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ISOZYME MARKERS IN COTTON BREEDING 1. STANDARDIZATION OF DIFFERENT ISOZYME SYSTEMS FOR IDENTIFICATION OF DIFFERENT CULTIVARS OF COTTON (*GOSSYPIUM HIRSUTUM*)

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Abstract

Isozyme peroxidase (Pox), shikimic dehydrogenase (Sdh), superoxide-dismutase (Sod), esterase (Est), phosphoglucoisomerase (Pgi) and catalase (Cat) were standardised for different varieties of cotton (*Gossypium hirsutum*) and one variety of *G. arboreum* using stacking and resolving polyacrlyamide gel electrophoresis (PAGE). Isozymes were extracted in different buffers using fresh leaves collected from different cotton cultivars growing in the field. Isozyme shikimate dehydrogenase and esterase have been studied in different varieties of *G. hirsutum* in addition to the remaining isozymes which have been reported only for *G. arboreum* and *G. herbaceum*. The objectives of the study were to standardize different isozyme systems for *G. hirsutum* varieties using material collected from field, identification of an isozyme system through which inter and intravarietal differences and genetic changes occurring in the plant growing under stress condition can be detected, and detection of a polymorphic locus which can be correlated with the stress prevailing in the area (s) of cotton cultivation. Electrophoretic profiles of isozymes showed generally monomorphic patterns for isozymes esterase and superoxide dismutase and thus detected minor inter and/or intravarietal differences. The remaining isozyme systems appeared polymorphic and differentiated different plants of a variety and different varieties from each other. Most of the isozymes appeared to be controlled by one or two loci except peroxidase for which 7 different loci were identified. Possibilities of using the observed isozyme polymorphism in persuing the prescribed objectives are discussed.

Introduction

Isozymes are multiple molecular form of an enzyme with similar or identical catalytic activities occurring within the same organism (Market & Moler, 1959). They are coded by different genes and each gene can have different allele at a single locus. Because isozymes are proteins, they can directly reflect alterations occurring in the DNA sequence through changes in the amino acid composition. Consequently, either the charge or the conformation of an enzyme would be altered. Such alterations can produce a change in the electrophoretic mobility of some of the isoform which may provide useful and reliable method of evaluating genetic differences among different cultivars (Jaaska, 1983).

Although isozyme profiles are independent of pleiotropic and epistatic interaction (Brown & Weir, 1983), in some cases however, significant quantitative and heritable variation can occur due to some of the environmental factors/stresses (Cullis, 1981). Such environmental induced variation can help understanding the behaviour of the particular plant species/variety towards that stress. Cotton leaf curl virus is also one of

the environmental stresses (Anon., 1995) which may affect the growth of a healthy (resistant) and diseased (susceptible) plants differently. Since isozymes exists in multiple forms (Weeden & Wandel, 1989) due to differences in their expression in a plant (Kay & Basil, 1987), it is possible that some of the isozymes in cotton plants may exhibit variation in some of the isoforms when they are exposed to the cotton leaf curl virus. The present paper documented the efforts that have been made to study such variation in the profiles of different isozyme systems in resistant and susceptible plants of a *G. hirsutum* cultivar CIM-1100 and in some other tolerant and susceptible varieties of cotton. The objectives have been i) to standardize different isozymes for *G. hirsutum*, ii) identification of a suitable isozyme that can detect inter and intravarietal differences and genetic changes occurring in plant growing under any of the prevailing stresses and iii) detection of a polymorphic locus or allozyme variation which can be correlated with cotton leaf curl virus disease.

Materials and Methods

Material used in this study comprised of cotton cultivars NIAB-78, NIAB-78 injected with exogenous DNA extracted from *G. arboreum*, CIM-1100 (both diseased and disease free plants collected from one cotton field at NIAB), Karishma, S-12, N-26 and *G. arboreum* (Detail of the material is given in Table 1).

Leaf samples were collected from the cotton field at NIAB in the month of August 1996 at the time when leaf curl virus disease was at peak. Since the isozymes are specific for tissue and stage of growth (Jones, 1984) and the observed spectrum of isozyme may vary in different plant tissues (Wendle & Weeden, 1989) therefore, special efforts were made to collect the material from the plants of similar age and stage of the growth and from the same field except for NIAB-78 (injected) that was collected from a different field located also at NIAB campus. The enzymes viz., peroxidase (Pox), shikimic dehydrogenase (Sdh), superoxide-dismutase (Sod), esterase (Est.), phosphoglucoisomerase (Pgi) and catalase (Cat) were extracted and electrophorased using the methods described by Davis (1964) and modified by Farooq *et al.*, (1996., 1997). The buffer systems and composition of gels used for electrophoresis were different for different enzymes (Table 2). After electrophoresis, the gels were stained according to the methods of Vallejose (1983). The banding pattern appearing on the gels were photographed using Eagle Eye still video system. The zymograms were prepared according to the number and intensity of the individual band either immediately upon staining (for isozymes of peroxidases) or after at-least half an hour in case of the other systems. Nomenclature of the isozymes and abbreviations were based on the standards set by the International Union of Biochemistry (Webb, 1984). The band migrated most anodal to the origin was designated as 1 and so on, while the allozyme and the specific locus was designated by numerical numbers superscripting the enzyme and the locus number (e.g., Pgi-1^4 means allele no. 4 at Pgi locus-1 and Pgi-2^3 means allele no. 3 at Pgi locus 2).

Table 1. Description of cotton material used in the study.

No.	Name of variety	Description of variety	Origin and reference
1.	NIAB-78	A short stature, high yielding and early maturing variety having good tolerance agaisnt CLCuV	NIAB, Faisalabad Anon., 1987).
2.	NIAB-78 produced by macro-injection of DNA extracted from *G. arboreum*	Variety possesses same characteristic as of parental NIAB-78. Macro-injection of DNA was given to possibly incorporate from *G. arboreum* the immunity that this variety possesses for CLCuV. The reason to use this material in this study was to see whether any of the isozyme systems could detect the changes (if any) produced after DNA macro-injection.	NIAB Faisalabad (Anon., 1997a)
3.	*G. arboreum*	A cultivated diploid (2n=26) perennial shrub also known as Indian cotton tree, it possesses immunity against CLCuV	India, (Hutchinson, 1950)
4.	CIM-1100 (healthy)	A high yielding variety with good fiber characteristics and ginning out turn (G.O.T.). It was developed as a variety resistant to CLCuV disease	CCRI, Multan Anon., 1996)
5.	CIM-1100 (infected with CLCuV)	Material possesses same character-istic but showed heavy symptoms of disease under the field conditions of NIAB	- do -
6.	Karishma	A high yielding, heat tolerant nectariless variety of cotton which also possesses tolerance for CLCuV disease	NIAB Faisalabad, (Anon., 1997b)
7.	S-12	A very high yielding variety with outstanding Ginning Out Turn now extremely susceptible to CLCuV disease	CRI, Multan (Anon., 1988)
8.	NIAB-26	A high yielding, early maturing, hairy and nectariless variety developed for insect resistance especially the bollworm. It is now largely susceptible to CLCuV	NIAB, Faisalabad (Anon., 1992).

Results and Discussion

1. **Standardization** of isozyme systems: Isozyme shikimic dehydrogenese (Sdh.) exhibited single band on the gel for each cotton variety except for diseased samples of CIM-1100, *G. arboreum* and Karishma which is a typical pattern of a monomeric enzyme. It is coded by one gene at locus Sdh-1 (Fig.1). Sdh-1 has three types of alleles (Sdh-1^1, Sdh-1^2 and Sdh-1^3) corresponding to bands differing in migration rate and staining intensity. Sdh-1^1 and Sdh-1^3 appeared to be the products of two alleles. Sdh-$1^{1(4)}$ expressed in Karishma only while Sdh-$1^{3(5)}$ expressed in *G. arboreum* and diseased samples of CIM-1100. Sdh-1^2 appeared in two collection of NIAB-78 while Sdh-1^4 appeared in CIM-1100 (healthy samples), S-12 and N-26.

Shikimic dehydrogenase has been studied in many plants including *Petunia* (Wijsman, 1983) and *Apium graveolens* (Orton, 1983) and is reported to be a monomeric enzyme (Weeden & Gottlieb, 1980). In cotton, shikimic dehydrogenase is being studied for the first time and in this study also it exhibited a typical monomeric pattern as only one band appeared in most of the varieties. The presence of two bands in infected CIM-1100, *G. arboreum* and Karishma indicated that these varieties may have in their pedigree the parents that are homozygous for different alleles at two loci.

Isozyme phosophoglucoisomerase (Pgi.) appeared to be coded by two loci: Pgi-1 and Pgi-2. Pgi-1 exhibited 6 and Pgi-2, 3 alleles in different varieties. These alleles were differentiated on the basis of intense isozymic activity and differences in their mobility. The banding pattern for most of the varieties was different and helped in differentiating all of them from each other either at Pgi-1 or at Pgi-2 locus (Fig. 2). The isozyme appeared as dimeric at Pgi-2 locus while it showed monomeric form at Pgi-1 locus.

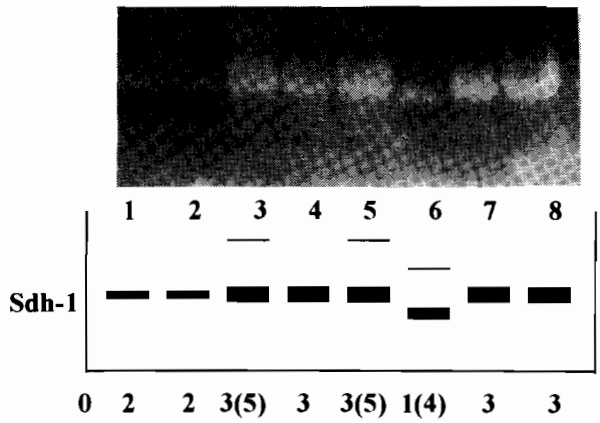

Fig. 1. Profile and zymograme of isozymes of the enzyme Shikimik dehydrogenase (Sdh) showing 3 types of alleles exhibited a single locus in cotton leaf curl virus resistant varieties *G. arboreum* and CIM-1100: healthy plants (3 & 4), tolerant varieties NIAB-78 and Karishma (1, 2, & 6) and susceptible varieties CIM-1100: infected plants, S-12 and N-26.

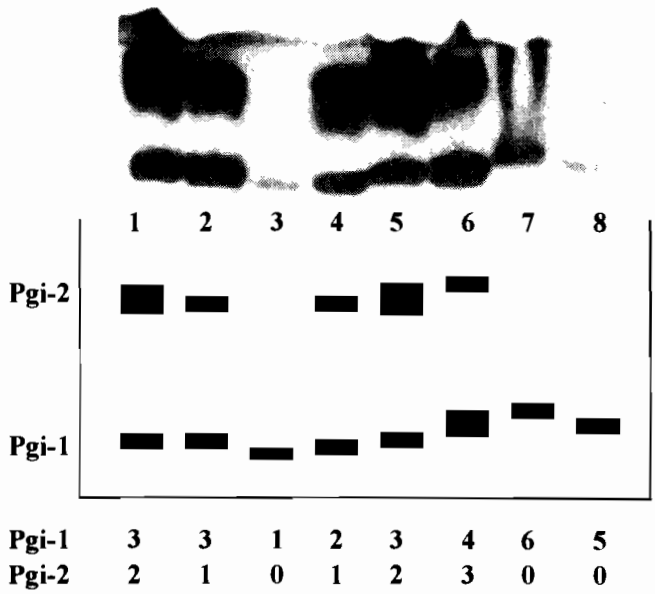

Fig 2. Profile and zymograme of isozymes of the enzyme Phospho-gluco-isomerase (Pgi) showing 6 types of alleles at locus Pgi-1 and 3 types of alleles at Pgi-2 differentiating resistant varieties *G. arboreum* and CIM-1100: healthy plants (3 & 4), tolerant varieties NIAB-78: both samples and Karishma (1, 2, & 6) and susceptible varieties CIM-1100: infected plants, S-12 and N-26.

Phosophoglucoisomerase is a known dimeric enzyme (Gottlieb, 1977) however, in different plant species it has been reported to have possessed different activities like in petunia (Wijsman, 1983), it is reported as monomeric, tomato (Tanksley, 1980) as dimeric, soybean (Gorman *et al.*, 1982) as dimeric, *Brassica oleracea* (Arus *et al.*, 1982) as mixture of monomeric and dimeric, in *Apium graveolens* (Orton, 1983) and cotton (Suiter, 1988) as dimeric. In the present study also, the isozyme at one locus appeared dimeric as evidenced by Pgi-2^2. These bands appeared to be composed of similar subunits and hence exhibited little variation while the pattern of isozyme at Pgi-1 locus appeared as typical of a monomeric enzyme. The enzyme therefore, appeared to possess a mixture of monomeric and dimeric activity. The absence of activity at Pgi-2 locus in *G. arboreum*, S-12 and N-26 indicated that their parents were homozygous for alleles that specify different allozyme.

Isozyme superoxide dismutase (Sod.) appeared to be coded by two genes at Sod-1 and Sod-2 loci. At both the loci, banding pattern was monomorphic with only one allele observed in all the test material except a slight difference in the mobility of Sod-2^1 in one of the tested lines which could not be clearly differentiated from the others. The banding pattern was like of a monomeric enzyme (Fig.3a).

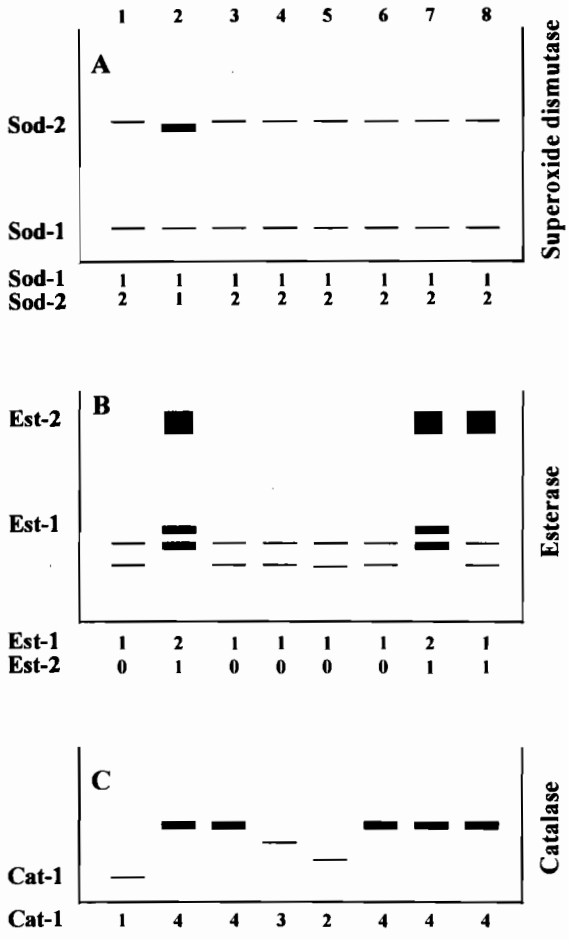

Fig.3. Zymograme of isozymes of the enzyme Superoxide dismutase (A), Esterase (B) and Catalase (C) showing different loci and alleles differentiating resistant (3 & 4), tolerant (1, 2, & 6) and susceptible (5, 7 & 8) varieties from each other.

Superoxide dismutase is known as a dimeric or a tetrameric isozyme (Baum & Scandalios, 1981). It has been studied in different varieties of cotton: *G. arboreum* and *G. herbaceum* (Suiter, 1988) and in many other plant systems including rye, wild grasses of the tribe *triticeae* (Jaaska & Jaaska, 1982) and in maize (Baum & Scandalios, 1981). In all these studies, it has been reported to be either a dimeric or a tetrameric or both. In rye, three heterozyme of different molecular structure and biochemical properties have been reported of which two were dimeric and the one as tetrameric (Jassaka & Jassaka, 1982). However, the loci detected in the present study clearly exhibited monomeric pattern. Situations like this have been observed in maize by different groups (Brown & Allard, 1969., Newton *et al.*, 1982 and Stuber & Goodman,

1983) and from 10 different loci of isozyme esterase of which some were assigned monomeric and others as dimeric structure. Isozyme Pgi in *Brassica oleracea* (Arus & Shield, 1983) has also been reported to be a mixture of monomer and dimer. In the present study monomeric activity of isozyme superoxide dismutase is being reported for the first time in *G. hirsutum* varieties. It is quite possible that *G. hirsutum* may have dimeric enzyme forms like that in *G. arboreum* and in *G. herbaceum* (Suiter, 1988) but were not detected in the present study.

Isozyme esterase exhibited two zones of activity. Est.-1 characterized by a fast moving high activity zone consisting of two sharp bands expressed in all the test material. These bands appeared to be the product of two alleles at Est.1 locus and are homozygous in all the varieties with a slight difference in mobility observed in NIAB-78 (injected samples) and in S-12. The Est.-2 locus comprised 2-3 barely separated and slightly slow moving bands present only in three of the eight cotton varieties (Fig.3b). Est.-1 showed a monomeric and monomorphic pattern indicating that all the varieties have in their pedigree, parents that are homozygous for different alleles at two different loci.

Esterases are involved in a host of ester hydrolases in a number of plant species and has been reported to possess either monomeric (Tanksley & Rick, 1980) or a dimeric activity (Wehling & Schmidt-Stohn, 1984). It has been studied in maize (Goodman & Stuber, 1982), wheat (Hart, 1983), barley, (Hvid & Nielsen, 1977), oats (Miller, 1977), tomato (Rick, 1983), potato (Mok, 1981), capsicum, (Mcleod *et al.*, 1979) and rice (Endo & Morishma, 1983). In all these studies, the structure of the enzyme is reported to be either a mixture of monomer and dimer (wheat, maize, tomato), dimer or tetramer (potato) or monomer only (capsicum). The esterase loci in different cotton varieties in the present study are being reported for the first time. The activity at Est.-2 locus with monomorphic profile appeared to be dimeric, as dimeric enzyme composed of similar subunits usually exhibit monomorphic profiles (Weeden & Marx, 1987) while, the pattern at Est.-1 locus appeared as monomeric. Hence like several others, esterase isozyme in cotton also exhibited a mixture of monomeric and dimeric activity.

Isozyme catalase also appeared as a monomeric enzyme coded by a single gene at locus Cat-1. Cat-1 exhibited 4 alleles as differentiated by differences in their mobility. Predominant allele among them was Cat-1^4 and was expressed in 5 of the 8 cotton varieties. Cat-1^1 expressed in NIAB-78 while Cat-1^2 and Cat-1^3 expressed in healthy and infected types of CIM-1100 (Fig.3c).

Catalase is known as tetrameric enzyme (Scandalios, 1974) and has been extensively studied in maize with 3 reported loci. These loci are associated with cytosol and mitochondria and all are tetrameric in structure (Goodman & Stuber. 1982). The catalase in *G. arboreum* and *G. herbaceum* on the other hand is reported to be controlled by one locus and like the present study of *G. hirsutum*, it is also reported to be of monomeric structure (Suiter, 1988). The presence of two different alleles in infected and healthy samples of CIM-1100 may indicate that it involved switching on of a catalase gene coding for a different enzyme form under the influence of virus infection.

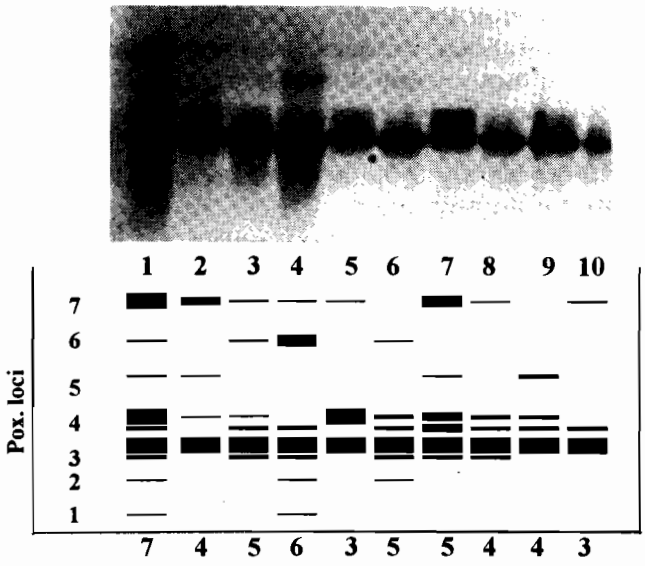

Fig. 4. Profile and zymogram of isozyme of the enzyme Peroxidase showing 7 loci in cotton varieties FH-682 (lanes 1-5) and BH-36 (lanes 6-10).

Isozyme peroxidase (Pox) exhibited most complicated zymogramme as it appeared to be controlled by 7 loci in *G. hirsutum*. Most of the loci are represented by one allele except for locus 3 and 4 exhibiting two alleles per locus. All the alleles have only one isoform and all of them do not express simultaneously in one cotton cultivar (Fig.4). Polymorphism was observed in locus-6 which in most of the varieties, appeared to have very strong activity (Fig.5) except in CIM-1100 (diseased samples), S-12 and N-26 that showed complete absence or light to very light activity, respectively, at this locus.

Peroxidases are known to exist both in monomeric or dimeric forms (Brewbaker *et al.*, 1985) and are often encoded by many different loci with the evidence of post translational modification (Jaaska, 1983). It has been studied in capsicum (Mcleod *et al.*, 1979), tobacco (Sheen, 1970), soybean (Brim *et al*, 1969; Buttery & Buzzell, 1968), maize (Brewbaker & Hasegawa, 1975), *Secale* (Jaaska, 1975), rice (Ida *et al.*, 1972), tomato (Tanksley, 1983a) and many other plants (Tanksley & Orton, 1983). In most of these studies, varied number of isozyme groups have been reported with number of isozymes ranged between 1 and 10 along with qualitative and quantitative differences. The high degree of polymorphism in this isozyme was believed to be due to heterogeneity in its primary structure (Mader *et al.*, 1980). In earlier studies on

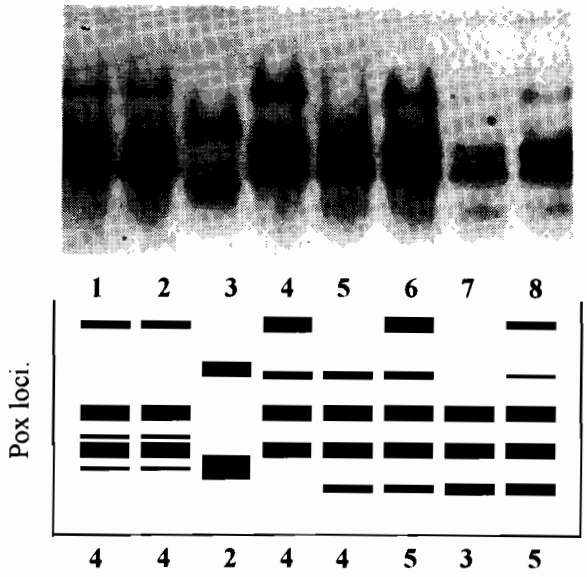

Fig.5. Profile and zymograme of isozymes of the enzyme Peroxidase differentiating resistant varieties and plants (3 & 4), tolerant varieties (1, 2 & 6) and susceptible varieties (5, 7 & 8).

peroxidase in *G. arboreum* and *G. herbaceum* (Suiter, 1988), this isozyme was reported as non-polymorphic and the enzymic structure as monomeric with only two coding loci. In *G. hirsutum*, it appeared to be controlled by 7 loci with qualitative polymorphism. The monomorphic patterns for Pox. of diffferent cotton varieties indicated that the parents involved in production of these varieties were homozygous for different Pox. alleles at different loci

Isozymes in plants have generally been studied either on seeds or seedlings raised under control conditions. The objectives in most of these studies were varietal identification (McLeod *et al.*, 1979), measuring genetic variability in plant population (Brown & Weir, 1983), identification of natural and parasexual hybrid (Lo Sehiavo *et al.*, 1980), characterization of alien genetic material (Hart & Tuleen, 1983), identification of alien material introgressed from wild species to cultivated varieties (Tanksley, 1983b), genetic purity of commercial seeds (Arus *et al.*, 1982) and in plant disease research (Burdon *et al.*, 1980). The present study was also made to pursue some of the similar objectives using leaf material collected from the field growing plants. The subunit structure of the isozymes was not specifically studied rather it was inferred through looking at the zymogramme phenotype. The idea was to detect any possible deviation from the earlier reported results. The observed results were however, generally similar to those reported earlier and thus helped us inferring that selection of material for isozyme extraction and the methodology used for characterization is reliable for studies of isozyme in the field plant populations.

Table 2. The buffer systems and acrylamide concentrations used for different isozyme systems.

Enzyme system	Gel buffer	Electrode buffer	Upper reservior buffer	Acrylamide concentration
Pox. Sdh. Sod.	Tris.Cl, pH 8.9 (resolving) and 6.7 (stacking).	Tris-glycine pH. 8.3	Tris-glycine pH. 8.3	8%
Est.	-	-	-	-
Pgi.	Tris-citric acid pH. 8.46	Boric acid- pH.7.8	Boric acid pH. 7.8	8%
Cat.	Tris-citric acid pH. 8.3	Boric acid- LiOH pH. 8.3	Boric acid pH 7.8	8% with 4% Starch

The isozyme extraction and analysis was according to the methods of Davis (1964) with slight modifications as mentioned in the table. The staining procedure was according to Vallejose (1983).

Varietal discrimination: Isozyme superoxide dismutase and esterase generally appeared monomorphic except a slight difference in mobility of Sod-2^1 which helped differentiating NIAB-78 (injected) from the remaining cotton cultivars.

Among the other polymorphic isozymes, shikimic dehydrogenase discriminated healthy CIM-1100 from deseased plant samples by the presence of Sdh-$1^{3(5)}$ in the later. It also discriminated Karishma from all the remaining varieties by the presence of Sdh-$1^{1(4)}$: an allele which is not present in any other variety. Catalase also discriminated healthy and diseased samples of CIM-1100 from each other and from rest of the varieties by the presence of Cat-$1^{2\,\&\,3}$. The two different types of NIAB-78 were also discriminated with the help of Cat-$1^{1\,\&\,4}$. Phosophog lucoisomerase discriminated two types of NIAB-78 by the presence of Pgi-2^2 in NIAB-78 (No-1). S-12, N-26 and *G. arborium* were discriminated from all the remaining varieties by the absence of activity at Pgi-2 locus and the presence of Pgi-1^6 in S-12 while Krishma was discriminated by the presence of Pgi-2^3 and Pgi-1^4. Both the samples of CIM-1100 were discriminated from each other by the presence of Pgi-2^1 in the healthy plants. Of the 11 alleles detected by the polymorphic isozymes, 3 are only for discriminating healthy and disease samples of CIM-1100 (Table 3).

Allelic variation or allozymic polymorphism at isozymic loci are of significant importance in plant breeding as this is a major mechanism through which plants can adapt to environmental changes (Weeden, 1983). Since the two allozyme possessing the same electrophoretic mobility may not necessarily be identical in structure or in many of their physiological properties (Bernstein *et al.*, 1973; Gottlieb & Greve, 1981) therefore, variation in allozyme number can play important role in studies of genetic diversity and in detection of inter and intraspecific differences in different crop

Table 3. Summary of the isozyme analyses made in the present study on different varieties of cotton (*G. hirsutum*).

Enzyme and code:	No. of loci per enzyme	No. of alleles per isozyme locus	Types of alleles per enzyme	Designation and allelic status of different varieties	Diagnostic allele (s)
Sdh. 1.11.2.5	1	3	3	Sdh-1^1 (6)** Sdh-1^2 (1.2) Sdh-1^3 (3,4,5,7,8)	Sdh-$1^{1.4}$ (6)**
Sod. 1.15.1.1	2	1 in Sod-1 2 in Sod-2	3	Sod-1^1 (All varieties) Sod-2^1 (2) Sod-2^2 (1,3,4,5,6,7,8)	Sod-2^1 (2)
Cat. 1.11.1.6	1	4	4	Cat-1^1 (1) Cat-1^2 (5) Cat-1^3 (4) Cat-1^4 (2,3,6,7,8)	Cat-1^1 (1) Cat-1^2 (5) Cat-1^3 (4)
Est. 3.1.1.2	2	2 in Est-1 1 in Est-2	3	Est-1^1 (1,3,4,5,6) Est-1^2 (2,7) Est-2^1 (2,7,8)	none
Pgi 5.3.1.9	2	6 in Pgi-1 3 in Pgi-2	9	Pgi-1^1 (3) Pgi-1^2 (4) Pgi-1^3 (1,2,5) Pgi=1^4 (6) Pgi-1^5 (8) Pgi-1^6 (7) Pgi-2^1 (2,4) Pgi-2^2 (1,5) Pgi-2^3 (6)	Pgi-1^1 (3), Pgi^2 (4). Pgi-1^4 (6), Pgi-1^5 (8) Pgi-1^6 (7) Pgi-2^3 (6)
Pox. 1.11.1.7	7	1: ach at loci 1,2,5,6 and 7. 2: each at loci 3 and 4	9	Pox-1 All varieties Pox-2 exhibited Pox-3a similar Pox-3b profiles for Pox-4a Pox isozyme Pox-4b while expression Pox-5 of alleles differs Pox-6 for different loci Pox-7 and in different varieties.	Diagnosis may be based on expression of different alleles

* and ** Number corresponds to the numbers given to different varieties as mentioned in Table.1.

cultivars. Such studies have been made in rice (Farooq *et al.*, 1996; Farooq, 1995; Glaszmann, 1987), maize (Brewbaker *et al.*, 1985) and sunflower (Kahler & Lay, 1985) and the observed allozymic variation were reported to be the consequences of post translational modification (Motojima & Sakaguchi, 1982; Beevers, 1982), mutation and selection in a particular locus or due to interference of the loci other than the one for which the enzyme is being processed (Kimura, 1983). In the present study, the observed allellic variations that have been used for varietal identification might have originated due to heterogeneity in parental lines used in the development of these varieties. The Cat. and other alleles discriminating healthy and diseased plants of CIM-1100 can play significant role in breeding for CLCuV disease. Such alleles can also be identified for other commercially important varieties by studying their healthy and susceptible samples and could possibly be used as a marker for identification of resistant plants of a particular variety in the segregating population without planting them under the disease screening nurseries. However, the Cat. alleles mentioned above may not be used for tagging disease resistance gene (s) in all the cotton varieties because of their absence in other varieties. Nevertheless, the study did indicate the potential of isozymes to be used for such purposes. Incidentally, the polymorphic enzymes in addition to other varieties, exhibited allelic variation in the cotton varieties CIM-1100, Karishma and S-12 which are claimed to be resistant, tolerant and susceptible respectively to cotton leaf curl virus disease (Anon., 1995). Since Karishma and another high yielding variety NIAB-78 (also possessed considerable tolerance for CLCuV) exhibited altogether different isozyme profiles with most of the polymorphic isozymes therefore, the allelic variation observed in these varieties may be considered as varietal and not due to the reaction of the specific variety towards CLCuV disease. The diagnostic alleles detected in different varieties can possibly be used for tagging the character(s) specific to these varieties e.g. heat tolerance in Karishma, high yield and disease susceptibility in S-12 and tolerance in CIM-1100.

The most interesting results were observed with the isozyme peroxidase which exhibited qualitative and quantitative differences. The former could be a consequence of difference in the function of a specific isozyme depending upon their response to external stimuli (Brewbaker *et al.*, 1985; Tanksley & Rick, 1980). For example heat induced expression of an isoform of esterase in self incompatible species of South American tobacco (Pandey, 1973) while quantitative differences could be due to environmental factor(s) such as disease infestation of plant (Stahmann & Demorest, 1973), nutrient imbalances (Culis, 1981), non-uniform irrigation and other prevalent biotic and abiotic stresses (Rahmat, 1995; Hashmi *et al.*, 1993) which may cause metabolic changes (Ahmad, 1995) and thus the variation in the enzymic profiles. Such quantitative variation in peroxidase have been reported in soybean (Brim *et al.*, 1969) and are controlled genetically (Buttery & Buzzel, 1968). Since in the present study, quantitative differences in peroxidase activity have been observed in few varieties growing only at one location having generally uniform environment therefore, it is not possible to certify the reason (s) of quantitative variation in Pox. activity unless large number of cotton varieties growing under different environmental conditions (with and/or without the prevalence of CLCuV) disease be studied. Such studies may reveal significant information on the role of peroxidase activity in leaf curl virus disease.

Acknowledgment

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