Student Minor Research Project

Biochemical methods for Identification and Genetic purity Testing of Cotton (Gossypium spp) Hybrids

Under RUSA 2.0 Scheme

(Through Ch.S.D.St.Theresa's College for Women (Autonomous), Eluru, AP).

Submitted by

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CERTIFICATE

This is to certify that the project work entitled **"Biochemical Methods for Identification and Genetic Purity Testing of Cotton (Gossypium spp) Hybrid"** is bonafied work carried out by Ms RVLS Padmavathi (Reg.No. 11710011), Ms I.Keziah (Reg.No. 11710002), Mr P.Ch.Sai Krishna (Reg.No. 11710010).submitted in Third year of the degree B,Sc in Biotechnology during the year 2019 – 2020 is an authentic work under my supervision and guidance.

To the best of my knowledge the matter embodied in the project work has not been submitted to any other College/Institution.

Date: 29-12-2019

Mr.G.SAM BABU

PROJECT ADVISOR

DECLARATION

We, the undersigned, declare that the project entitled "**Biochemical methods for Identification and Genetic Purity testing of Cotton (Gossypium spp) Hybrids**", being submitted in Third Year of Bachelor of Science in Biotechnology, Sri Y N College (Autonomous), is the work carried out by us.

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CONTENTS

OBJECTIVES

Considering the above facts, the present study Application of RAPD and SSR Markers for purity testing of F1 hybrid seed in Cotton was undertaken with following objectives.

1. Differentiation of cotton hybrid from its parents using molecular markers
2. To distinguish the hybrid from off type

Introduction

Cotton is the king of fibre crop that fulfilled man's basic needs for fibre and food since its domestication more than 3,500 years ago. Today, cotton remains one of the world's most important economic crop species and a renewable resource providing raw material for textile manufacturing, mulch and cattle (Ali et al, 2008). Taxonomically cotton is described under the order Malavales, family Malavaceae and genus Gossypium. According to the Percival and Kobel (1990), the genus Gossypium includes 49 species. Four of these are cultivated, 43 are wild diploid and two wild tetraploid. Of the four cultivated species Gossypium hirsutum and Gossypium barbadense are tetraploid (2n=2x=52), commonly known as new world cotton whereas, Gossypium arboreum and Gossypium herbaceum are diploid (2n=2x=26) and commonly known as old world or Asiatic cotton (Noormuhammadi et al, 2011). India has a pride place in the global cotton scenario due to several distinct features such as largest cotton growing area, cultivation of all the four cultivated species, large area under tetraploid cotton, one of the largest producers of long and extra-long staple cotton, possibly the only country to grow hybrid cotton, native home of old cultivated cotton and wide diversity in agro climatic conditions under which cotton is grown (Basavaraddi, 2007; Macha, 2010). Cotton is grown in more than one hundred countries, of these, ten countries accounts for as much as 80 per cent of the total cotton production. It is grown globally over 30.4 mha with a total production of 23.6 mt and the productivity is 691 kg/ha. Since the introduction of BT cotton in the year 2002, there has been almost 2-fold cotton production from 2.3 mt in 2002- 03 to 5.4 mt in 2007-08 though the area has just increased from 7.7 mha to just 9.4 mha. During these years, the area under BT hybrids has expanded to more than 80% of the total cotton area and the yields have increased from 302 kg/ha to 567 kg/ha. Not

surprisingly, the number of farmers growing cotton has shown a phenomenal increase. The ratio of cotton production has increased with a peak reaching to 5.5 mt in 2010-2011 to 5.8 mt in 2011-2012 (till December) respectively (Karihaloo and Kumar, 2009; Anonymous, 2012a; Anonymous, 2012b). The increased production and productivity is credited to the release of high yielding hybrids for commercial cultivation which occupy around 22 per cent of the total area under cotton. The yield of these hybrids in many places is about 50 per cent higher than the pure line varieties (Rana et al, 2006). Maintaining genetic purity is of utmost importance that helps to exploit the full potential of hybrids. When a seed lot is passed from one generation to another, some form of genetic contamination is likely to occur, which cannot be detected morphologically and may go on accumulating unnoticed in the population finally leading to deterioration of genetic worth of that variety (Tafvizei et al, 2010). Therefore, a reliable method to discriminate between cultivars as well as to assess genetic purity of seed samples will enable seed producers to monitor and maintain adequate levels of genetic purity at each generation of seed production and multiplication. This will ultimately ensure high quality seeds. With the introduction of Indian legislation on Protection of Plant Varieties, the new crop varieties should be distinct from other varieties, uniform in their characteristics and generally stable over the years. Farmers and seed growers need an assurance that they are being supplied with correct seed material having known identity of a specific variety and assured quality (Macha, 2010). Thus, there is a need to search a rapid and reliable method of varietal identification and genetic purity testing of cotton seed. In this context, varietal description for identification of crop varieties has assumed a critical importance in national and international seed programmes and there is a considerable need for the development of reliable methods and identifiable characters for the purpose. The characters for which a variety is distinct from other

could be morphological, chemical and biochemical/physiological in nature which aids in varietal identification. According to International Union for Protection of New Plant Varieties (UPOV), any new characteristic used in varietal characterization should be clearly defined, accepted and should have standard method of observation and not affected by environment, accessible to breeders, associated with reasonable costs and efforts. The interesting fact is that most of the currently used morphological characteristics do not fulfill all these criteria (Macha, 2010; Dongre et al, 2011). To test the conformity of hybrid seed, one must be able to distinguish the true hybrid resulting from cross between selected male and female parents and one coming from self-pollinated female parent. To meet the demand of genetically pure seed, the Certification Agencies are following a Grow Out Techniques (GOT) where morphological characters are scored at various stages of plant growth, which has been used extensively in purity control mechanism of hybrid seed and for the purpose of identification of varieties (Selva kumar et al, 2010). For the improvement of agronomically and economically important traits, plant breeding generally recombines traits present in different parental lines of cultivated and wild species. Conventional breeding programmes reach this goal by generating an F1 hybrid and F2 segregating population and then screening the phenotypes of pooled or individual plants for presence of desirable traits, which is followed by a process of repeated backcrossing, selfing and testing. During this process, breeder depends on accurate screening methods and availability of lines with proper phenotypic characters, which is time consuming and difficult to achieve with classical methods (Beckmann & Soller, 1986). Conventional GOT requires one full season thus excluding the immediate cultivation of the hybrid seed produced. In addition, expenditure incurred on storage, increases the hybrid seed cost (Nanda kumar et al, 2004). These limitations of conventional GOT demands a new technique which must be

environmental independent, quick and reliable. The alternative way to overcome this limitation and to speed up the testing procedures is to use DNA markers in addition to morphological markers (Ali et al, 2008; Rakshit et al, 2008). Use of molecular markers facilitate these breeding processes, since it can provide means to detect and resolve complications and accelerate the generation of new varieties and allow association of phenotypic traits with genomic loci (Jiang et al, 2000). Ideal molecular markers must be stable, abundant and detectable in plant tissues regardless of growth, differentiation and defence status. These properties make molecular markers indispensable for crop improvement. A number of DNA fingerprinting techniques are available for detection of polymorphism. RFLPs are reliable markers in linkage analysis and crop breeding. However, it is time consuming, expensive and requires large quantity of DNA for restriction and hybridization analysis (Paterson et al, 1993). Most of the DNA marker assays that use PCR, among them are RAPD, SSR, AFLP and SNPs (Liu et al, 2000; Semagn et al, 2006; Dongre et al, 2011). RAPD is much faster and cheaper than RFLP analysis and uses only minute quantity of DNA (Williams et al, 1990). Microsatellites are typically the repeat units of 1-6 nucleotides and SSR analysis is performed by using pairs of specific primers flanking tandem arrays of microsatellite repeats. SSR markers are co-dominant and extremely polymorphic (Liu et al, 2002). AFLP is robust and reliable for DNA fingerprinting of varied genomes because it combines the use of restriction enzymes and PCR amplification (Vos et al, 1995). The AFLP system is technically intricate and expensive to set up, but it detects a large number of loci. SNPs are the single base substitutions or small insertions and deletions in homologous genomic regions. SNPs are more frequent and co-dominant in nature (Lindblad et al, 2000). Recent developments of molecular techniques and application of molecular markers have brought a new dimension into the traditional area of plant breeding. Molecular

markers not only allow the easy and reliable identification of breeding lines, hybrids and cultivars (Bastia et al, 2001; Asif et al, 2005, 2006; Tabbasam et al, 2006) but also facilitate the monitoring of introgression, Marker Assisted Selection (MAS) (Ribaut and Hoisington, 1998; Zhang et al, 2003), high-density genetic linkage maps (Guo et al, 2007; He et al, 2007) and estimation of genetic diversity (Mukhtar et al, 2002; Rahman et al, 2002). Hence, the present study was undertaken to identify cotton hybrid and its parental lines based on RAPD and SSR markers

Review of Literature

Gossypium hirsutum varieties have been developed from crosses between closely related ancestors but only limited increases in productivity is obtained. Pressure for higher productivity in cotton farming has stimulated the search for more exotic germplasm, but although breeding methods have increased the efficiency of transferring alleles from exotic germplasm sources to cotton breeding gene many germplasm sources still remain underused. The genetic diversity ensures protection procedures against diseases and pests and thus provides a basis for future genetic gains (Esbroeck et al, 1998). Hybrid seed production in cotton is usually taken up by hand emasculation and pollination. Being often cross pollinated crop, the genetic purity of cotton hybrid seeds is adversely affected by the foreign pollen. Hence, in order to get better returns from the hybrids, greater seed purity and quality are emphasized elsewhere. Traditionally, it has been the practice to carry out GOT to analyse the genetic purity of hybrid seeds using morphological traits (Tatineni et al, 1996; Gumber, 2003; Ankaiah et al, 2005 and Patel et al, 2005). However, GOT involves growing plants to maturity and assessing several morphological characteristics that distinguish the hybrids. The environmental influences on morphological characters and time factor make it difficult to collect the morphological data, besides other limitation in unambiguous differentiation of genotypes (Ali et al, 2008; Dongre et al, 2011). Alternatively, other markers such as chemical, biochemical, isozymes and seed storage proteins have been suggested for genetic purity determination (Dadlani et al, 1997; Mehetre and Dahat, 2001; Borle et al, 2007 and Rakshit et al, 2008). Nevertheless, the main weaknesses of biochemical marker are its low abundance and sensitivity to environmental and experimental conditions. Therefore, it is necessary to develop a rapid, reliable and reproducible technique to assess the genetic purity of cotton hybrids. With the

advent of the molecular marker technology, it is now possible to test the purity of the hybrid seed immediately after harvesting and processing by DNA markers (Selva kumar et al, 2010). The chemical tests are spot tests and useful in identification by change in seed colour as well as solution due to added chemicals. Simple chemical tests viz., phenol test, peroxidase test, NaOH, KOH test, seedling response to various chemicals have been proved quite useful in detecting varietal mixtures and grouping of large number of genotypes into distinct classes. Electrophoresis of seed storage proteins show promising results in genetic purity determination of cotton hybrid (Macha, 2010). It is suggested that recent breakthrough in molecular markers can be employed in genetic purity analysis. Molecular markers have been widely used in genetic analyses, breeding studies and investigations of genetic diversity and the relationship between cultivated species and their wild parents because they have several advantages as compared with morphological markers, including high polymorphism and independence from effects related to environmental conditions and the physiological stage of the plant (Bertini et al, 2006). DNA markers such as RFLP (Pendse et al, 2001; Dongre and Parkhi, 2005) RAPD (Gent et al, 1995; Venu, 2001; Rao et al, 2002; Mehetre et al, 2007), AFLP (Rana and Bhat, 2004), SSR (Rana, 2003; Dongre and Parkhi, 2005; Saravanan et al, 2007) and ISSR (Dongre and Parkhi, 2005; Rana et al, 2006) have been used to rapidly screen genetic purity of hybrid seed lots. Most widely used RAPD was utilized for hybrid identification and assessment of genetic diversity in pearl millet (Rao et al, 2001), pepper (Ilbi, 2003), rice (Sonti et al, 2003; Haiyuan et al, 1998) muskmelon (Park and Crosby, 2004), tomato (Rom et al, 1995; Ilbi et al, 2004) chilli (Mongkolporn et al, 2004), maize (Iva et al, 2005), corn (Andreoli et al, 2006), cabbage, leucadendron (Lui et al, 2007), cucumber (Li et al, 2008) and wheat (Awan et al, 2008). Genetic purity and diversity of cotton hybrids and their parental lines using PCR based molecular marker technique were assessed by

many scientists (Yadav et al, 2001; Mehetre et al, 2004; Dongre and Parkhi 2005; Rana et al, 2006; Vamadevaiah et al, 2006; Sharma et al, 2007;) and indicated that the RAPD method might be an alternative to the time consuming GOT. Molecular techniques overcome most of these limitations. It is rapidly being used by the research community in various fields of plant improvement, Studies of the genetic diversity of cultivated cotton have generally reported low genetic diversity (Brubaker and Wendel 1994; Tatineni et al, 1996; Iqbal et al, 1997) thus more than one marker, likely to be promising for genetic purity testing of cotton. Recently, Selva kumar et al (2010) assessed genetic purity of three cotton hybrids were clearly distinguished from their parents using six SSRs. Hence, it is proposed that these SSR markers can be used in efficient analysis of hybrid seed purity Noormohammadi et.al (2011) studied thirteen F1 and F2 cotton genotypes by using 19 RAPD and 8 ISSR primers. (Dongre et al, 2011) demonstrated genetic purity in Gossypium hirsutum F1 hybrid its parents with 20 RAPD, 19 ISSR and 33 SSR primers. Result indicated that, using all the three markers in combination is more reliable than using the three in isolation for identification and testing of genetic purity of cotton hybrids. Cotton F1 Hybrid and its parents were analysed by the RAPD and ISSRs DNA markers. However, a combination of two PCR based markers can be used for testing the genetic purity of cotton seeds which will be more reliable (Dongre et al, 2012).

MATERIALS AND METHODS

The experiments were conducted within the objective to determine the usefulness of chemical tests and biochemical and molecular markers for identification and genetic purity testing of cotton (Gossypium hirsetum) hybrids. For the purposes of identification, genotypes were characterized on the basis of seedling response to modify nutrient medium under controlled conditions, color of the seed extract in the organic solvents and biochemical (proteins and isoenzymes) and molecular (RAPD) markers.

The genetic purity of the commercial seed samples of cotton hybrids was evaluated by field grow out trials and laboratory evaluation using standardized electrophoreses technique. The results obtained in the laboratory tests were compared with those of the field grow out trial were testing reliability of the techniques.

To reduce the sample size for genetic purity analysis, sequential sampling procedure was tested.

Seed material

The seed material for the present study consisted of five inter-hirsutum cotton (Gossypium hirsetum) hybrids and their parental lines. Seeds were collected

through the project coordinator (cotton), CICR from the following sources. Parentage of the hybrids used for characterization is given below.

Hybrid	Female parent	Male parent	Source
Savitha	T-7	M-12	CICR, regional station, Coimbatore
PKHy-2	AK-32	DHY-286-2	P.D.K.V,Akola
NHH-44	BN-1	ACC-738	Cotton Research Centre,Nanded
H-10	BC-68/2	LRA-5166	CICR,Regional station, Coimbatore
PHH-316	PH-93	PKV-081	P.D.K.V,Akola

Of these, hybrid PHH-316 is based on CMS system of the female parent PH-093.

Identification of cotton hybrids and their parental lines

Chemicals tests

Seedling response to modified nutrient medium and chemical applications under controlled conditions.

Experiments were conducted growth cabinet and the selected tests were performed in the glass house, Division of seed Science and Technology, IARI, New Delhi. Seeds of five hybrids and their female parents were shown in sand medium in three replications of 15 seeds per pot. Sterilized sand was filled 3/4th in plastic pots of 4.0'' height. Growth conditions were maintained at 25^{o}C and continuous light. The

inert sand was 70% saturated with normal or modified Hoagland solutions to create.

1. Deficiency and toxicity of sodium
2. Deficiency and toxicity of Calcium, and
3. Toxicity to 2,4-D (5ppm and10ppm).

Morphological descriptors of cotton hybrids and their female parental lines.

Characters	**Savitha (H)**	**T-7 (F)**	**PkHy-2 (H)**	**AK-32 (F)**	**NHH-44 (H)**	**BN-1 (1)**	**H-10 (H)**	**BC-68/2 (F)**
Plant habit	Open Short, internodes	Pyramid	Bushy	Semi erect	Bushy Open	Open	Erect	Open
No. of monopodia	0-3	1-3	3-4	2-4	2-3	1-2	3-4	2-3
Leaf color	Light green	Dark green	Light green	Dark green	Light green	Light green	Light green	Light green
Leaf hairness	Medium	Moderate	Dense	Short dense	Medium hairy	Medium hairy	Medium hairy	Medium hairy
Leaf nectarines			Present	Present	Present	Present		
Leaf lobes	3-5	3-5	3-5 Broad	3-4 Medium	3-5	3-5	3-5	3

Petal color	Cream	Cream	Sulphur yellow	Yellow	Light yellow	Cream	Creamy white	Basal color
Anther color	Yellow	Yellow	Pale yellow	Pale yellow	yellow	yellow	yellow	Yellow
Petal spot	Absent	Absent	Purple	Purple	Absent	AB	AB	AB
Bract			Less	Medium				
Boll shape and size	Medium ovate,beaked tip	Medium ovid	Medium ovid	Medium	Medium round	Slightly elongaged	Oval Pointed tip	Big oval

The sand was moistened with respective solutions on every fourth day. Ten seedlings were maintained per pot and data were recorded on 20th day on shoot length, proportion of shoot root growth, leaf area, leaf shape, leaf color, trichome density.

Colour intensity of the seed extract in organic solvents
Anthocyanin intensity

Estimation of anthocyanin content (Oleze – Karow and Mohr,1978)

200 mg of the seed material, hydrated for 72 hrs and decoated, was crushed in a pestle and mortar and transferred to 10 ml test tubes. To this 5ml of acidified methanol (conc.HCL was added to make up 1% acidified methanol reagent) was added. Test tubes were wrapped in black paper and maintained at 4^{o}C over night. The supernatant was decanted and sediments, if any, were separated by centrifugation.

The colour variation of the methanolic extract (ranging from yellow to wood) was recorded by visual comparison (Ridgway, 1912) and documented photographically for quick comparison of cultivars. The intensity of the anthocyanin coloration in the methanolic extract was determined by measuring by the OD of decanted extract at 653 nm in a spectrophotometer.

Gossypol intensity

Estimation of Gossypol content (Sadasivam and Manickam, 1997)

Reagents

Phloroglucinol reagent: 5gms Phloroglucinol was dissolved in 100ml of 80% ethanol.

The Gossypol content was estimated following the methods of (**Sadasivam and Manickam, 1997)** 5gms of seed imbibed in water for 48hrs was decoated and

homogenized in 95% ethanol in a pestle and mortar.homogenized material was transferred into clean test tubes and heated in boiling water bath for 5 minutes.

Extract was collected by filtering through whatmann filter paper. The same was repeated until the extraction from the residues was complete. The extract was cooled and the pH was adjusted to 3.0 using 1N HCL and it was diluted with 40% ethanol to a volume of 10ml.1.5 volumes of diethyl ether was added to the ethanolic extract and incubate at 10^{O} c for an Hour. Ether phase was separated following several washes with distilled water. Ether extract was evaporate to dryness and redissolved in 95% ethanol to a volume of 5ml.

The color variation (from pale buff to purple) was recorded by visual comparison (Ridgway, 1912) and documented photographically quick comparison of cultivars. 1ml of extracts was pipetted out in test tubes and added with 0.5ml of phloroglucinol reagent, followed by conc.HCl to each test tube. Samples were incubated for 30 minutes. At room temperature with occasionally stirring. Volume was made up to 10ml with 80% ethanol. The relative amount of gossypol was estimated by measuring by the OD of the samples at 550 nm against a ethanol blank.

Biochemical markers

Electrophoresis of soluble seed proteins and isoenzymes were performed following standard techniques.

Total soluble seed proteins

Total tris soluble proteins were electrophoresd by the modified procedure of Lammaeli (1971) described by Dadlani and Varier(1993).

Reagent for extraction and electrophoresis

Tris soluble seed proteins

1. Defatting solvent mixture:

   Chloroform, methanol and acetone mixed in a 2:1:1 ratio

2. Stock protein extraction solution:

   2gm SDS and 10µg Pyronin G dissolved in 10.4 ml of 0.6 M Tris-HCL buffer(pH 6.6) and 7.9 ml distilled water and 10ml glycerol, warmed gently and mixed well.

3. Working protein extraction solution:

This was prepared by mixing 4.25 ml stock protein extraction solution, 0.75ml

Beta-mercaptoethanol and make up to 10ml by adding distilled water.

Reagents for Gel Electrophoresis

1. 30% acrylamide for running gel

   75 gm acrylamide and 1 gm bis acrylamide dissolved in distilled water and make up to 250ml.

2. 30% acrylamide for stacking gel

   75 gm acrylamide and 2g gm bis acrylamide dissolved in distilled water and make up to 250ml.

3. Stock buffer for running gel

   1.875 M Tris HCL (pH 8.8); 22.69 gm tris dissolved in 50 ml distilled water and pH adjusted to 8.8 by adding conc.HCL drop by drop. The volume was make up to 100 ml distilled water.

4. Stock buffer stacking gel

   0.6 M tris HCL(pH 6.8) ; 7.26gm dissolved in 50 ml distilled water and pH adjusted to 6.8 by adding conc.HCL drop by drop. The volume was make up to 100 ml distilled water.

5. Stock SDS Solution (10%)

   10 gm SDS dissolved in distilled water with constant stirring and gently heating. The volume was make up to 100 ml distilled water.

6. Ammonium per sulphate (5%)(freshly prepared)

   0.5gm ammonium per sulphate dissolved in 10ml distilled water

7. SDS Tris Glycine (pH 8.3) 9.0 gm tris ,42.3gm glycine and 3gm SDS dissolved in distilled water and make upto 3litres.
8. Fixing solution (15% TCA)

   150gm trichloroacetic acid dissolved in distilled water to make up to 1 litre.
9. Staining solution

1gm commassie blue was dissolved in 100 ml methanol and 10 ml of it was added to 100ml of 15% TCA solution to make a final staining solution.

Preparation of gel:

1. Separating gel/ running gel (15%)

Tris buffer (pH 8.8)	12.0ml
Water	7.4ml
30% running gel acrylamide	20.0ml
10% SDS	0.4ml
5% APS	0.4ml

   0.04ml of TEMED was added just before pouring the gel mixture.

   All the reagents were mixed well and poured between the plates of the cassettes. Care was taken to avoid air bubbles to be trapped in the gel solution.casesstes was filled 3/4th and gel was allowed to set.
2. Stacking gel (4%)

After the running gel gets polymerized the following solutions were mixed and poured above it carefully.

Tris buffer (pH 6.8)	1.5ml
Water	6.0ml
30% running gel acrylamide	2.0ml
10% SDS	0.10ml
5% APS	0.40ml

0.04ml of TEMED was added just before pouring the gel mixture.

After pouring the stacking the gel solution, an acrylic comb having required number of wells was set, without trapping any bubble and gel was allowed to polymerize. The comb was removed and the wells were washed with tank buffer.

10 seeds from each genotype were decoated, powdered and defatted using 20 ml defatting solvent mixture (A1) for 48 Hrs with atleast 5 solvent changes. 50mg of ground material air dried at room temperature was taken in a clean 1.5ml Eppendorf tubes to which 0.5ml of working protein extraction solution (A-3) was mixed well and kept overnight at room temperature. The samples were heated in a boiling water bath for 10 minutes, cooled and centrifuged at 15,000 rpm for 10 min. the supernatant was collected for electrophoresis.

Electrophoresis

Electrophoresis was conducted in a Bio-Rad Protean II vertical Electrophoresis Unit, 20 µl of protein extract was carefully loaded on a 15% denaturing polyacrylamide gel using a micro syringe. A tracking dye (Bromophenol blue) was added to the upper tank buffer. The gel was run at maximum volt and constant power of 30amp per plate till the tracking dye reached the bottom of the gel.

Fixing and staining

The gel was fixed overnight in 15% Trichloroacetic acid (TCA), after fixing the gel was rinsed with distilled water and immersed in a mixture of 15ml of 2% coomassie blue(rectified spirit) and 100 ml of 15% TCA. Staining was done till the bands developed. Distaining was done in distilled water till the background was clear. Later the gels were photographed.

Evaluation and Documentation

The gels were scanned in Epson GT-950 scanner and prints were taken. The electrophoregrams were prepared by observing gels over a transilluminator measuring the distance of each band from the point of loading.

Relative mobility (Rm) of each band was calculated as

$$Rm = \frac{\text{Distance travelled by the band}}{\text{Distance travelled by the tracking dye}}$$

Bands were numbered on the basis of increasing Rm values.

Salt soluble Globulins

Globulins were analysed by the procedure described by Anisimova et al. (1991)

Reagents for extraction and gel electrophoresis

Globulins

1. Extraction buffer

   5% NaCl in 50mM Tris HCl (pH 8.0)

   Dissolve 0.605g Tris in 50ml distilled water, adjust the pH to 8.0 with conc.HCl and make up the volume to 100ml with distilled water.

Tris HCl buffer (pH 8.0) 50 mM	25ml
Nacl	1.25g

   The above mentioned components are mixed thoroughly.

2. 25 mM Tris Glycine buffer(pH 8.3)

   Dissolved 3gm Tris in 50ml of distilled water adjust the pH to 8.3 with conc. HCl. dissolve 2.075 gm glycine in Tris HCl (pH 8.3) and make up the volume to 100ml with distilled water.

3. Stock working sample buffer ,62.5nM Tris HCl buffer (pH 6.8)

   Dissolved 0.756g of Tris in 50ml of distilled water, adjust the pH 6.8 with conc.HCl and the volume made upto 100ml with distilled water.

4. Working sample buffer

Tris HCl buffer (pH 6.8) 62.5mM	25ml
SDS	0.5gm
B-mercaptoetanol	1.25ml
Urea	9gm
Sucrose	2.5gms

All the above components are mixed thoroughly

Electrophoresis

Electrophoresis was conducted using Bio-Rad protean II vertical electrophoresis unit.

10-15µl of the sample (globulin extract) was carefully loaded denaturing polyacrylamide gel using a microsyringe. The gel was run at maximum voltage and constant power of 30amp per plate till the tracking dye reached the bottom the gel.

Fixing,staining and evaluation was done

Isoenzmye markers

For the purpose the identification of hybrids and parental lines,polymorphism among the genotypes with the respect to the following isoenzymes was analyzed.

A. Acid phosphastase
B. Esterase
C. Superoxide dismutase

D. Alcohol dehydrogenase

E. Malatate dehydrogenase

F. Peroxidase

G. Catalase

Preparation of sample

For extraction of ADH isoenzyme, five seeds of each genotype soaked in distillled water in air tight tubes for 48 hrs. outer seed coat was removed and seed were ground using chilled mortar and pestle over ice with 0.30ml extraction buffer. after fine grinding, the sample was taken in a clean 1.5ml appendorf tubes and centrifuged at 12000rpm for 30minutes at 4^{o}C. The supernatant was transferred to another 1.5ml appendrof tube and centrifuged at 12000rpm for 10 minutes at 4^{o}C.the clear supernatant was used for electrophoresis.

For the rest of the isoenzymes,seedling were raised at 25oc , 5 to 7 days old coleoptiles were used for extraction of enzymes 5' coleoptiles were crushed with 0.25 to 0.3 ml extraction buffer at 4oC in a chilled pestle and mortar. The extract was centrifuged at 10000rpm for 20 minutes at 4oC.the clear supernatant was collected and used for loading.

DNA Isolation

DNA was isolated from the seeds following the methods west and Krishna with some modification. This method is particularly suitable for single seed analysis in such crops where the quality of DNA that adverse affected during the process of isolation due to presence polyphenols and polysaccharides etc due their ability to bind with nucleic acids. The present method gave good quality DNA following the simple protocol.

Protocols

1. Seeds were soaked overnight at room temperature
2. Seed coat was removed and 3 to 4 gm of cotyledons tissue was crushed in a prechilled mortar and pestle.
3. 15 ml of DNA extraction buffer(solutionA) and 1.5ml of 20% SDS (solutionB) was added to the sample which was transferred to centrifuged tube and pulverized.
4. The sample was incubated at 65^{o}C for 10 minutes with occasionally gentle swirling and then cooled on ice for 10 minutes.
5. 5.0ml of potassium acetate(solution C) was added and mixed thoroughly
6. The mixture was centrifuged at 13000rpm for 20min at 4^{o}C.
7. 700µl of clear supernatant was collected in 1.5ml eppendorf tube.
8. Same quantity of isopropanol and ammonia acetate (3:1) mixture (solution D) was added to precipitate nucleic acids.
9. Content were mixed thoroughly and centrifuged at 10000 rpm for 20 min to pellet the DNA.
10. Supernatant was discarded and DNA pellets were washed twice with 70% alcohol to remove the salts.
11. Pellet was air dried at room temperature in aseptic conditions and dissolved in 500µl of TE buffer(solution F)

Purification of DNA

Inclusion of SDS at the time of DNA extraction helps in the precipitation of proteins. Potassium acetate facilitates the SDS protein precipitation. RNA was removed by treating the sample with RNAse.

Treatment of RNAse

1. RNAse was added to DNA sample @ 50µg/500µl and incubated at 37^{o}C for one hour.
2. An equal volume of phenol-chloroform (1:1) was mixed and the tubes were centrifuged at 10000 rpm for 5 min at room temperature.
3. The aqueous phase was transferred into a fresh micro centrifuge tube.
4. Extraction with chloroform: isoamyloalcohol (24:1) was done twice and aqueous phase separated out.
5. 0.5 volume of 3M sodium acetate buffer (pH 4.8) was added and mixed properly.
6. 2.5 times chilled absolute alcohol was added and mixed to precipitate the DNA.
7. DNA was pelleted was centrifugation at 10000rpm for 5min.
8. The supernatant was decanted carefully and the pellet was washed with 70% ethanol, dried aseptically and dissolved in 50 to 100µl buffer.

Checking the quality and quantity of DNA

This was done by agarose gel electrophoresis

0.8% gel was prepared by mixing 1.2gm of agarose in 150ml of distilled water. It was heated in microwave oven till agarose was dissolved. After cooling to room temperature, ethidium bromide was added at the 50µg/ml. this was poured into the gel casting tray in which coombs was set. After 1 hr, the gel was solidified and combs were removed to form the wells.

To 2µl of DNA samples, 2.5µl of dye and 10.5µl TE buffer was mixed and loaded into the wells. Gene ruler DNA ladder plus loaded as control in the corner well (3µl of marker DNA ladder plus and 2 µl of Dye). Gel was run at 50V for 1 hr. the quality of DNA was judged by the nature of the band at the corresponding position of the control. Presence of a single compact band indicated that isolated DNA was of high molecular weight and good quality. The approximate quantity of the DNA was estimated in the sample by comparison with control.

After quantifying the DNA of each genotype, the samples were diluted with TE buffer so that final concentration of DNA was 12.5µg/ml.depending on the quantity of DNA in the sample, different volumes of TE buffer was added to get the above mentioned concentration of DNA.

Results

Characterization of fifteen cotton genotypes ,comprising of five hybrids and their parental lines, was done on the basis of rapid chemicals tests and proteins,isoenzymes and molecular markers. Genetic purity tedsting of commerical seed lots of three cotton hybrids was tested on the basis of proteins markers and results were compared with those of field grow out test. The results of these studies are detailed below.

Identification of cotton hybrid and their parental lines

Characterization based on seeding response to nutrient medium and chemical applications.

Pot culture experiments were conducted and controlled growth conditions in the glass house.seedlings were raised in nutrient media,which were deficient or toxic for certain elements or containing a low dose of herbicide.

Seeding characters of the hybrids and their female parents grown under controlled conditions were recorded. The observations on seedling characters viz., foliage colour, leaf area, shoot length and stem diameter for different hybrids and their female parents are presented.

Morphological descriptors of cotton hybrids and their female parental lines.

Characters	Savitha (H)	T-7 (F)	PkHy-2 (H)	AK-32 (F)	NHH-44 (H)	BN-1 (1)	H-10 (H)	BC-68/2 (F)
Plant habit	Open Short, internodes	Pyramid	Bushy	Semi erect	Bushy Open	Open	Erect	Open
No.of monopodia	0-3	1-3	3-4	2-4	2-3	1-2	3-4	2-3
Leaf color	Light green	Dark green	Light green	Dark green	Light green	Light green	Light green	Light green
Leaf hairness	Medium	Moderate	Dense	Short dense	Medium hairy	Medium hairy	Medium hairy	Medium hairy
Leaf nectarines			Present	Present	Present	Present		
Leaf lobes	3-5	3-5	3-5 Broad	3-4 Medium	3-5	3-5	3-5	3
Petal color	Cream	Cream	Sulphur yellow	Yellow	Light yellow	Cream	Creamy white	Basal color
Anther color	Yellow	Yellow	Pale yellow	Pale yellow	yellow	yellow	yellow	Yellow
Petal spot	Absent	Absent	Purple	Purple	Absent	AB	AB	AB
Bract			Less	Medium				
Bollshape and size	Medium ovate,beaked tip	Medium ovid	Mediu m ovid	Medium	Medium round	Slighty elongaged	Oval Pointed tip	Big oval

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.00														
2	1.00	1.00													
3	1.00	1.00	1.00												
4	1.00	1.00	1.00	1.00											
5	1.00	1.00	1.00	1.00	1.00										
6	1.00	1.00	1.00	1.00	1.00	1.00									
7	1.00	1.00	1.00	1.00	1.00	1.00	1.00								
8	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00							
9	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	1.00						
10	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.53	1.00					
11	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	1.00	0.53	1.00				
12	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.53	1.00	0.53	1.00			
13	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	1.00	0.53	1.00	0.53	1.00		
14	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	1.00	0.53	1.00	0.53	1.00	1.00	
15	0.57	0.57	0.57	0.57	0.57	0.57	0.57	0.57	1.00	0.53	1.00	0.53	1.00	1.00	1.00

Similarity Matrix of Peroxidase isoenzyme of cotton through native PAGE1

1=V 797; 2=G Cot 13; 3=A.D.C-1; 4=GV hv 473; 5=GV hv 235; 6=GV hv 715; 7=Dhumad; 8=DLSA 24; 9=G Cot 21; 10=Kutch Selections; 11=9726; 12=Jaydhar; 13=Bagesera ghed sel; 14=G Cot 25; 15=G Cot 23.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.00														
2	0.64	1.00													
3	0.50	0.36	1.00												
4	0.50	0.45	0.53	1.00											
5	0.64	0.50	0.46	0.45	1.00										
6	0.80	0.69	0.53	0.43	0.69	1.00									
7	0.80	0.57	0.53	0.43	0.69	0.73	1.00								
8	0.73	0.50	0.47	0.58	0.62	0.67	0.79	1.00							
9	0.69	0.69	0.35	0.33	0.47	0.73	0.53	0.47	1.00						
10	0.47	0.44	0.41	0.40	0.44	0.50	0.50	0.53	0.59	1.00					
11	0.28	0.29	0.58	0.45	0.29	0.29	0.38	0.40	0.29	0.64	1.00				
12	0.67	0.47	0.53	0.35	0.47	0.61	0.61	0.56	0.61	0.76	0.56	1.00			
13	0.35	0.50	0.46	0.33	0.38	0.47	0.47	0.31	0.47	0.53	0.64	0.56	1.00		
14	0.80	0.69	0.53	0.43	0.57	0.86	0.73	0.67	0.73	0.59	0.38	0.71	0.47	1.00	
15	0.87	0.64	0.50	0.40	0.53	0.80	0.69	0.63	0.80	0.56	0.35	0.76	0.44	0.93	1.00

Similarity Matrix of total protein of cottonseed through SDS-PAGE

1=V 797; 2=G Cot 13; 3=A.D.C-1; 4=GV hv 473; 5=GV hv 235; 6=GV hv 715; 7=Dhumad; 8=DLSA 24; 9=G Cot 21; 10=Kutch Selections; 11=9726; 12=Jaydhar; 13=Bagesera ghed sel; 14=G Cot 25; 15=G Cot 23.

Table: Peroxidase and polyphenol oxidase activity different genotype of cotton

Genotype	Peroxidase (µmol/min/mg)	Polyphenol oxidase (µmol/min/mg)
V797	1.47	2.08
G Cot 13	1.56	2.06
A.D.C.1	1.75	2.21
Gv Hv 473	1.67	2.34
Gv Hv 235	1.83	2.38
Dhumad	1.64	1.68
DLSA 24	1.72	2.31
G Cot 21	1.58	1.93
Kutch Selection	2.30	1.78
9726	1.75	1.84
Jaydhar	1.44	2.57
Bagasara Ghed selection	2.28	2.64
G Cot 25	1.67	2.43
G Cot 23	2.24	2.66
S.E.m	0.119	0.156
CD %	0.343	0.449
CV %	11.97	12.36

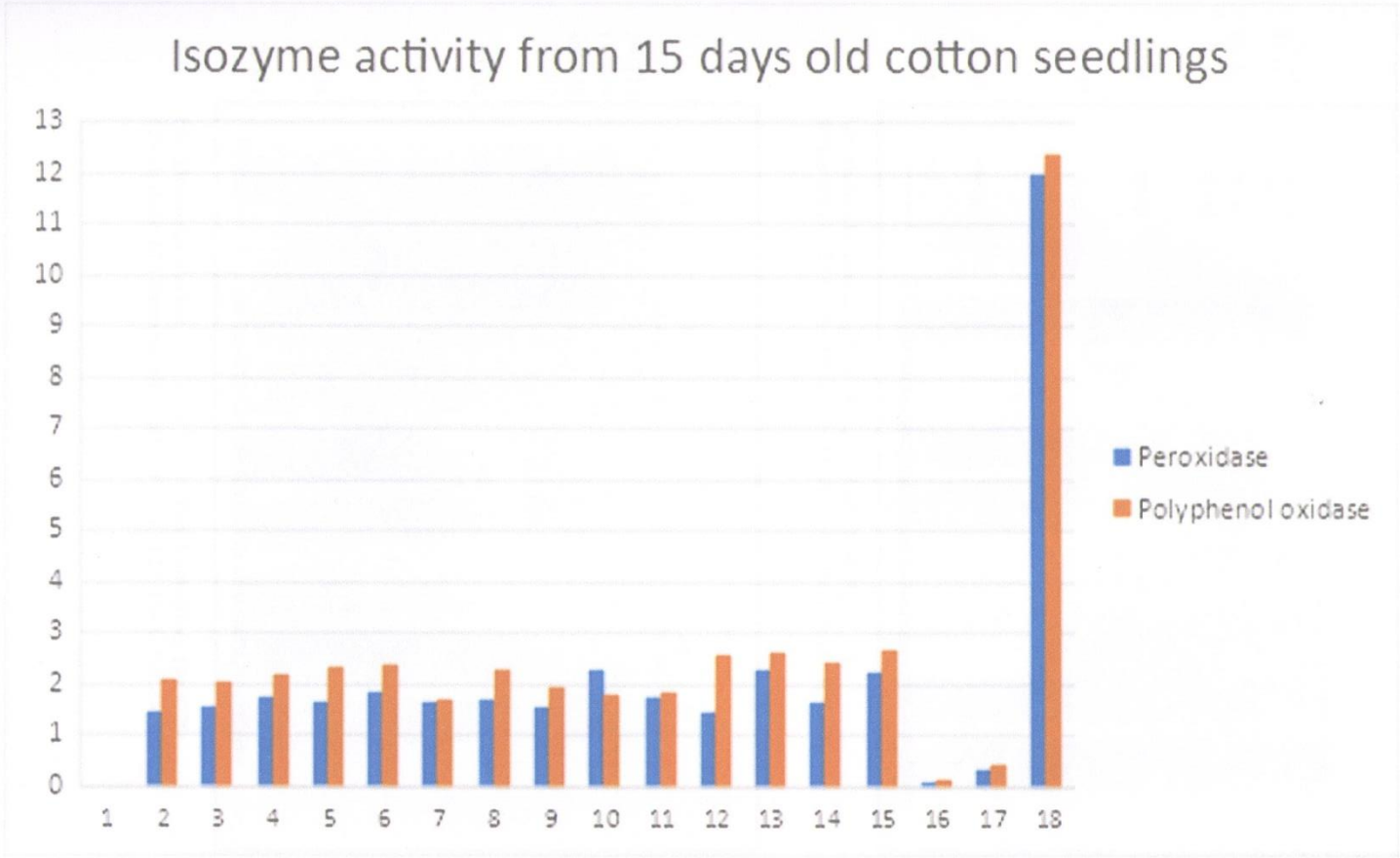
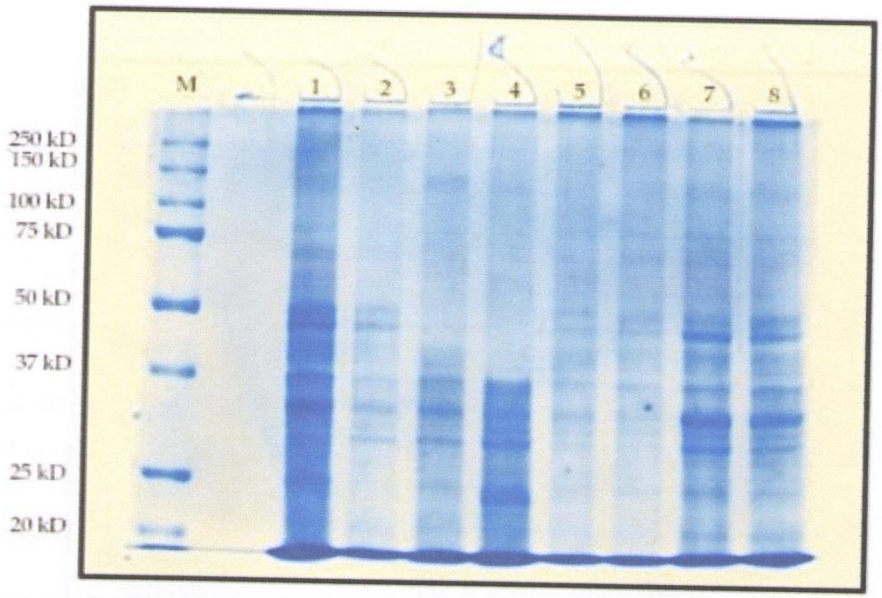
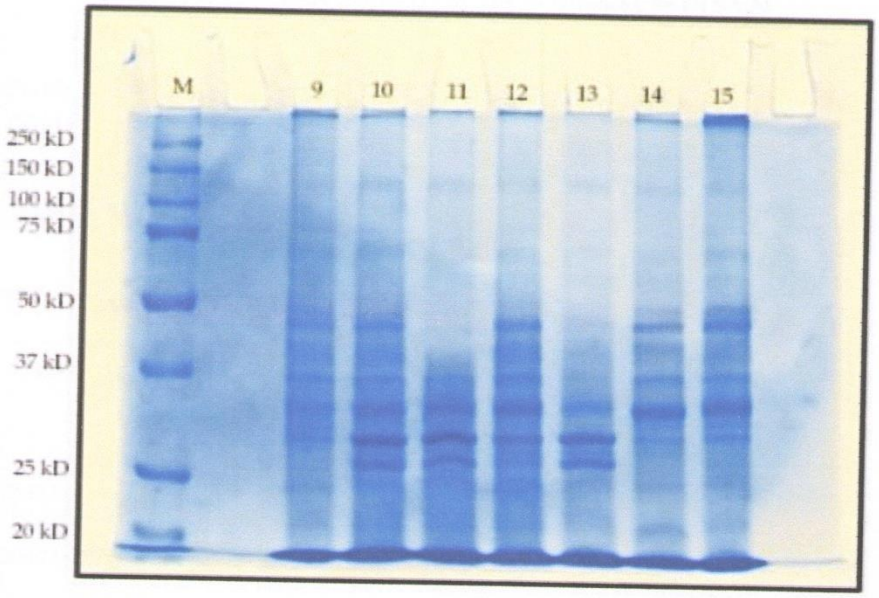

Isozyme activity from 15 days old cotton seedlings
13
12
11
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Peroxidase
Polyphenol oxidase

Plate 4.1 **Total protein profiling through SDS-PAGE.**

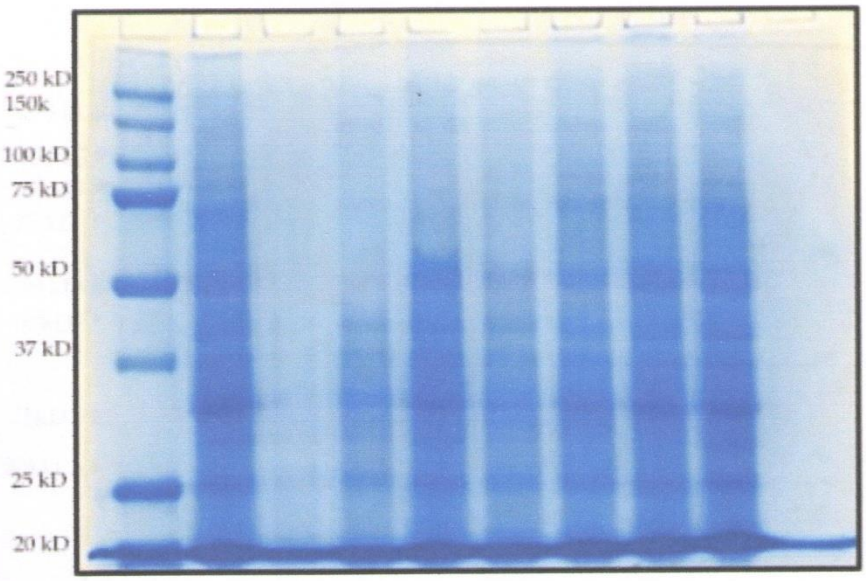


M= Molecular marker ; 1= V 797; 2= G Cot 13; 3= A.D.C - 1; 4= Gv hv 473; 5= Gv hv 235; 6= Gv hv 715; 7= Dhumad; 8= DLSA 24

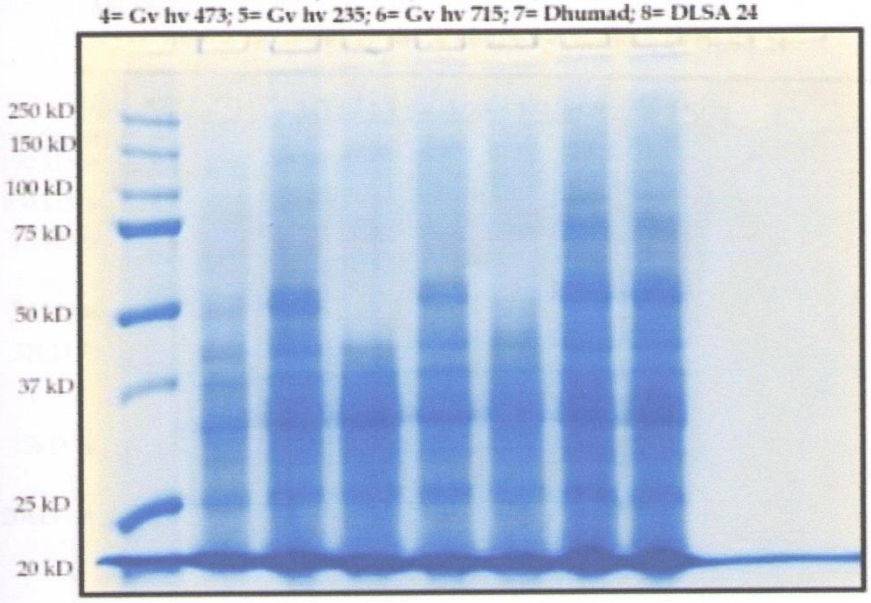


M= Molecular marker ; 9= G Cot 21; 10= Kutch selection; 11= 9726; 12= jaydhar; 13= Bagesera ghed sel.; 14= G Cot 25; 15= G Cot 23.

Plate 4.2 Albumin fraction from cottonseed

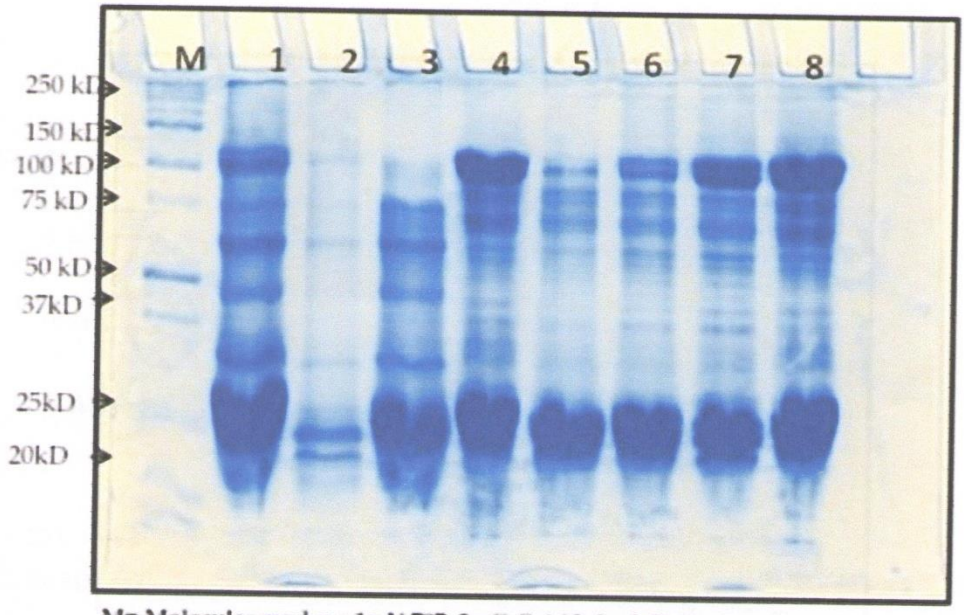


M= Molecular marker ; 1= V 797; 2= G Cot 13; 3= A.D.C – 1;
4= Gv hv 473; 5= Gv hv 235; 6= Gv hv 715; 7= Dhumad; 8= DLSA 24

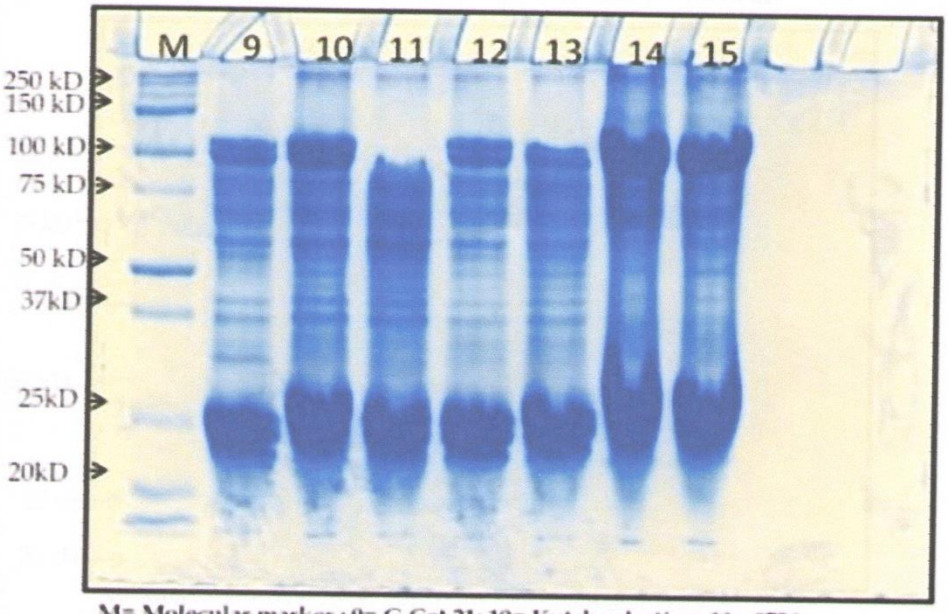


M= Molecular marker ; 9= G Cot 21; 10= Kutch selection; 11= 9726;
12= jaydhar; 13= Bagesera ghed sel.; 14= G Cot 25; 15= G Cot 23.

Plate 4.3 Globulin fraction from cottonseed

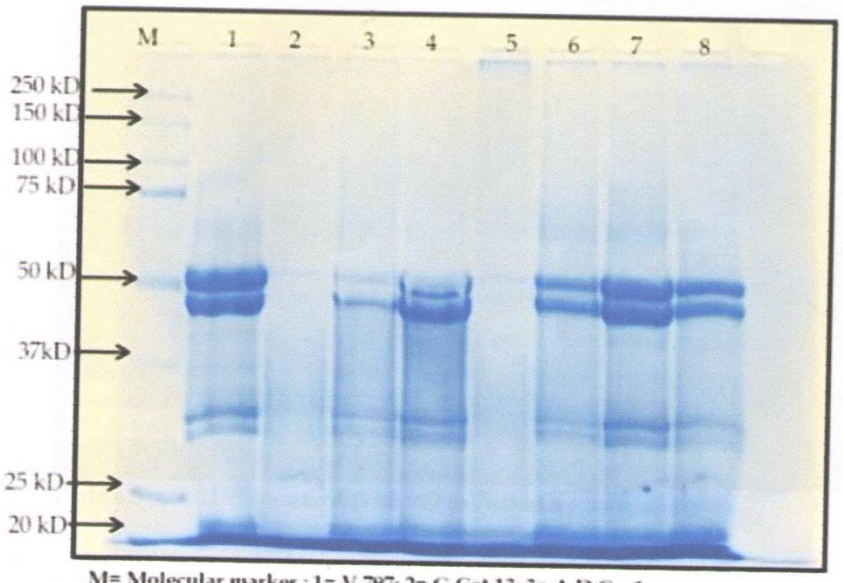


M= Molecular marker ; 1= V 797; 2= G Cot 13; 3= A.D.C - 1; 4= Gv hv 473; 5= Gv hv 235; 6= Gv hv 715; 7= Dhumad; 8= DLSA 24

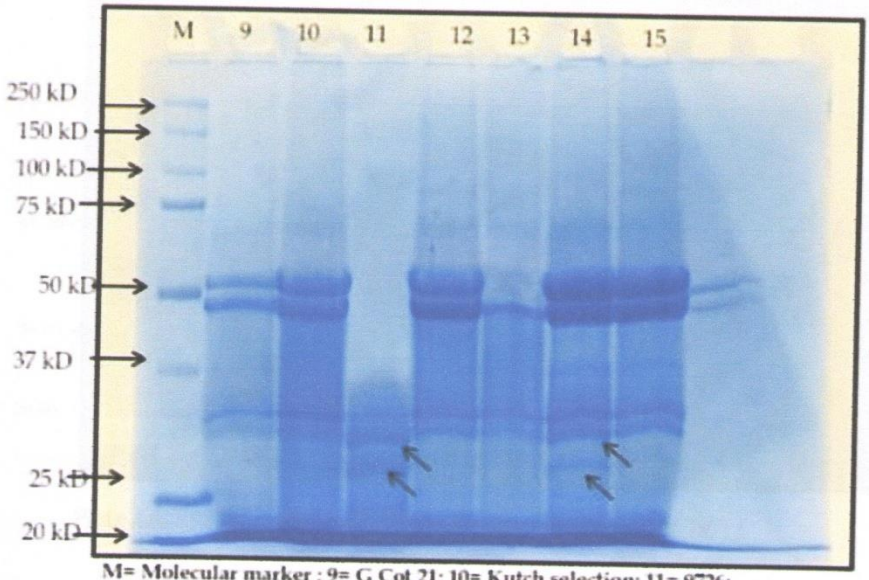


M= Molecular marker ; 9= G Cot 21; 10= Kutch selection; 11= 9726; 12= jaydhar; 13= Bagesera ghed sel.; 14= G Cot 25; 15= G Cot 23.

Plate 4.4 Glutelin fraction from cottonseed



M= Molecular marker ; 1= V 797; 2= G Cot 13; 3= A.D.C - 1; 4= Gv hv 473; 5= Gv hv 235; 6= Gv hv 715; 7= Dhumad; 8= DLSA 24



M= Molecular marker ; 9= G Cot 21; 10= Kutch selection; 11= 9726; 12= jaydhar; 13= Bagesera ghed sel.; 14= G Cot 25; 15= G Cot 23.

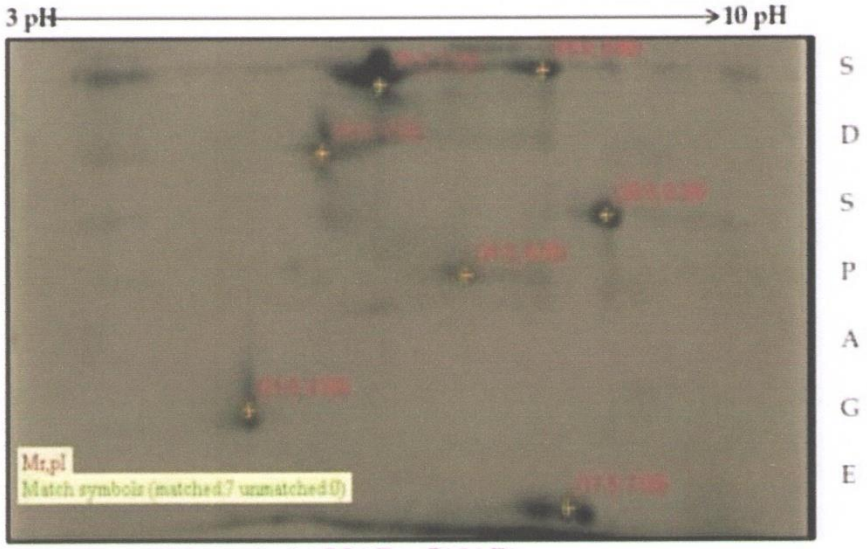


Plate 4.5 2DE standard (17.5 kD to 76.0 kD)

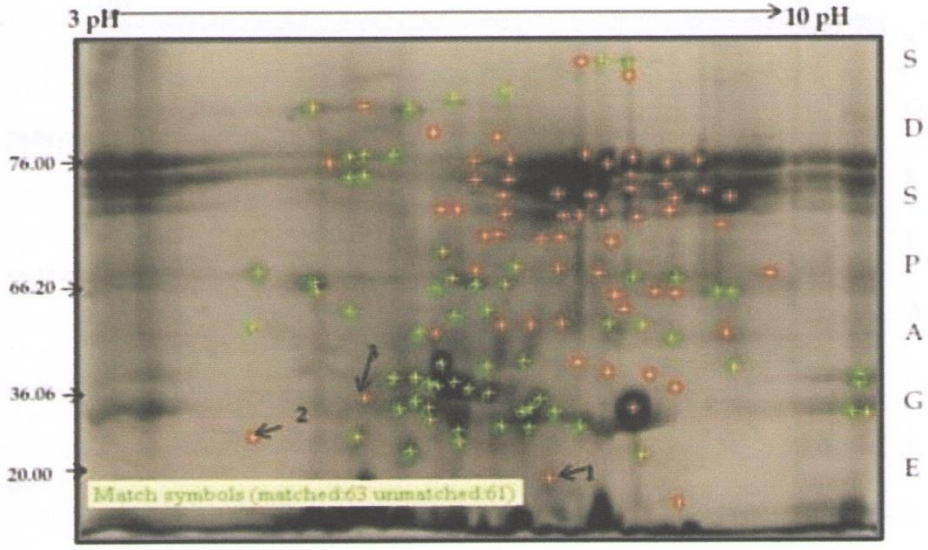


Plate 4.6 2D protein profiling of cottonseed meal (V 797)

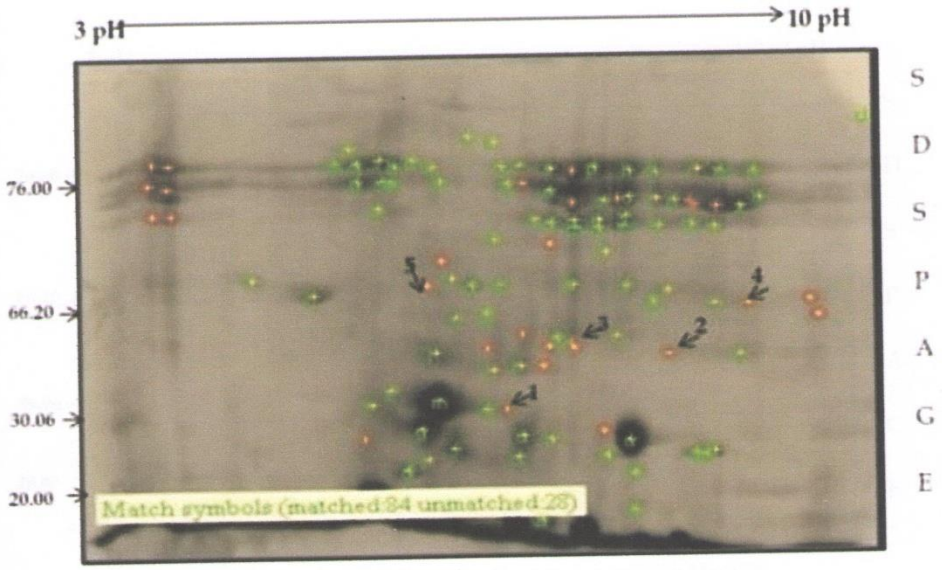


Plate 4.7 2D protein profiling of cottonseed meal (G Cot 13)

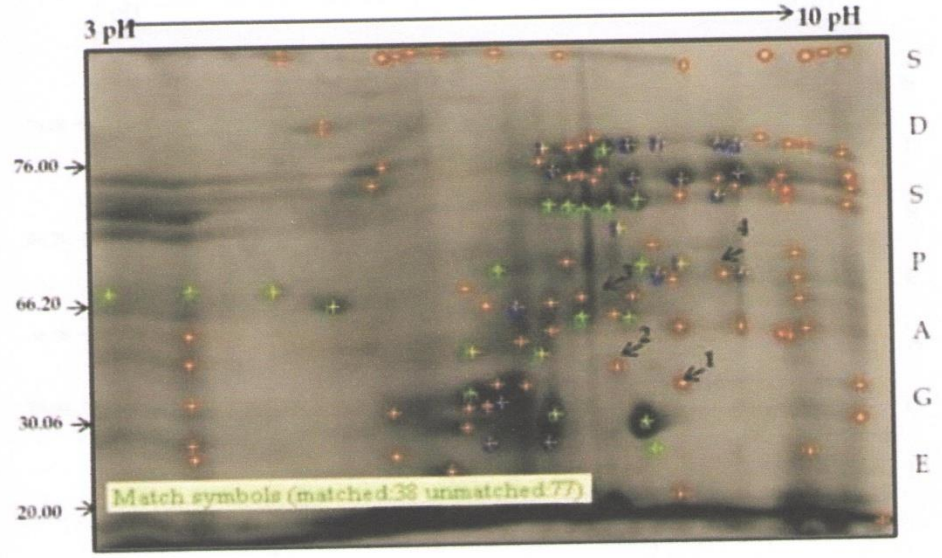


Plate 4.8 2D protein profiling of cottonseed meal (A.D.C 1)

Plate 4.9 2D protein profiling of cottonseed meal (Gv Hv 473)

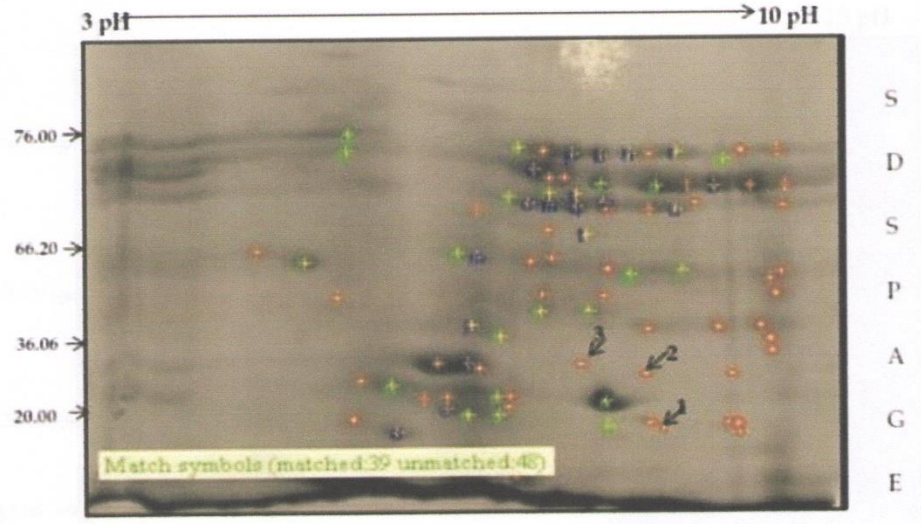


Plate 4.10 2D protein profiling of cottonseed meal (Gv Hv 235)

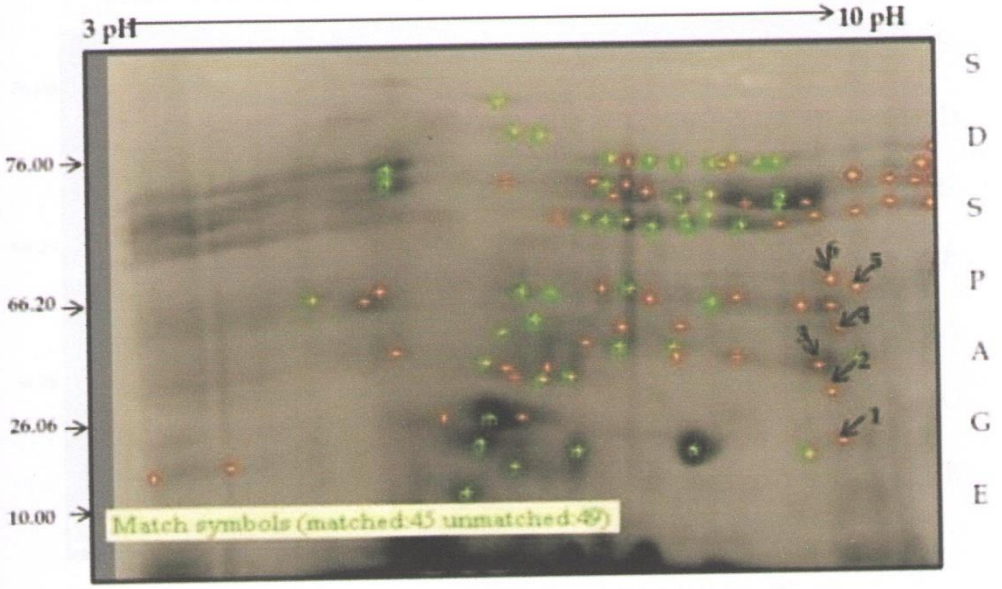


Plate 4.11 2D protein profiling of cottonseed meal (Gv Hv 715)

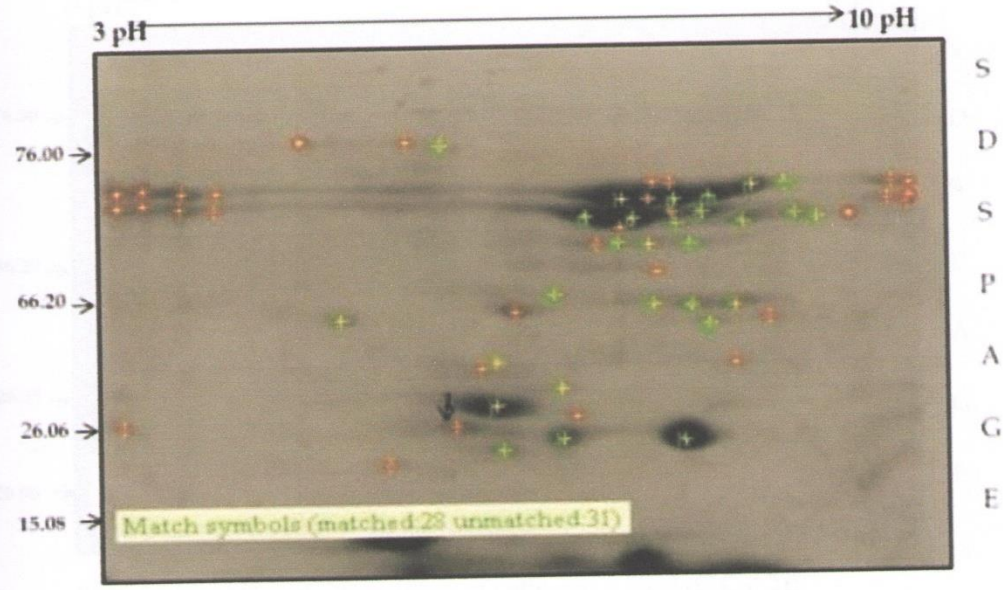


Plate 4.12 2D protein profiling of cottonseed meal (Dhumad)

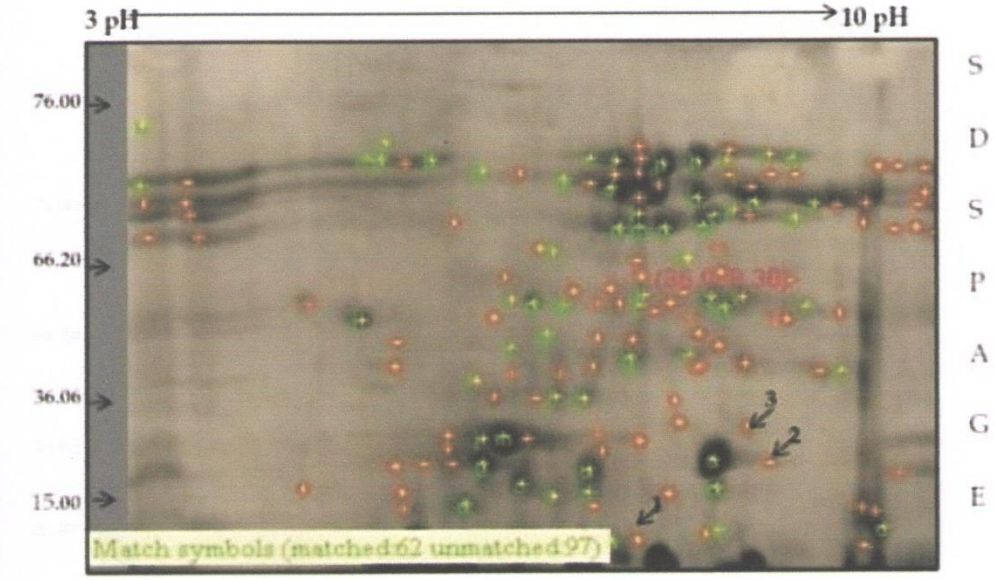


Plate 4.13 2D protein profiling of cottonseed meal (DLSA 24)

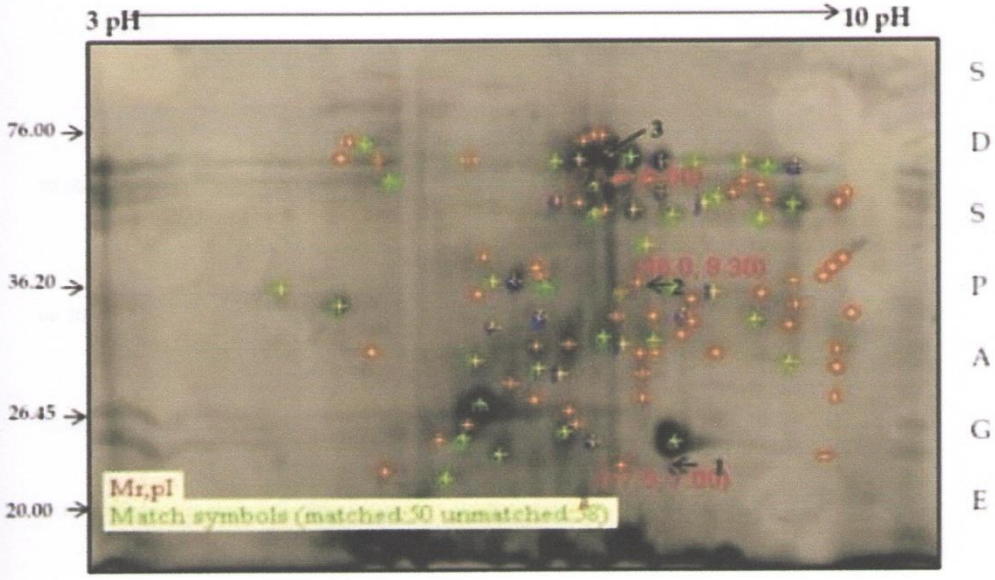


Plate 4.14 2D protein profiling of cottonseed meal (G Cot 21)

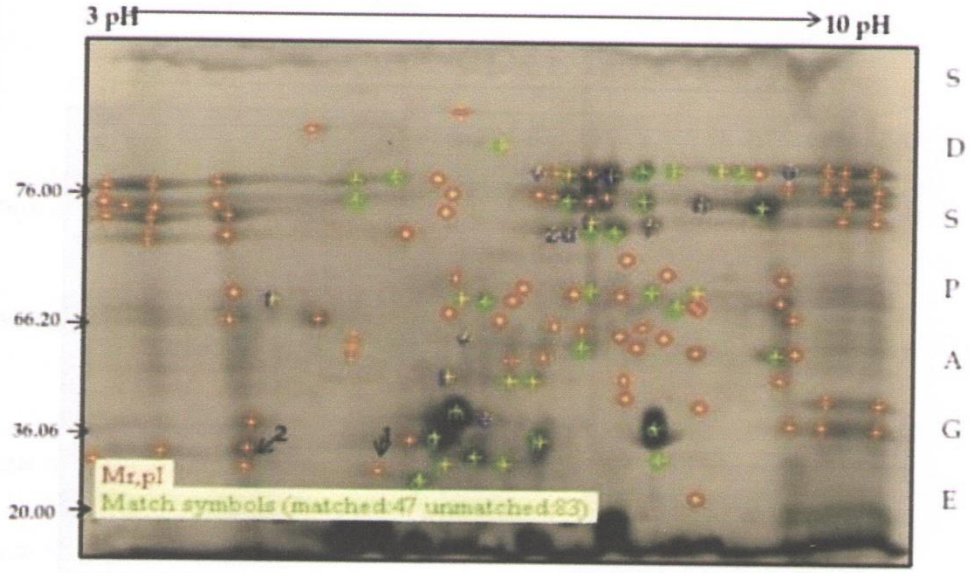


Plate 4.15 2D protein profiling of cottonseed meal (Kutch selection)

Plate 4.16 2D protein profiling of cottonseed meal (9726)

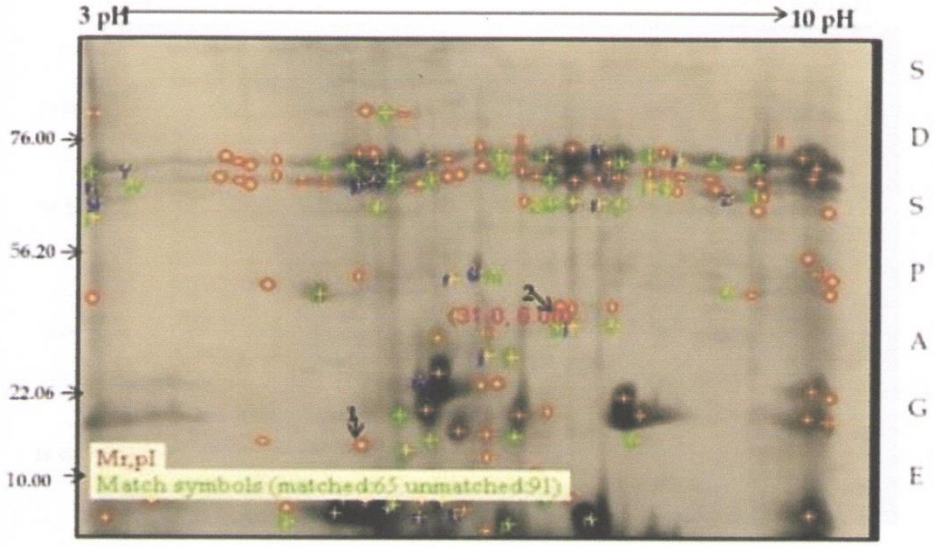


Plate 4.17 2D protein profiling of cottonseed meal (Jaydhar)

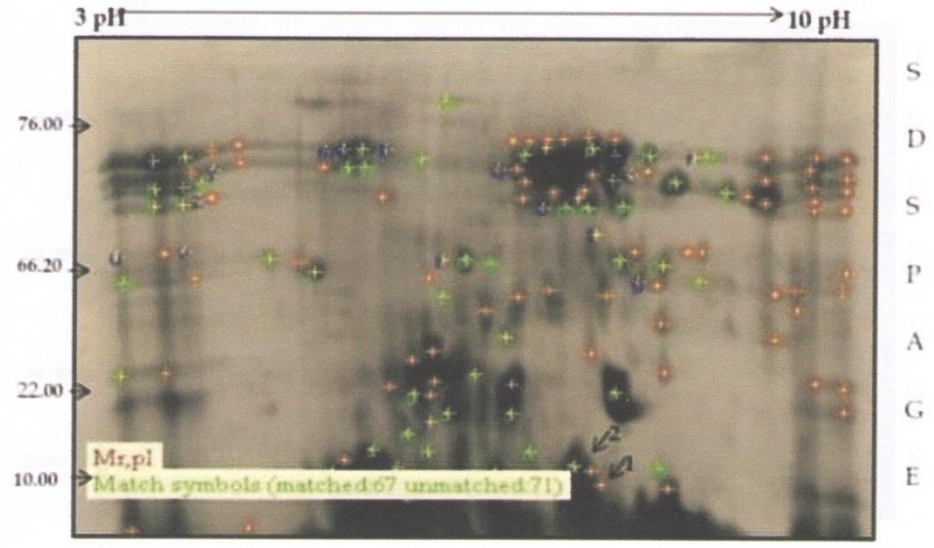


Plate 4.18 2D protein profiling of cottonseed meal (Bagasara ghed selection)

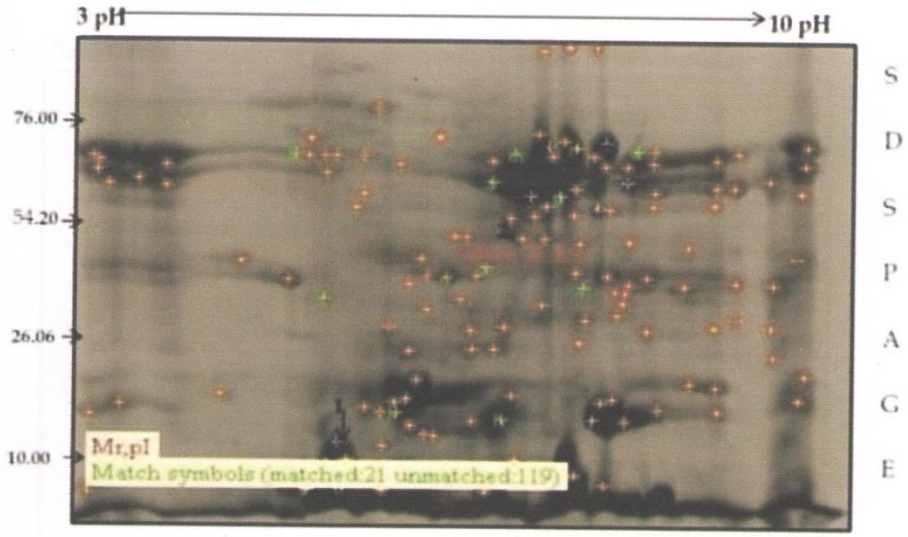


Plate 4.19 2D protein profiling of cottonseed meal (G Cot 25)

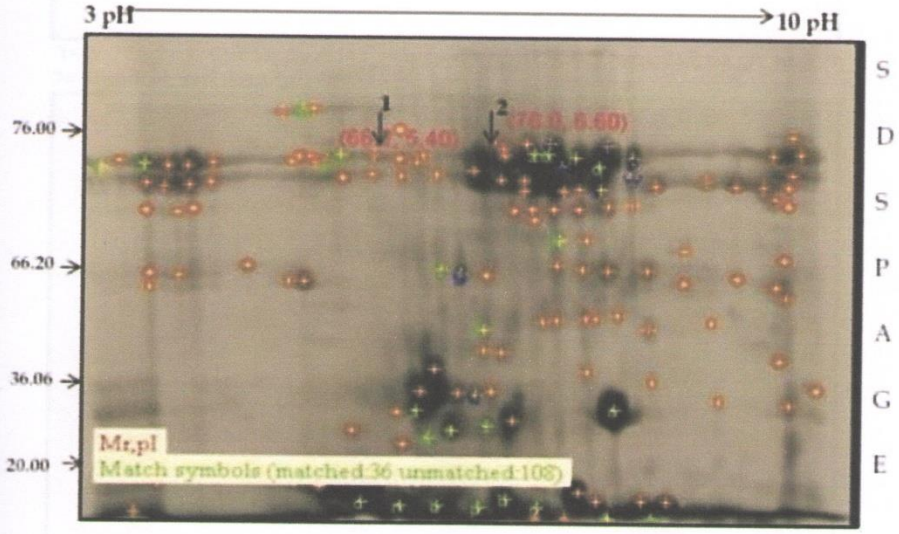
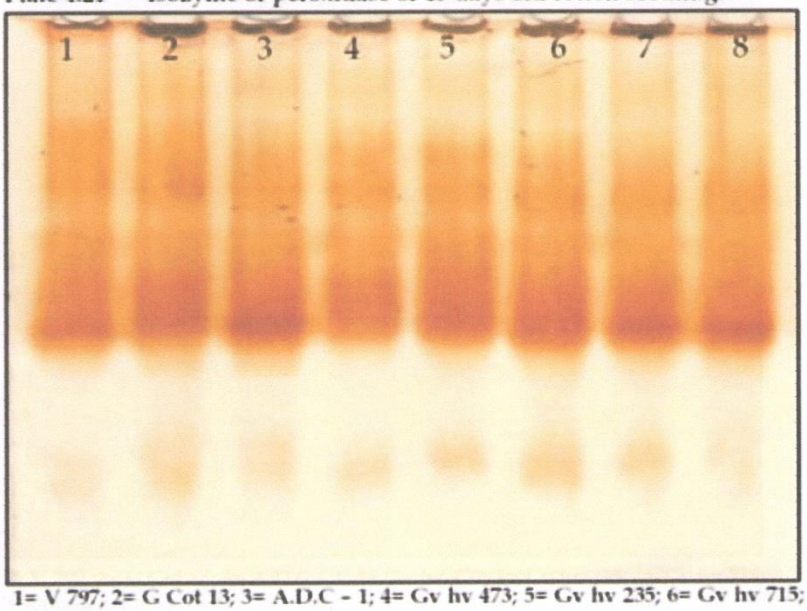
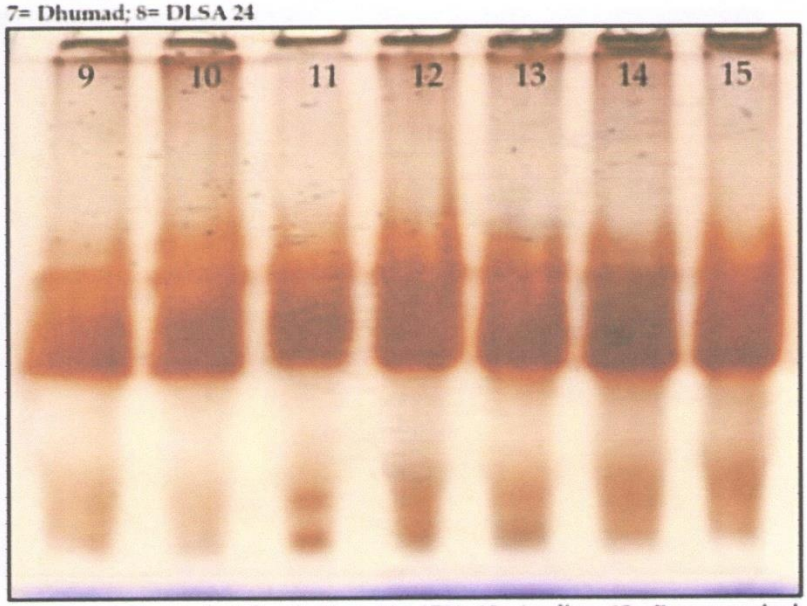


Plate 4.20 2D protein profiling of cottonseed meal (G Cot 23)

Plate 4.21 Isozyme of peroxidase of 15 days old cotton seedlings

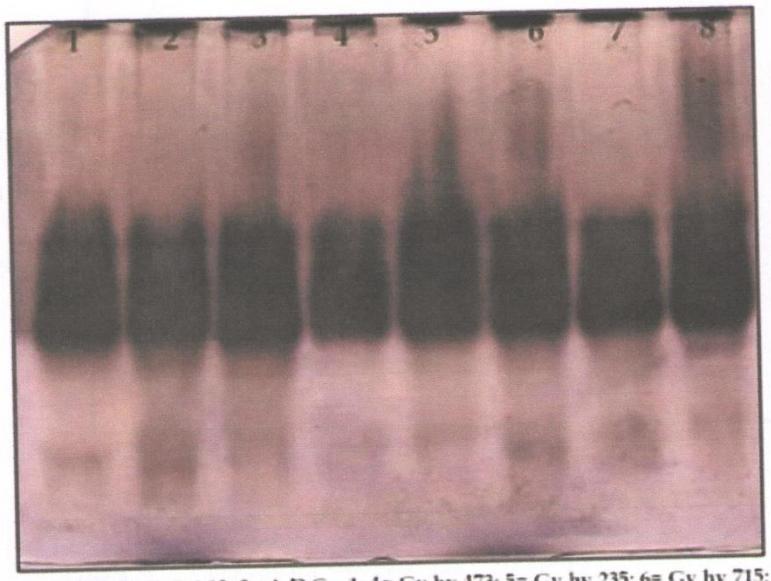


1= V 797; 2= G Cot 13; 3= A.D.C - 1; 4= Gv hv 473; 5= Gv hv 235; 6= Gv hv 715; 7= Dhumad; 8= DLSA 24

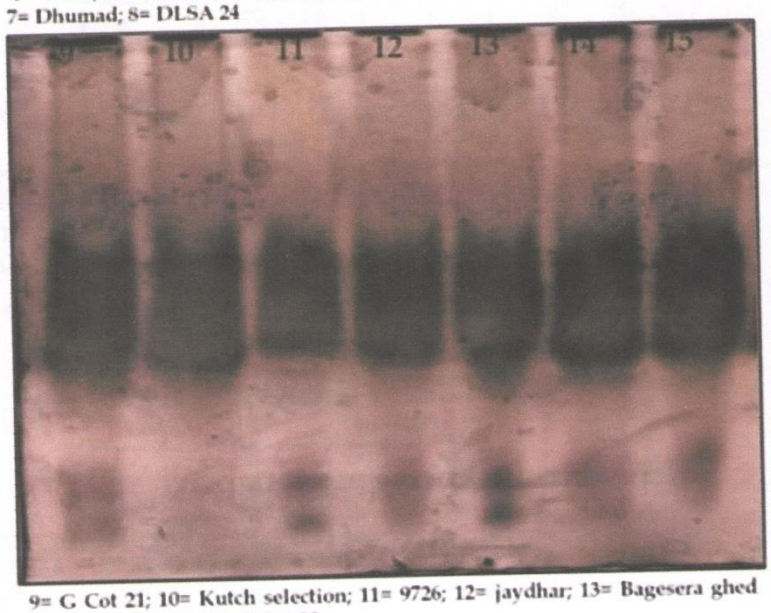


9= G Cot 21; 10= Kutch selection; 11= 9726; 12= jaydhar; 13= Bagesera ghed sel.; 14= G Cot 25; 15= G Cot 23.

Plate 4.22 Isozyme of polyphenol oxidase of 15 days cotton seedlings

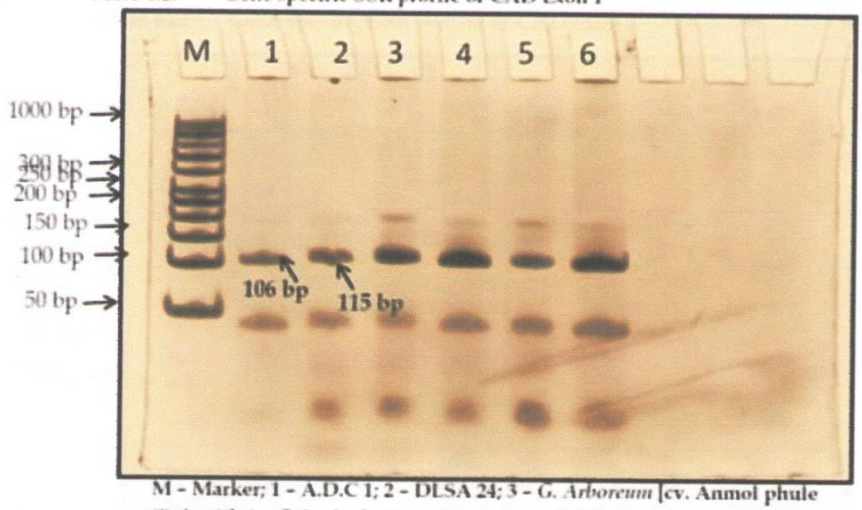


1= V 797; 2= G Cot 13; 3= A.D.C - 1; 4= Gv hv 473; 5= Gv hv 235; 6= Gv hv 715; 7= Dhumad; 8= DLSA 24



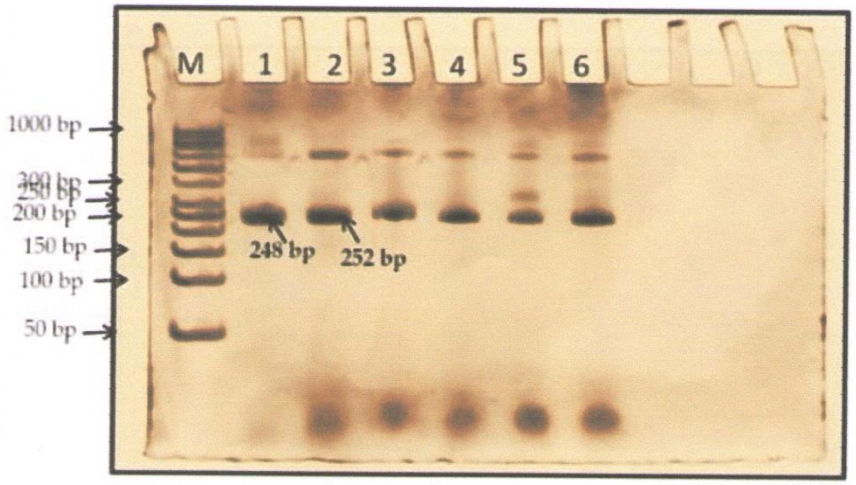
9= G Cot 21; 10= Kutch selection; 11= 9726; 12= jaydhar; 13= Bagesera ghed sel.; 14= G Cot 25; 15= G Cot 23.

Plate 4.23 Gene specific SSR profile of CAD Exon 1



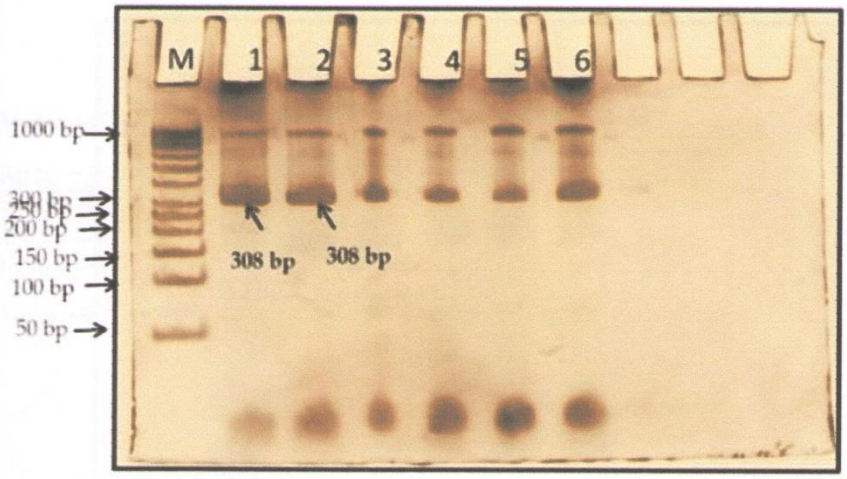
M – Marker; 1 – A.D.C 1; 2 – DLSA 24; 3 – *G. Arboreum* [cv. Anmol phule (Rahuri)]; 4 – *G. barbadance* (cv. Suvin); 5 – *G. hirsutum* (cv. G.cot 10); 6 – Kidney cotton

Plate 4.24 Gene specific SSR profile of CAD Exon 2



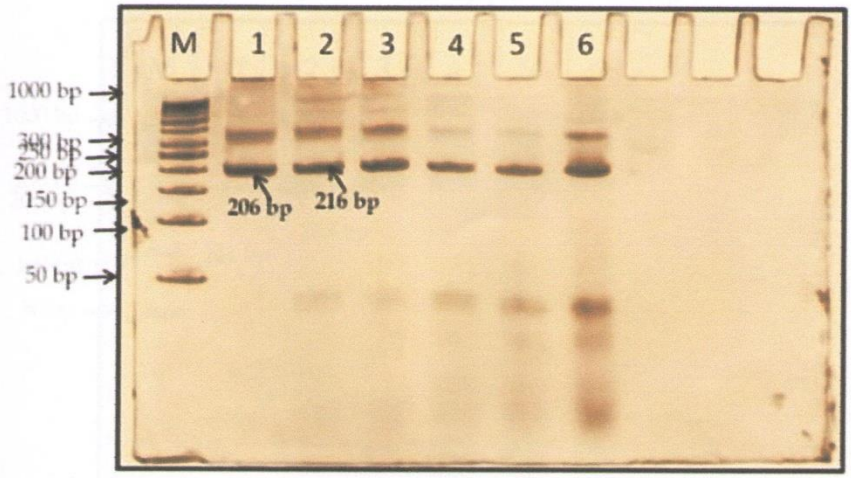
M – Marker; 1 – A.D.C 1; 2 – DLSA 24; 3 – *G. Arboreum* [cv. Anmol phule (Rahuri)]; 4 – *G. barbadance* (cv. Suvin); 5 – *G. hirsutum* (cv. G.cot 10); 6 – Kidney cotton

Plate 4.25 Gene specific SSR profile of CAD Exon 3



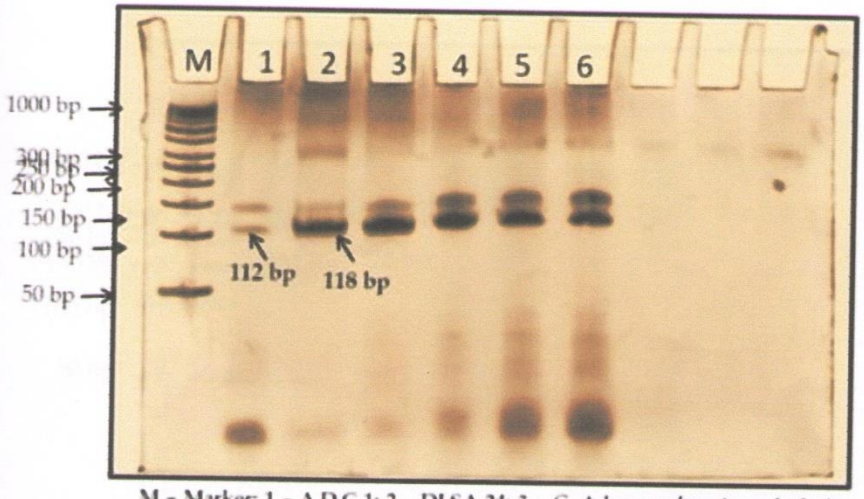
M – Marker; 1 – A.D.C 1; 2 – DLSA 24; 3 – *G. Arboreum* [cv. Anmol phule (Rahuri)]; 4 – *G. barbadance* (cv. Suvin); 5 – *G. hirsutum* (cv. G.cot 10); 6 – Kidney cotton

Plate 4.26 Gene specific SSR profile of CAD Exon 4



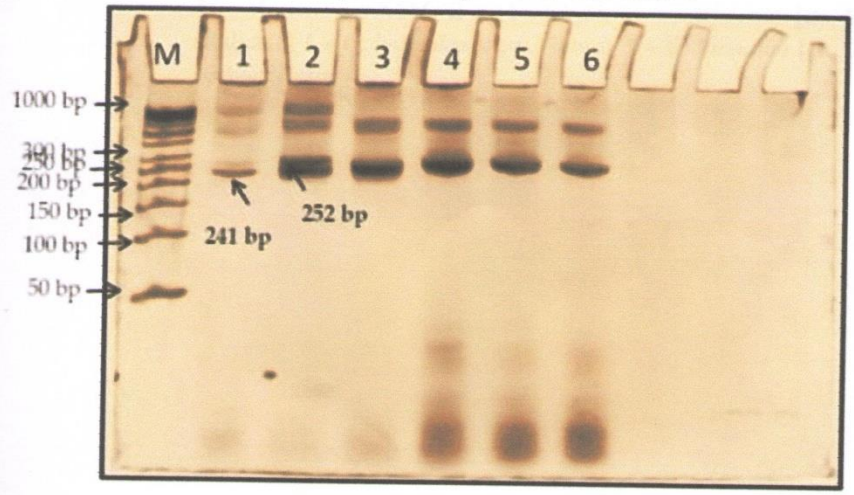
M – Marker; 1 – A.D.C 1; 2 – DLSA 24; 3 – *G. Arboreum* [cv. Anmol phule (Rahuri)]; 4 – *G. barbadance* (cv. Suvin); 5 – *G. hirsutum* (cv. G.cot 10); 6 – Kidney cotton

Plate 4.27 Gene specific SSR profile of CAD Exon 5



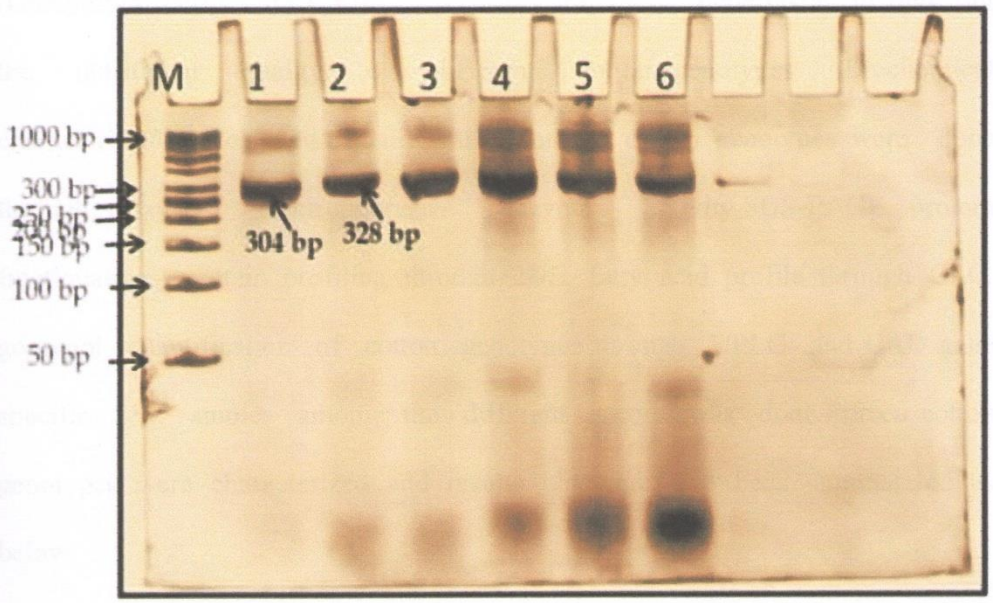
M – Marker; 1 – A.D.C 1; 2 – DLSA 24; 3 – *G. Arboreum* [cv. Anmol phule (Rahuri)]; 4 – *G. barbadance* (cv. Suvin); 5 – *G. hirsutum* (cv. G.cot 10); 6 – Kidney cotton

Plate 4.28 Gene specific SSR profile of CAD Exon 6



M – Marker; 1 – A.D.C 1; 2 – DLSA 24; 3 – *G. Arboreum* [cv. Anmol phule (Rahuri)]; 4 – *G. barbadance* (cv. Suvin); 5 – *G. hirsutum* (cv. G.cot 10); 6 – Kidney cotton

Plate 4.29 Gene specific SSR profile of CAD Exon 7



M – Marker; 1 – A.D.C 1; 2 – DLSA 24; 3 – *G. Arboreum* [cv. Anmol phule (Rahuri)]; 4 – *G. barbadance* (cv. Suvin); 5 – *G. hirsutum* (cv. G.cot 10); 6 – Kidney cotton

Summary And Conclusion

The present investigation entitled “Biochemical characterization of cotton (Gossypium herbaceum L.) for seed quality traits”was undertaken to find out the nutritional quality of different cotton genotypes. Biochemical characterization for seed quality traits among cotton genotypes were done through isozyme electrophoresis, enzyme activity,SDS-PAGE, protein fractionations, protein profiling through 2DE, fatty acid profile through GLC, gossypol quantification of cotton genotypes through UPLC and CAD gene specific SSR studies among the different species was done.Fifteen cotton genotypes were characterized and results obtained have been summarized as below:

Cotton seed

Proximate composition

Major nutritional components of cotton genotypes have been analyzed from defatted cottonseed meal and oil.Moisture percent in cottonseed of different genotypes varied from 5.2 to 7.4% with an average of 6.3%. However,it was recorded maximum in Gv Hv 715 (7.50%) and minimum in G Cot 21(5.21%). The ash content was varied from 3.32 to 5.73%. Among all the studied genotypes, G Cot 25 (5.73 %) showed the highest ash content,

whereas total carbohydrate content was varied in the range of 34.76 to 43.67%.Higher amount of total carbohydrate was present in DLSA 24 (43.67%) genotype while lower was recorded in 9726 (34.76%) genotype. In all the genotypes,oil content was in the range of 14.10 to 21.79%. Genotype G Cot 25 (21.79%) had the highest value for oil content.Cottonseed mealis a good source of protein and characterized by presence of total protein content and it was in the range of30.36 to 42.38%. The total protein content was found high in Gv Hv 715(42.38%) and low in G Cot 13(30.36%).The limiting amino acids lysine, methionine and tryptophan were quantified in different cotton genotypes and they were in the range of1.39% to 2.44%; 0.53% to 1.38% and0.35% to 0.78%,respectively. The highest lysine content was found in genotype Bagasara ghed selection and methionine and tryptophan were maximum in genotype G Cot 13.

Gossypol quantification through UPLC

Free gossypol was quantified from different genotypes of cotton and it was found in the range of2.34 to 6.90%.The highest gossypol content was found in A.D.C 1 (6.90%) genotype whereas in DLSA 24 (2.34%) genotype showed the lowest gossypol content.

Electrophoretic study of total protein through SDS –PAGE.

Seed protein of cotton genotypes have been analyzed through electrophoresis. Variation for the protein banding pattern and band intensity were observed in all the genotypes.Electrophoresis studies produced total19 bands. Most of the genotypes could be identified on the basis of specific banding pattern and the presence or absence of bands.Variations in the intensity of bands were also observed within each genotypes.

Jaccard's similarity coefficient was calculated for all possible pairs of 15 genotypes of cotton.The highest similarity index value 0.93 was found between G Cot 25 and G Cot 23, while the least similarity index value 0.28 was found between V 797 and 9726.

When comparing different protein fractions on PAGE, it was observed that albumin and globulin showed major difference inbanding pattern among cotton genotypes, while in glutelin fraction genotype 9726 and G Cot 25 showed two specific bands of molecular weight 28.17 kD and 23.94 kD with Rm value 0.78 and 0.84 respectively. Which differentiate it with all other genotypes.

Thus, it can be concluded that protein electrophoresis and its fractions are useful to differentiate cotton genotypes. Variation in banding pattern and band intensity could be used for varietal identification.

2DE protein profiling of cottonseed

Protein profiling through 2-D gel electrophoresis showed total 1773 protein spots from fifteen cotton genotypes, from which 726 spots were matched among the genotypes while 1047 spots were unmatched. Genotype G Cot 23 has the highest number of matched spots (84 spots). Each genotype was found many differential protein spots which was quantified by their molecular weight and isoelectric pH through PDquest 8.0.1 software. In the present investigation attempts weremade to separate proteins on the basis of their molecular weight and isoelectric pH from each cotton genotype.

Cottonseed oil

Qualitative parameters

Measurement of seed oil quality of cotton has been studied,properties of the oil suchas acid value, iodine value, saponification value and peroxide value which gives the structural, stability and quality information about the oils were compared among the cotton genotypes.

Saponification value was found within the range of (178.56-195.43),which indicated the presence of highe rmolecular weight fatty acids in oil and it was not good for human health.The iodine value was found in the range of(82.81 –98.07), which was almost similar to those for olive oil and sunflower oil. Iodine value

increases as the degree of unstauration increases. The results indicated that the oil possesses high degree of unstauration, therefore the oil possesses high proportion of unsaturated fatty acid.Acid values of cotton seed oil was found to varies from 7.30 to9.56 which indicated the presenceof high proportion of free acid. If the concentration of free fatty acid in a fat or oil is very high, then it is considered that the oil is hazardous for human health (Roy et. al.,2007). Peroxide values of cotton seed oil was found withinthe range of19.69 to25.69. Itshowed that the oil had morefree active oxygen enabling its autoxidation, which indicated that oil wasvery sensitive to rancidity.

The oil of genotype G Cot 25was found higher in iodine value (97.54) and lower in saponification value (178.83) which indicated that oil contains high amount of unsaturated fatty acids. Whereas oil of genotype Kutch selection was found higher in saponification value(195.43), acid value(9.56)and peroxide value(25.69), which indicated that oil contains high molecular weight fatty acids, high proportion of free acid and reactive oxygen which lowers the oil quality.

Fatty acid profiling through GLC

GLC analysis showed that linoleic acid (47-57%) was the major fatty acid found in the cottonseed oil,other unsaturated fatty acids were oleic acid (18-

22%) and linolenic acid (0.06-2.6%).The saturated fatty acids present in the oil sample mainly palmitic acid, stearic acid and arachidic acid were found in the range of19to25%, 3to4%and0.079to3.45%,respectively.

The highest(linoleic acid + oleic acid) contentwas found in genotype G Cot 25 followed by G Cot 23 and G Cot 13,respectively whereas high saturated fatty acid content was present in genotype V 797, G Cot 23 and G Cot 21.

Cotton seedlings

Electrophoretic study of isozyme

The peroxidase isozyme showed 6-7 isoforms with Rm values ranged from 0.24 to 0.86. The Jaccarrd's similarity index (SI) of peroxidase isozyme ranged between 0.57 to 1.00. Themaximum similarity value 0.86 was observed between genotypes G Cot 13 & G Cot 23 and G Cot 25 & G Cot 13 whereas, minimum value 0.57was observed between G Cot 21& V 797 and G Cot 21 & A.D.C 1. While polyphenol oxidase isozyme showed only 6 isoforms with very less variation between the genotypes.Therefore,peroxidase isozyme was found useful for varietal identification of cotton genotypes.

Enzyme activity

Enzyme activity (POX and PPO) was recorded in all the studied genotype of cotton.Significantly higher peroxidase activity was observed in Kutch selection (2.30 µmol/min/mg)which was at par with genotypes Bagasara ghed selection (2.28 µmol/min/mg) and G Cot 23 (2.24 µmol/min/mg as compared tothe rest of the genotypes.Significantly higher PPO activity was found in G Cot 23 (2.66 µmol/min/mg) which was at par with genotypes Bagasara ghed selection (2.64 µmol/min/mg),Jaydhar (2.57 µmol/min/mg)and A.D.C 1 (2.21µmol/min/mg)(Released by AAU, Anand). Results suggest that decrease in activities of peroxide scavenging enzymes may be due to the cotton seed deterioration during accelerated ageing.

(+)-δ-cadinene synthase gene specific SSR

In the present study, preliminary attempt was made to correlate the gossypol content with genic sequences of CAD synthase gene. The primers designed from Cad1C subfamily of this gene were able to generate valuable information. It was observed that large fragments were amplified in low gossypol content cultivar DLSA24. The mechanism of this enzyme involve three different processes viz., isomerisation, cyclization and deprotonation to convert FDP to (+)-δ-Cadinene. Hence forth the results in the present study

indicated that insertion of nucleotides into exonic regions of CAD synthase gene may impart any of these reactions. This may be a possible reason which can be used to explain the low gossypol content in this cultivar.This can be

Further conformed through gene sequencing study.

From the above findings, it can be concluded that:

1.Genotype Gv Hv 715 had maximum protein content therefore seed meal of this genotype could be a good source of protein for animal feed.

2.The oil composition of genotype GC ot 25 was found superior in nutritional quality due to high MUFA & PUFA content,low saponification value and high iodine value.

3.Genotype A.D.C 1 was found good sourceof gossypol. This couldbe utilized for pharmaceutical purpose.

4.Protein electrophoresis and its fractions albumin and globulin banding pattern and band intensity could be used for varietal identification.

5.(+)-δ-cadinene synthase gene specific SSR showed larger band size in low gossypol content genotype DLSA 24 and smaller band size in high gossypol content genotype A.D.C 1 whichmay indicate that the insertion of

nucleotides take placein exon region of the gene responsible for (+)-δ-cadinene synthase enzyme.

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