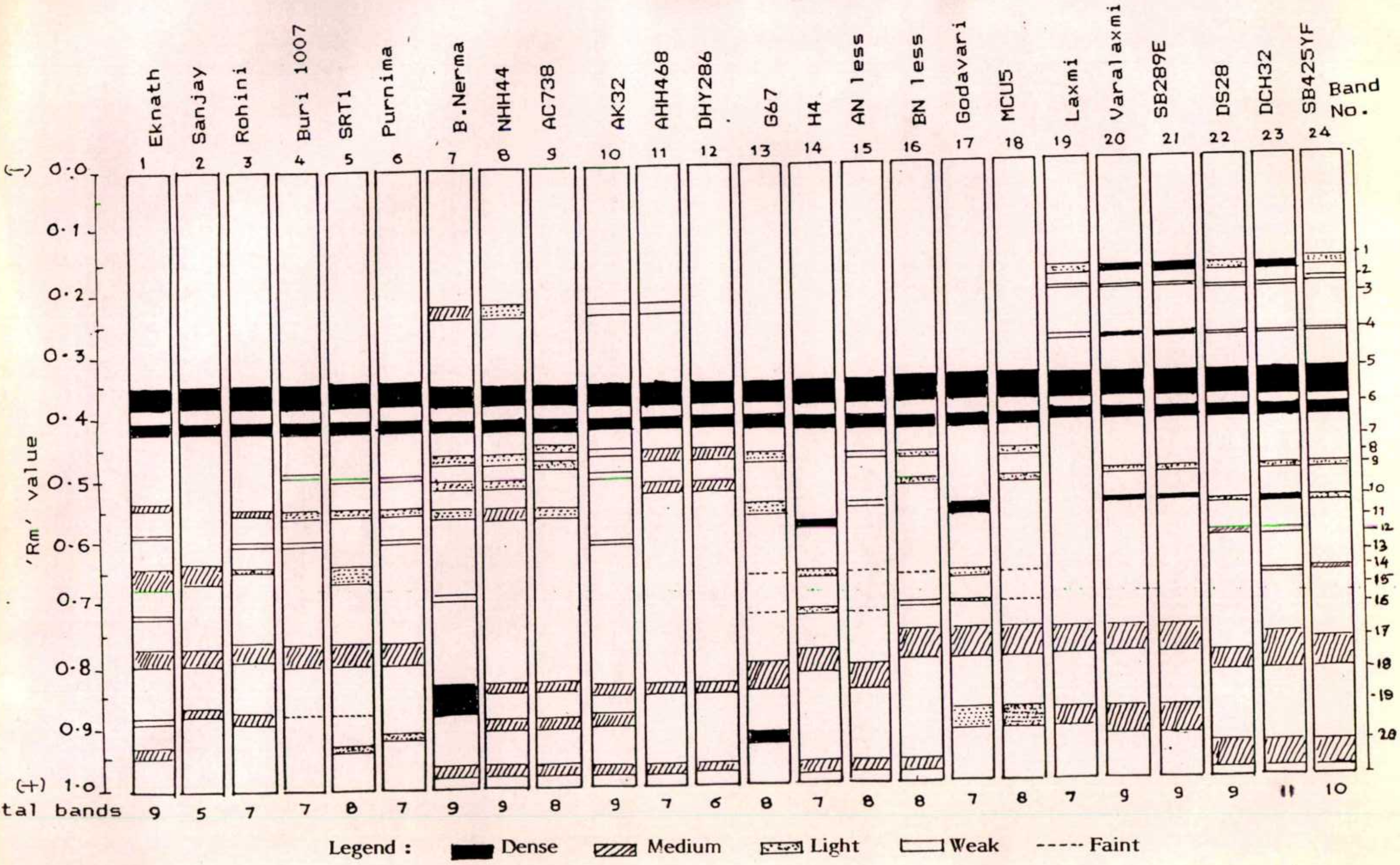

Fig. 47 Schematic diagram of the SDS-PAGE profiles of seed globulins in cotton varieties, hybrids and their parents

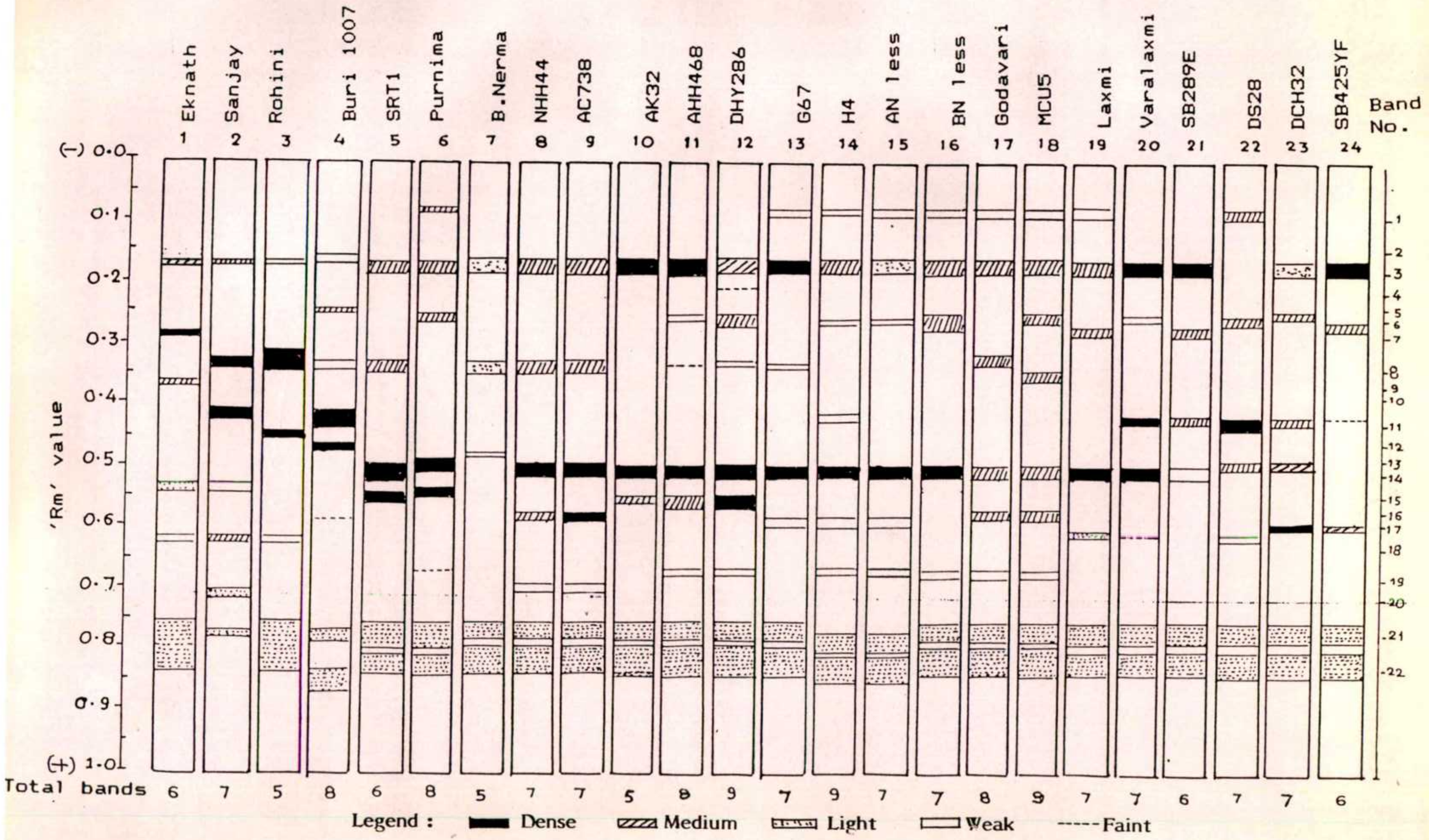

Fig. 48 Schematic diagram of the PAGE patterns of seed esterases in cotton varieties, hybrids and their parents

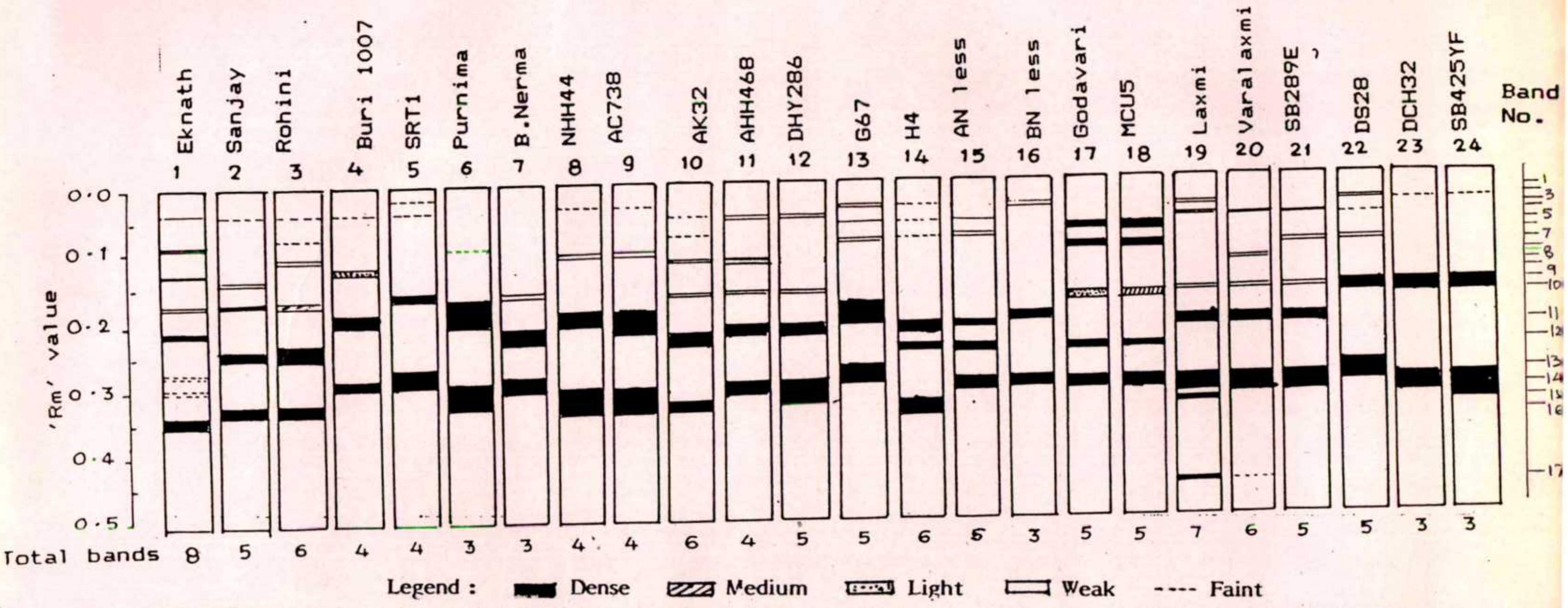

Fig. 49 Schematic diagram of the PAGE patterns of MDH isozymes in seed of cotton varieties, hybrids and their parents

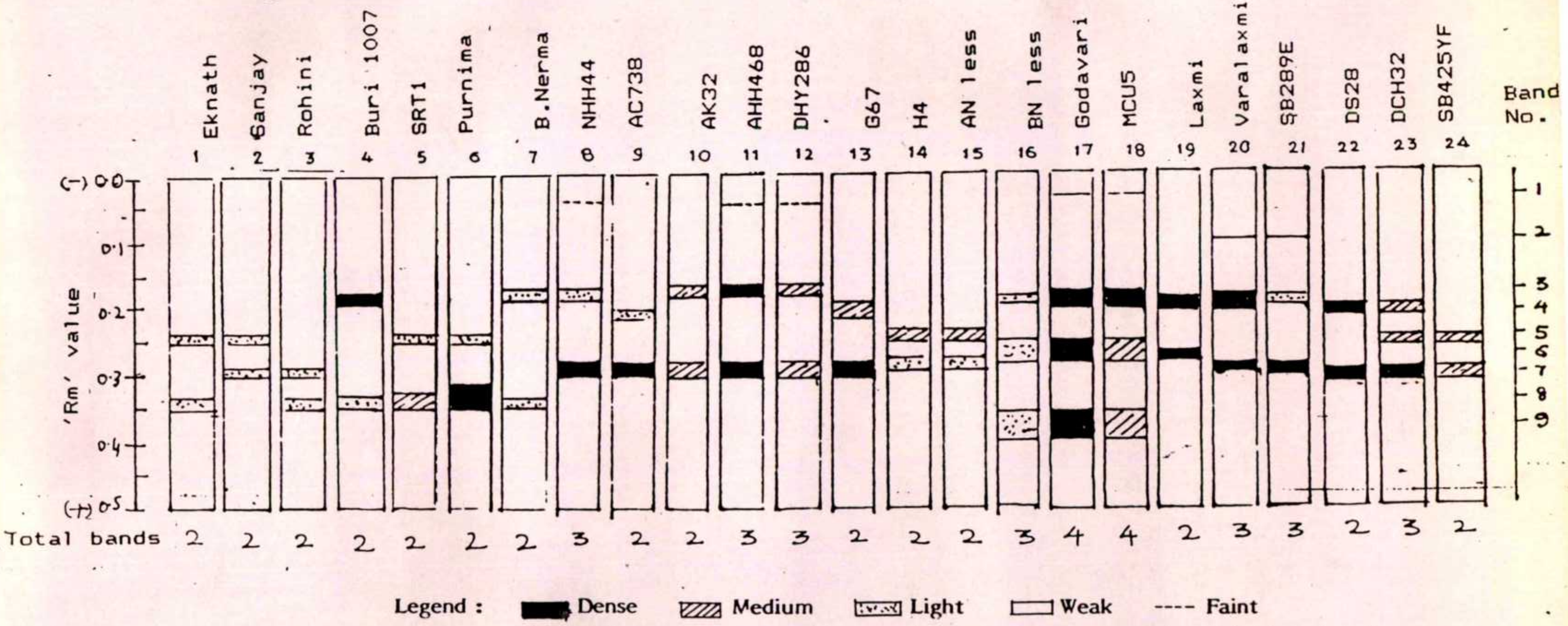

Fig. 50 Schematic diagram of the PAGE patterns of ADH isozymes in seed of cotton varieties, hybrids and their parents

5.7 Isozyme patterns

5.7.1 Esterase isozyme patterns

The overall differential isozyme patterns of seed esterases reveal the qualitative and quantitative variation among the cotton cultivars (Fig. 48). The number of bands in each cultivar ranged from 5 to 9. A total of 22 bands were recognised for the 24 genotypes studied, with no band common in them. Critical study revealed that no two genotypes were exactly alike in their EST patterns. Thus, this system of study could be considered suitable for the determination of genetic purity of cotton cultivars.

Similar results on EST isozymes were reported in cotton (Cherry _et al_., 1972) and other crops such as wheat (Cubadda _et al_., 1975), barley (Kahler and Allard, 1970), rice (Nakagahra _et al_., 1975), sorghum (Tripathi _et al_., 1983), maize (Goodman and Stuber, 1980), beans (Mittal _et al_. 1987), _Brassica_ (Suurs, 1987) and alfalfa (Quiros, 1980).

5.7.2 MDH isozyme patterns

The Fig. 49, representing the overall differential isozyme patterns of MDH reveals variation in the number and staining intensity of bands among the different cotton cultivars. The number of bands in each cultivar ranged from 3 to 8. A total of 17 bands were recognised in the material under study; 3 pairs of cultivars (i.e. the hybrid and respective male parent) including NHH44 and AC738, Godavari and MCU5 and DCH32 and SB425YF were similar to each other. The remaining 18 cultivars could be identified individually. This enzyme system aided in discriminating the hybrids from their respective female parents. Hence it can be considered as a useful system to determine the genetic purity of hybrids and other varieties of Gossypium species.

The study of MDH isozyme system has not been reported so far in Gossypium species. However, similar results are reported in other crops such as rice (Second, 1982), maize (Cardy, 1981), Brassica (Wills et al., 1979), Nicotiana (Wilkinson et al., 1985) and peaches (Arulsekar et al., 1986).

5.7.3 ADH isozyme patterns

From Fig. 50, the overall differential banding pattern of ADH isozyme reveals variation in the number and intensity of the bands among the cotton genotypes. The number of bands in each cultivar ranged from 2 to 4. A total of 9 bands were recognised in the material, with no band common in them. Each cultivar exhibited an unique banding pattern except hybrid H4 and its male parent American Nectariless. However, qualitative variation was not sufficient to aid in discrimination of the cultivars. Hence, this system will not be sufficiently useful in determination of genetic purity of cotton cultivars.

Hancock (1982) reported variations in ADH patterns among different species of Gossypium. However, this isozyme study was useful in identifying the varieties of other crops such as wheat (Fejer et al., 1979), rice (Second, 1982), sorghum (Tripathi et al., 1983), maize (Hunter and Kannenberg, 1971) and tomato (Tanksley and Jones, 1981).

5.7.4 PER, CAT and ACP isozyme patterns

The banding patterns of the PER, CAT and ACP isozymes are represented in Fig. 51, Fig. 52 and Fig. 53 respectively. These figures reveal the existence of similarities to a greater extent than the variations in the isozyme patterns among the cotton cultivars. Thus, these isozyme systems could not be considered useful in the determination of genetic purity of the cotton cultivars.

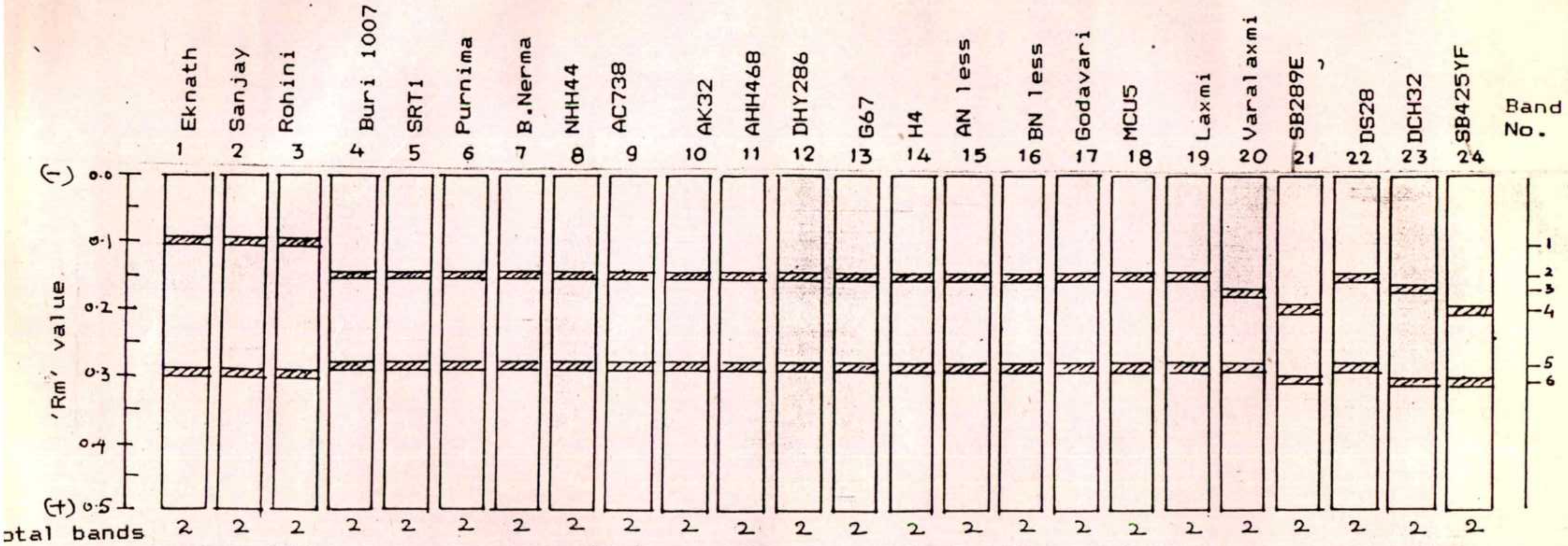

Fig. 51 Schematic diagram of the PAGE patterns of PER isozymes in seed of cotton varieties, hybrids and their parents

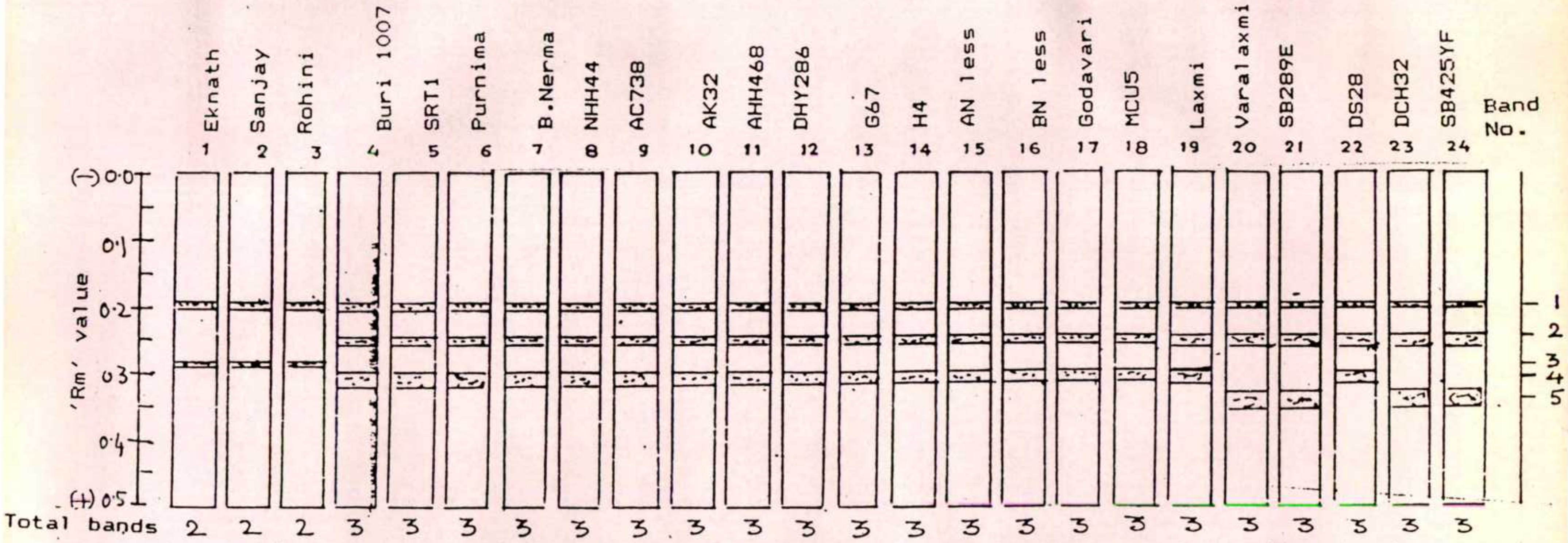

Fig. 52 Schematic diagram of the PAGE patterns of CAT isozymes in seed of cotton varieties, hybrids and their parents

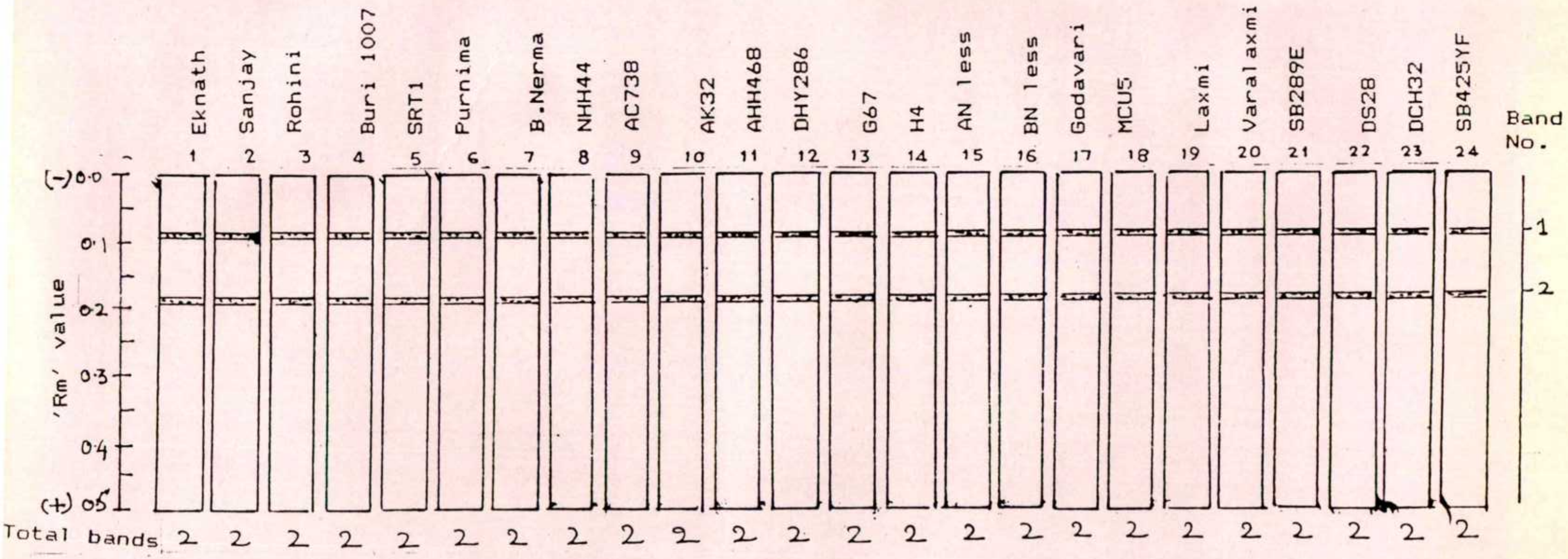

Fig. 53 Schematic diagram of the PAGE patterns of ACP isozymes in seed of cotton varieties, hybrids and their parents

Such similarities in the banding pattern of the CAT isozyme are reported in different varieties and species of _Gossypium_ by Cherry _et al_. (1972). Several other workers have also reported such similarities for PER, CAT and ACP isozyme patterns in different varieties of other crops such as barley (Bassiri, 1976), sorghum (Phul _et al_., 1987), beans (Bassiri and Adams, 1978b and Mittal _et al_., 1987) and _Brassica_ (Yadava _et al_., 1979 and Kumar and Gupta, 1985).

5.8 Conclusions and future line of work

The present study revealed the possible use of the gel electrophoretic study of seed albumins, globulins, EST and MDH isozymes in the determination of genetic purity of the cotton cultivars. This technique is reliable and quick as compared to field tests.

Seed globulin patterns were the most useful in distinguishing the cotton cultivars. This sytem exhibited the polymorphism to aid in identification of _arboreum_ and _hirsutum_ varieties as well as H4, Godavari and DCH32 hybrids from their respective female parents. Seed albumin patterns were most useful in identification of the hybrid Varalaxmi from its parents. The EST isozyme patterns were useful in distinguishing the NHH44 and AHH468 hybrids from their respective female parents.

However, the technique can be refined by studying the densitometer tracings of the banding patterns in order to reveal the smaller differences which could not be detected by the visual screening of the gels. This may be useful in identifying the varieties/hybrids more precisely and easily as reported by Siddiq et al. (1972) in rice, Tripathi et al. (1983) in sorghum and De Prins and Van de Weghe (1983) in rye grass. The densitometric data can also be used for the statistical analysis as suggested by Sarkar and Bose (1984).

SUMMARY

6. SUMMARY

The present investigation was undertaken with a view to determine the genetic purity of cotton cultivars using electrophoretic patterns of seed albumins, globulins and some enzymes. The experimental material consisted of three varieties in each of *Gossypium arboreum* and *G. hirsutum*, four intra-*hirsutum* hybrids, two *hirsutum* x *barbadense* hybrids and the respective parents of the hybrids. The SDS-PAGE method (using vertical slab gel) was followed to separate the seed albumin and globulin proteins in single seed extracts. PAGE method (using disc gel) was followed to separate isozymes of six enzyme systems, viz., EST, MDH, ADH, PER, CAT and ACP. Findings of these investigations are summarised below :

1. The three *arboreum* varieties, viz., Eknath, Sanjay and Rohini had same number of bands for seed albumins. However, qualitative and quantitative differences were observed among three genotypes, which were helpful in discriminating one from the other. Each genotype exhibited certain specific bands as their uniqueness. Seed globulin patterns proved most useful in distinguishing the three genotypes. Among the six enzyme systems analysed, EST and MDH provided discriminative banding patterns followed by

ADH to aid in identification of the genotypes. The PER, CAT and ACP isozyme patterns could not provide any polymorphism to aid in distinguishing the three genotypes.

2. The three hirsutum varieties, viz., Buri 1007, SRT1 and Purnima could be distinguished from one another on the basis of seed albumin and globulin banding patterns. Seed albumins proved to be the most useful in identifying them individually. Among the six enzyme systems analysed EST and MDH isozymes could provide sufficient polymorphism to aid in discriminating the genotypes, followed by ADH isozyme patterns. The variations in PER, CAT and ACP isozyme patterns were not sufficient to discriminate the three genotypes.

3. The hybrid NHH44 could be identified from the female parent (Bikaneri Nerma) on the basis of three bands of the seed albumins which are absent in the latter. Seed globulins were not of much help in discriminating the hybrid and the female parent. EST and MDH provided most discriminative patterns to aid in distinguishing the hybrid from its female parent. However, the patterns are more or less similar in both the hybrid and the male parent (AC738). Even with less number of bands, ADH was useful in identifying the hybrid from both of its parents. The PER,

CAT and ACP isozyme patterns could not exhibit sufficient polymorphism to aid in identification of the genotypes.

4. The hybrid AHH468 could be discriminated from its female parent (AK32) based on the qualitative and quantitative variations in the banding patterns of seed albumins and globulins. The polymorphism was also sufficient to distinguish the hybrid from its male parent (DHY286). Among the enzyme systems analysed, EST proved to be the most useful followed by MDH to aid in identifying the hybrid from their parents. The ADH isozyme pattern was useful in distinguishing the hybrid only from its female parent. The PER, CAT and ACP isozyme patterns could not be considered as useful systems in identifying the genotypes.

5. To identify the hybrid H4 from its female parent (G67) seed albumin patterns were useful. Seed globulins provided most discriminative banding patterns to aid in identifying the hybrid from both of its parents. Among the isozymes studied, EST could provide sufficient polymorphism to distinguish the hybrid from its parents. The MDH and ADH isozyme patterns were helpful in differentiating the hybrid from its female parent. However, these banding patterns were more or less similar in the hybrid and the male parent (American Nectariless). The PER, CAT and ACP isozyme

patterns were not useful in identifying the genotypes individually.

6. The hybrid Godavari could be distinguished from its female parent (Buri Nectariless) by the seed albumin banding patterns. The banding patterns of the hybrid and the male parent (MCU5) were similar except some quantitative differences in three of their bands. The seed globulins proved useful in identifying the hybrid from both of its parents. Among the enzyme systems studied, EST exhibited qualitative and quantitative variations and proved to be the most useful in discriminating the hybrid from both of its parents. The MDH and ADH were helpful in distinguishing the hybrid from its female parent, but these isozyme patterns were similar in both hybrid and the male parent. The PER, CAT and ACP isozyme patterns have not exhibited sufficient polymorphism to aid in identification of the hybrids or the genotypes.

7. The hybrid Varalaxmi could be distinguished from its female parent (Laxmi) on the basis of the banding pattern of seed albumins, globulins, EST, MDH, ADH, PER and CAT isozymes. The hybrid could also be discriminated from its male parent (SB289E) on the basis of banding pattern of seed albumins and MDH isozyme pattern, while for the other

systems, the hybrid and the male parent were more or less similar. The ACP isozyme pattern was not useful in identifying these genotypes.

8. The hybrid DCH32 could be identified from its female parent (DS28) on the basis of seed albumin and globulin profiles. The EST, MDH, ADH and PER isozyme patterns were also useful. However, seed albumins and EST provided most discriminative banding patterns to aid in identifying the hybrid from its female parent. The hybrid could be distinguished from its male parent (SB425YF) by the quantitative difference in certain bands of seed albumins and esterases.

9. The present investigation has clearly indicated that the profiles of water soluble (albumins) and salt soluble (globulins) proteins from single seeds would be used as quick and reliable technique in identification and determination of the genetic purity of cotton varieties and hybrids.

Among the enzyme systems studied in the single seed extracts, EST and MDH were proved to be most useful in identification and determination of the genetic purity of cotton cultivars. The ADH, PER and CAT were useful in discriminating the interspecific hybrids and cultivars of different species of <u>Gossypium</u>, while ACP patterns were not useful for such purpose.

The three varieties in each of <u>arboreum</u> and <u>hirsutum</u>, the hybrids H4, Godavari and DCH32 were easily distinguishable from their seed globulin patterns. The hybrid Varalaxmi was identifiable from its parents by seed albumins, while NHH44 and AHH468 by the EST isozyme patterns.

Thus the seed protein profiles and the isozyme patterns serve as laboratory genotyping or fingerprinting of cotton cultivars and can as well be used for determining cultivar genetic purity or uniqueness. This technique is thus preferable over field grow-out tests to obtain the phenotypic descriptions.

10. However, it is necessary to study the densitometer tracings of the profiles in order to reveal the smaller differences which could not be detected by the visual screening of the gels. This may be useful in identifying the hybrids and cultivars more precisely and densitometric data can be statistically analysed over different environments.

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