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Biochemical Evaluation of Cotton Genotypes using Soluble Protein, Esterase (EST), Peroxidase (POX) And Polyphenol Oxidase (PPO) and their Role in Plant Disease Resistance

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ABSTRACT

Isozyme analysis is a powerful biochemical technique that has numerous applications in plant biology. It has long been used by geneticists to study the population genetics. The isozyme esterase, peroxidase and polyphenol oxidase were standardized for upland cotton (*Gossypium hirsutum* L.) germplasm lines collected from all over the country. The knowledge of nature and magnitude of genetic diversity present in the germplasm is most important pre-requisite for the success of any breeding program. The thirty-four cotton germplasm lines were screened for prime three isozymes based on quantification assay and qualitative PAGE profiling. Among the material, the genotype AKH – 24 (190.60 mg ml^{-1}), AKH – 053 (189.42 mg ml^{-1}) and VIKAS (184.53 mg ml^{-1}) recorded high protein content, whereas the enzymatic activities of esterase, peroxidase and polyphenol oxidase exhibited remarkable differences along with the protein content. The genotype LRA–5166 exhibited high esterase (462.68 µM mg $protein^{-1}$ min^{-1}) and peroxidase activity (250.97µM mg $protein^{-1}$ min^{-1}), while AKH – 24 recorded the maximum polyphenol oxidase activity (131.45 µM mg $protein^{-1}min^{-1}$). The banding pattern of biochemical markers revealed that the maximum number of bands were recorded in esterase analysis (fifteen) followed by protein (twelve) whereas, only five bands each were detected in peroxidase and polyphenol oxidase analysis indicating limited polymorphism. The Relative Mobility (Rm) values were ranged from 0.083 to 0.883 (protein), 0.100 to 0.971 (esterase), 0.033 to 0.283 (peroxidase) and 0.048 to 0.206 (polyphenol oxidase). The present study demonstrated that cotton genotypes could be differentiated by their quantity and quality through electrophoretic banding profiles. These results could be of practical value for cultivar identification, purity testing along with associated prediction of pest and disease resistance. However, the major constraint is that these biochemical markers do not able to reproduce the similar kind of variation pattern, but can provide strong distinguishing polymorphism each time.

Keywords- Isozymes, Cotton, Esterase, Disease Resistance, Peroxidase, Polyphenol oxidase, Soluble Seed Protein.

I. INTRODUCTION

Isozymes are molecular forms of an enzyme. Plant scientists prominently interested in cellular differentiation is to discern the biochemical steps that accompany the development of cells, resulting about the diversification and specialization characteristic of multi-cellular organisms. Its, well documented that as cellular differentiation progresses to bring about morphological development and functional specialization there is a continuous synthesis and/or degradation of specific enzymes and structural proteins. A common factor in cellular development and differentiation is the ability of cells to lose and acquire specific biochemical characteristics. Two principal alterations encountered in the life of most organisms are the quantitative and qualitative changes in protein (enzymatic) activity within their cells. The appearance of new or increased enzyme activity in a developing organism may result from either the de novo synthesis of the enzyme molecule or from the

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activation of a pre-existing enzyme precursor (Scandalios, 1974).

These forms usually have similar, if not identical, enzymatic properties but slightly different amino acid sequences. Only those isozymes with amino acid compositions of different net charge, or those that result in large differences in the shape of an enzyme, can be differentiated by electrophoresis. It has been estimated to represent about one- third of all the possible isozymes that may present within a genetic system (Marshal and Brown, 1975). Biochemical markers correlating with such important traits as tolerance to drought and salinity, tolerance to wilt, earliness, evaluation of varietal purity and morphological homogeneity were developed in experiments (Shadmanov et al, 2018) Cultivated cotton has a unique origin and history among cultivated crops. The wild ancestors of modern cotton species were perennial vines that inhabited several distinct geographic areas, including Africa, Arabia, Australia and Mesoamerica. During the past several centuries, people native to these regions developed four distinct species of cultivated cotton, including upland cotton (*Gossypium hirsutum* L.) (Anonymous, 2007)

Cotton is a unique natural fiber producing most common fiber crop of the world, which provides humanity with cloth and vegetable oil, medicinal compounds, meal and hull for livestock feed, energy sources, organic matter to enrich soil, and industrial lubricants. Assessment of genetic markers and diversity form an integral part of any successful breeding program as the knowledge of nature and magnitude of genetic diversity present in the germplasm is most important pre-requisite. Morphological features are indications of the genotype but are represented by only a few loci because there are not a large enough number of characters available. Moreover, they can also be affected by environmental factors and growth practices. Biochemical markers such as isozymes have been used to study the genetic distances and estimate the level of genetic variability of cotton varieties and accessions (Wendel et al., 1989; Percy and Wendel, 1990; Abdel-Tawab et al., 1990 & 1993; Melchinger et al., 1991; Wendel et al., 1992; Sukumar and Allan, 1998; Farooq et al., 1999, Kharade et al.2016, Ujjainkar and Patil, 2020) These advanced biochemical and molecular techniques can now supplement to speed up the conventional breeding programmes along with improvement in precision as these biochemical and molecular markers are adequate to judge the dissimilarity among the genotypes which will help the activities under Marker Assisted Selection (MAS). (Ujjainkar et al., 2020)

The proteins and enzymes are the primary product of the genes and hence are most suitable for determination of genetic purity and polymorphism. (Nijenhuis, 1971). The changes in coding base sequences results in corresponding replacement of amino acid and thus in the primary structure of protein and enzymes (Cherry *et al.,* 1970). In the presence of electric field and while passing through a semi-porous gel medium, these differences cause dissimilar forms of protein and enzyme profiles (Markert and Moller, 1959). Therefore, for characterization and identification of elite, diverse cotton lines it is necessary to examine them by the use of SDS-PAGE method of protein and enzymes at preliminary level. The biochemical markers may complement the efforts to reduce the burden to a greater extent, as these are estimates devoid of environmental factor which a cause of error. Especially in the selection of parents based on biochemical and molecular polymorphism, there is marked improvement in the screens for the quantitative traits and understanding their architecture. Therefore, it is an imperative need to understand the genetics of constantly emerging accessions. Hence, the study on the biochemical characterization-based quantity as well as quality in form of profiling for soluble protein and three isozyme viz., carboxylase esterase, polyphenol oxidase and peroxidase in thirty-four upland cotton genotypes was undertaken.

II. MATERIALS AND METHODOLOGY

The experimental material comprising of thirty-four genetically diverse elite upland cotton germplasm lines were used for present investigation provided by Senior Research Scientist, Cotton Research Unit, Dr. PDKV, Akola (MS) their details are given in the Table 1.

Table 1: Source of experimental material used for present investigation

Zone	State	No of genotypes	Genotypes
Central India	Maharashtra	22	AKH 84365, AKH 8835, NH 545, JLH 1594, AKH 91, AKH 44, AKH 9312, AKH 24, AKH 62, AKH 8801, JK 119, AKH 107, AKH 87, AKH 32, AKH 081, PH 93, AKH 053, AKH 023, AKH 1234, AKH 3456, AKH 8660 and PH 348
	Gujarat	02	GA B and G.COT 10
	Madhya Pradesh	01	KH 118
North India	Punjab	02	LCMS 5 and LCMS 2
	Uttar Pradesh	01	Vikas
	Rajasthan	01	IC 1547

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South India	Tamil Nadu	04	LRA 5166, NS 95, Narsimha and Sahana
	Andhra Pradesh	01	Sumagla
Total		**34**	

Preparation of Plant Sample: The plant samples of cotton were prepared as per the procedure given by Krishina and Jawali (1997).

Quantification of Protein: Soluble protein estimation in seed of different elite cotton lines was done by Lowry's method (Lowry *et al*. 1951). This method based on contrasting colour obtained from the reaction of protein with Folin-Ciocalteau regent. The colour so formed was due to the reaction of the alkaline copper with the protein and the reduction of phosphomolybdate and phosphotungestate by tyrosine and tryptophan present in the protein. The intensity of the colour depends on the amount of these aromatic acids (tyrosine and tryptophan) present and thus varies for different protein.

Quantification of Isozymes: The quantity of isozyme produced is also controlled by the genetic constitution of the plants. Hence the plant with varying genome will produce the different amount of isozymes, which can be utilized for discrimination of the population. (Salisbury and Ross, 1978). Following spectro-photometric assay were used for quantification of major isozymes. Peroxidase catalysis the oxidation of various hydrogen donors like P – cersol, benzidine, guaicol, ascorbic acid nitrite and cytochromes in presence of H_2O_2. The peroxidases are present in all plant species. The enzyme has iron as prothetic group, which can be dissociated readily. The tissue enzyme extract when added to pyragallol in the presence of H_2O_2 as oxidized to a coloured derivative, which can be measured on spectrophotometer. Peroxidase activity was determined as per procedure given by Retig (1974), the appearance of the brownish – pink colouration resulting from guaicol oxidation in the presence of H_2O_2. Polyphenol oxidase (Trosinase) is a bifunctional, copper containing oxidase having both catecholase and cresolase activity. Polyphenol oxidase oxidizes phenol to quinone in the presence of methyl catechol. The polyphenol oxidase activity was determined by procedure given by Giber Vela *et al* (2003).

Electrophoresis and Gel Analysis: Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis method was followed for the separation of total seed proteins for the identification of cultivars as described by Varier and Dadlani (1992). Whereas, Alkaline Polyacrylamide Gel Electrophoresis followed by staining was adopted for identifying isozyme bands in different cotton cultivars (Dadlani and Varier, 1993). After completion of electrophoresis, the gel sandwich put into staining solution for staining as per the specified laboratory protocols as mentioned below.

Staining of gels:

[a] Esterase: The gel was incubated in staining solution of α - napthyl acetate (30 mg in 3 ml of acetone). α - naphtyl acetate was first dissolved in acetone and then the solution of Fast Blue BB salt (100mg in 100 ml of 0.1 N SPB pH 7) was used. The blackish brown esterase bands were developed after 15 minutes (Vallejos, 1983).

[b] Peroxidase isozyme: The isozyme bands of peroxidase were localized by procedure given by Novacky and Hamptom (1968). First incubated the gel in 0.25 % guiacol for 30 minutes followed by incubation in 0.3 % H_2O_2 for 15 minutes which showed the appearance of pinkish brown bands of peroxidase.

[c] Polyphenol oxidase isozyme: Park *et al.,* (1980) have suggested the procedure of staining the polyphenol oxidase bands. The gel was incubated in 0.03 M catechol containing 0.05 % phenylene diamine in citrate phosphate buffer pH 6.0 (0.1 M solution citric acid, 2.10 g in 100 ml + 0.2 M solution of dibasic sodium phosphate, 3.56 g in 100 ml) for one hour.

III. RESULT AND DISCUSSION

Soluble Protein: The protein being direct gene products, reflect the genomic composition of varieties and hence are good indicators for varietal distinctness. Seed storage proteins are found in abundant and have proved an important tool in biochemical analysis. The first report on seed storage protein was appeared in 1979 (Cherry *et al.*,). In cotton seed, two major classes of storage proteins are globulins and albumins, which differ in their solubility properties. Both globulins and albumins are synthesized and compartmentalized in protein storage vacuoles during cotton seed maturation. Globulins can be further classified based on sedimentation rate of their aggregated forms into the 7 S vicilins (or α-globulin) and 11/12 S legumins (or β-globulin) (Shutov et al, 1995 and Liu et al 2012). Both vicilin and legumin families comprise the major (60–70%) components of cotton seed proteins revealed by the proteomic profiles of mature cotton seeds (Hu et al., 2011). There are also some functional proteins in cottonseed. For example, oleosins in cottonseed play dual physiological roles, by acting as protectors for stabilizing the oil bodies in developing and mature seeds and as the recognition signal for lipase binding in germinating seeds (Shutov et al, 1995). Involvement of protein components in plant diseases resistance has been documented in many plant pathogenic interactions (Tornero et al. 2002; Carvalho et al. 2006). In non inoculated plants, total soluble protein content was significantly higher in leaves of susceptible genotype (Zeeshan Siddique et al., 2014). Usually, infected plants show a high protein content, which could be due to both the activation of the host defense mechanisms and the pathogen attack mechanisms (Agrios 1997)

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The total soluble protein was estimated by employing Lowery's Method (1951). The results obtained from quantification assay revealed that total soluble proteins in genotypes studied ranged from 85.37 to 190.60 mg ml^{-1} indicating substantial variability. The maximum soluble protein content was estimated in AKH–24 (190.60 mg ml^{-1}) followed by AKH–053 (189.42 mg ml^{-1}) and VIKAS (184.53 mg ml^{-1}) respectively. While the genotype JLH–1594 exhibited lowest soluble content of 85.37 mg ml^{-1} during the study (Table 2, Plat 1). Total twelve bands were observed in the SDS–PAGE Gel Cassette having the range of Relative mobility (Rm) from 0.083 to 0.883. The genotypes AKH–62, GA–B, AKH–023, IC –1547 and AKH–3436 exhibited all twelve bands in the present investigation. Whereas, the genotypes AKH–8835, JK–119 and AKH–8660 had shown the minimum number of bands i.e. six only. The wide range of relative mobility values indicates the broad spectrum of protein fraction have been successfully utilized and detected by gel electrophoresis. Also, the plates reveals that the soluble protein fraction of cotton crop gives better response for the electrophoretic techniques.

The seed protein expression is usually controlled by homologous multigene families exhibiting monogenic segregation with co – dominance for molecular weight variants and presence of polypeptide bands being completely dominant over case of absence. Deletion or mutation of structural gene coding for the polypeptides or their regularity loci results in inhibition of transcription or translation of polypeptide leading to the lack of expression of concerned polypeptides. This kind of variation, revealed that in the electrophoretic banding pattern could lead to the detection of genotype specific bands. Therefore, the polypeptides varying from their presence could be used as reliable biochemical marker for distinguishing the population. This fact confirms the congruence between the protein and genetic constitution of the individual. Dongre *et al.*, (2001), Prasad *et al.*, (2001), Namrata Idnani (2002), Satija *et al.*, (2002), Kulkarni and Patil (2003).

Table 2: Quantification of Protein and Isozymes in Cotton

Genotypes	Soluble Protein content (Mg/ml)	Isozyme activity (μM mg protein^{-1} min^{-1})			Genotypes	Soluble Protein content (Mg/ml)	Isozyme activity (μM mg protein^{-1} min^{-1})		
		EST	POX	PPO			EST	POX	PPO
AKH.84365	120.2	363.8	131.9	92.1	**LCMS 5**	139.0	269.7	181.3	76.1
AKH 8835	162.1	287.2	154.4	41.9	**LCMS 2**	155.0	242.1	211.3	42.3
KH 118	155.8	326.2	143.7	110.8	**AKH 87**	91.4	209.8	185.5	86.4
NH 545	118.9	394.8	111.5	55.4	**AKH 32**	170.4	198.4	149.1	85.6
JLH 1594	85.4	356.4	222.0	61.2	**AKH 081**	88.7	256.2	204.9	49.5
LRA 5166	102.5	462.7	251.0	110.2	**PH 93**	127.1	316.8	177.0	36.9
AKH 91	98.9	331.5	135.1	113.5	**AKH 053**	189.4	298.7	186.6	81.6
NS 95	102.6	274.4	166.2	73.1	**AKH 023**	100.1	288.5	192.0	64.7
AKH 44	94.1	304.6	193.1	90.7	**IC 1547**	88.4	258.9	167.3	79.7
AKH 9312	97.3	249.5	152.3	41.1	**AKH 1234**	114.7	261.6	205.9	45.0
AKH 24	190.6	299.9	215.6	131.5	**AKH 3456**	164.0	277.7	189.8	66.6
AKH 62	101.8	283.1	200.6	57.1	**SUMAGALA**	137.5	244.1	165.2	71.2
G.COT 10	154.7	326.8	182.3	117.3	**NARSIMHA**	124.0	224.6	158.7	54.3
AKH 8801	92.8	283.8	197.3	69.9	**VIKAS**	184.5	453.9	129.8	62.8
AH 107	131.5	236.1	110.5	60.9	**SAHANA**	125.4	174.2	174.8	50.4
JK 119	87.9	230.0	178.0	43.5	**AKH 8660**	161.5	205.8	145.9	88.2
GA B	164.4	222.6	175.9	59.7	**PH 348**	176.7	306.7	127.6	75.0

Isozyme analysis: Isozymes are differentially charged protein molecules that can be separated by using eletrophoretic procedures. The quantity of isozyme produced is also controlled by the genetic constitution of the plants. Hence the plant with varying genome will produce the different amount of isozymes, which can be utilized for discrimination of the population. (Salisbury and Ross, 1978). The enzyme activity of individual isozymes in a specific tissue is dependent, among other factors, on maturity and cellular environment. Changes in isozyme activity during development of a tissue can be detected most conveniently by pattern shifts on

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zymograms subsequent to electrophoresis (Scandalios, 1974).

[a] Carboxylase Esterase: The esterase activity (μM mg protein^{-1} min^{-1}) of the cotyledonory tissues obtained by raising the seedlings of upland cotton up to fortnight. The EST activity ranged from 174.18 to 462.68 μM mg protein^{-1} min^{-1} as shown in Table 2. The highest esterase activity was observed in the genotype LRA–5166 (462.68 μM mg protein^{-1} min^{-1}) followed by the Vikas (453.94 μM mg protein $^{-1}$ min^{-1}) and NH–545 (394.76 μM mg protein^{-1} min^{-1}) respectively. The lowest activity estimate for EST was estimated in the genotype Sahana (174.18 μM mg protein^{-1} min^{-1}) accompanied by AKH–32 (198.39 μM mg protein^{-1} min^{-1}). Total fifteen bands were observed having the relative mobility in range of 0.100 to 0.971, indicating the wide range of esterase activity. The maximum number of bands were exhibited by the genotypes AKH–24 and PH–93 (eight each) followed by NH–545, AKH–62, AKH–023 and AKH–1234 showing seven bands. Only one band was observed in genotype AH–107 in the present study. The esterase PAGE had shown the highest number of polymorphic bands among all the biochemical techniques employed. (Table 2, Plat 2)

Isozymes are not only quicker and less labor intensive than traditional methods but are also more reliable since the expression of isozymes loci are co-dominant (Arus, 1983, Ryan and Scowcroft, 1987). In the present investigation, six isozyme systems of peroxidase (PRX), esterase (EST), malate dehydrogenase (MDH), acid phosphatase (ACP), glutamate-oxalo-acetate transaminase (GOT) and superoxide dismutase (SOD) were used to detect the genetic variability among nine cotton (*Gossypium barbaradense* L.) varieties namely: Dandara, Giza-75, Giza-83, Giza-85, Giza-86, Giza-88, Giza-89, Giza-90 and Giza-91 (Fig. 1). In present investigation it has been found that esterases possess very sensitive nature of enzymatic reactions, hence the results were not reproducible. Also, there is limited scope for screening as they are more prone to fluctuations, as reported by Roy *et al.*, (2001) in grasspea, Chandrashekhar (2001), Sapna Joshi (2005) and Shadmanov et al (2018) in upland cotton.

[b] Peroxidase: Peroxidase **(**POX) is one of the first enzymes responding and providing fast defense against plant pathogens (Sulman et al. 2001). POXs are involved in the lignification, suberification, polymerization of hydroxy-proline-rich glycoproteins, regulation of cell wall elongation, wound healing, and resistance against pathogens in plants (Hammond-Kosack & Jones 1996; Yoshida et al. 2003; Maksimov et al. 2014). POX activity was significantly higher in noninoculated plants of both susceptible genotypes, as compared to resistant genotype. Peroxidases are versatile plant enzymes for electrophoresis study that have wide distribution in plant species and tissues. Peroxidase activity was assayed in five organs of the cotton plant. Total activity was in the order: leaf > ovary wall (boll) > petiole > stem = root. Peroxidase isozymes were separated from extracts of the young leaf, mature leaf, stem, and boll, using acrylamide gel disc electrophoresis. Five isozymes were found in the cotton plant, a major one occurring in each of the organs examined. One isozyme was present only in the boll, and another only in leaves; two isozymes, not present in the young leaf, were present in the old leaf. Sub cellular fractionation by differential centrifugation suggested that the cotton leaf peroxidase occurs primarily in the cytosol (Wise and Morrison, 1971).

The POX activity among the genotypes was ranged from 110.47 to 250.97 μM mg protein^{-1} min^{-1}. The genotype LRA–5166, among the genotypes under study exhibited the highest peroxidase activity (250.97 μM mg protein^{-1} min^{-1}) followed by JLH–1594 (222.01 μM mg protein^{-1} min^{-1}) and AKH–24 (215.57 μM mg protein^{-1} min^{-1}) respectively. Whereas, the genotype AH–107 has shown lowest peroxidase activity (110.47 μM mg protein^{-1} min^{-1}) along with NH–545 (111.54 μM mg protein^{-1} min^{-1}) among the study material (Table 2, Plat 3).

The results revealed that total five bands were observed in thirty-four cotton lines. Very narrow range of relative mobility values, from 0.033 to 0.283 was observed, indicating that the differentiation of POX activity was not clear in cotton lines under investigation. The maximum three bands were observed in AH–107 and Vikas, whereas in most of the genotypes exhibited single band of different relative mobility. Limited polymorphism was observed for banding pattern of POX for cotton lines analyzed. Misagi (1982) reported that the POX possibly contributes to resistance by catalyzing oxidative polymerization of simple phenols to lignin and synthesis of antimicrobial oxidized phenols. An increased peroxidases activity in various plant host parasite interactions has been correlated with the disease resistance. Hence on the basis findings of the present study it can be revealed that the genotypes LRA–5166, JLH–1594, AKH–24 and LCMS–2 may have resistance power against pathogenic reactions on the basis of Peroxidase activity, as a factor of antibiosis kind of mechanism for plant disease resistance.

[c] Polyphenol oxidase: Polyphenol oxidase, another important plant isoenzyme has been studied successfully in present investigation. Phenol oxidases are copper proteins of wide occurrence in nature, which catalyze the aerobic oxidation of certain phenolic substrates to quinones, which are autoxidized to dark brown pigments generally known as melanins. PPO is important in the initial stage of plant defense where membrane damage causes release of phenols such as chlorogenic acid. PPO catalyzes the oxidation of phenolics to free radicals that can react with biological molecules, thus creating an unfavorable environment for pathogen development (Jockusch 1966; Mohamed et al. 2012). The PPO activity among the studied genotypes ranged from 36.92 to 131.45μM mg protein^{-1} min^{-1}. The genotype AKH–24 has shown maximum activity (131.45 μM mg protein^{-1}

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min^{-1}) for polyphenol oxidase isozyme, followed by G Cot–10 (117.30 µM mg $protein^{-1}$ min^{-1}), AKH –91 (113.51 µM mg $protein^{-1}$ min^{-1}), KH–118 (110.75 µM mg $protein^{-1}$ min^{-1}) and LRA–5166 (110.23 µM mg $protein^{-1}$ min^{-1}) respectively. Whereas, the lowest activity of PPO was exhibited in the genotype PH–93 of 36.92 µM mg $protein^{-1}$ min^{-1} (Table 2, Plat 4)

The PAGE analysis for polyphenol oxidase revealed that there is very limited polymorphism among the genotypes under investigation. Total five PPO bands overlapping each other were detected having the relative mobility in the range of 0.04 to 0.21. It reveals that there is limited polymorphism within the material under study for polyphenol oxidase activity. Among the thirty-four genotype, the genotype GA–B, LCMS–5, LCMS–2, Vikas, AKH–8660 and PH–348 has shown all the five bands whereas the genotypes viz., AKH–8835, KH–118, NH–545, LRA–5166, JK–119 and AKH–081 exhibited single band each of varying relative mobility.

Role of isozymes in Plant Disease Resistance: Plants unlike animals due to the fact, that they are tightly bound to the habitats, during long term evolution and changing environmental conditions, had developed a wide range of defense mechanisms. In the process of plants domestication the necessity in some of these defense mechanisms eliminated, but they was preserved in genome (Metlitskiy and Ozertskovskaya, 1985). When plants are attacked by pathogens, they defend themselves with an arsenal of defense mechanisms, both passive and active. The active defense responses, which require de novo protein synthesis, are regulated through a complex and interconnected network of signaling pathways that mainly involve three molecules, salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), and which results in the synthesis of pathogenesis-related (PR) proteins. Microbe or elicitor-induced signal transduction pathways lead to (i) the reinforcement of cell walls and lignification, (ii) the production of antimicrobial metabolites (phytoalexins), and (iii) the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Among the proteins induced during the host plant defense, class III plant peroxidases (EC 1.11.1.7; hydrogen donor: H2O2 oxidoreductase, Prxs) are well known. They belong to a large multi-gene family, and participate in a broad range of physiological processes, such as lignin and suberin formation, cross-linking of cell wall components, and synthesis of phytoalexins, or participate in the metabolism of ROS and RNS, both switching on the hypersensitive response (HR), a form of programmed host cell death at the infection site associated with limited pathogen development. The present review focuses on these plant defense reactions in which Prxs are directly or indirectly involved, and ends with the signaling pathways, which regulate Prx gene expression during plant defense (Almagro et al, 2009).

Moreover, it is reported that the processes plants adopt in order to defend themselves against pathogens are indeed complex. Some defense enzymes, including Peroxidase (POD), Superoxide Dismutase (SOD), Catalase (CAT), Polyphenol oxide (PPO) and Esterase (EST) play a very important role in these processes. The correlations between aggravation of diseases and enzymes activity were significant (Silva et al., 2004; Jetiyanon, 2007). The mechanism by which defensive enzymes mitigated pathogen-induced oxidative stress is complicated. Generally, an appropriate intracellular balance between ROS generation and scavenging exists in all cells. This redox homeostasis requires the efficient coordination of reactions in different cell compartments and is governed by complex signal transduction pathways. Plants possess an array of antioxidants that can protect cells from oxidative damage by scavenging Reactive Oxygen Species (ROS). The scavengers include ascorbate, glutathione and hydrophobic molecules (tocopherols, carotenoids and xanthophylls) and detoxifying enzymes that operate in the different cellular organelles (Noctor and Foyer, 1998). These enzymes include SOD, CAT and POD, which work together with other enzymes of the ascorbate-glutathione cycle to promote the scavenging of ROS (Hernandez et al., 2001). SOD catalyzes the dismutation of O_2 to H_2O_2 and O_2. CAT is present in the peroxisomes of nearly all aerobic cells (Dionisio-Sese and Tobita, 1998). It can protect the cell from H_2O_2 by catalyzing its decomposition into O_2 and H_2O (Foyer and Noctor, 2000). POD is widely distributed in all higher plants and protects cells against the destructive influence of H_2O_2 by catalyzing its decomposition through the oxidation of phenolic and enodiolic co-substrates (Asada, 1992; Borsani et al., 2001)

Thipyapong and Steffen (1997) found that PPO has a wide range of responses to multiple induction signals such as infection of bacteria or fungi, physiological stress, mechanical damage and signal molecules (methyl jasmonate, salicylic acid, ethylene and cAMP). PPO is also involved in the oxidation of polyphenols into quinones using molecular oxygen as an electron acceptor and lignification of plant cells during microbial infection (Chittoor et al., 1999). Alterations in oxidative processes and phenolic metabolism are still debatable because it is not certain whether they can play roles in hypersensitive plant cells or not. A number of studies suggested that PPO may participate in defense reactions and confer hypersensitivity to plants resistant to diseases, for example, potato, cotton and vicia. The new generation of PPO isozymes and the performance of disease resistance for organization are positively correlated.

Verticillium wilt is one of two vascular wilt diseases affecting cotton, the other being Verticillium wilt. Vascular pathogens have the ability to colonize plant roots and penetrate to the vascular tissues, where they are contained and proliferate within the xylem vessels, eventually becoming distributed throughout the plant. Under optimal conditions for infection, the susceptible host is normally killed by a combination of toxic fungal

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metabolites, accumulated fungal material and host responses to infection, leading to vascular occlusion and moisture deficit (Shadmanov et al, 2018). Further Juan Zhao et al., (2012) reported that the defensive enzymes' activities and isozymes are closely related to disease resistance of fruit, and they play an important role in the process of tissue biochemical defense. As such, it is a kind of performance for plant system to acquire resistance ability

IV. CONCLUSIONS

Isoenzymes are proteins which catalyse the same reaction as that of the same enzyme class but have a different molecular structure as apparent through differential electrophoretic mobility. The metabolism of plant defense response including its physiological and biochemical reactions catalyzed by enzymes is very complex. Isozymes were the first molecular markers to be used in plant genetics to map polygenes and were successfully used for genomic characterization. However, the use of isoenzymes is limited due to the paucity of adequate number of isozymes to map an entire genome. The banding pattern of biochemical markers revealed that the maximum number of bands were recorded in esterase analysis (fifteen) followed by protein analysis (twelve) whereas, only five bands each were detected in peroxidase and polyphenol oxidase analysis indicating limited polymorphism. Further, the biochemical study further revealed that although electrophoretic proteins and isozyme profiles could be efficiently used for distinguishing the genotypes but qualitative differences could not be reproduced, as they were not consistent when experiment was repeated. Moreover, it's cumbersome to identify and count the faint bands being the presence or absence of bands as the criteria for discrimination; the method needs to be used very critically. Further, it could be concluded that the utilization of biochemical markers on seed allozymes level, researchers can able to develop genotypes with tolerance to unfavorable factors and to increase homogeneity and cultivar purity. This kind of biochemical markers based on biological polymers' polymorphism, can be applied as unique phenotypic markers reflecting allelic state of corresponding genes.

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REFERENCES

[1] Agrios G.N. 1997. Plant pathology. 4th ed. San Diego (CA): Academic Press., Cambridge, Massachusetts

[2] Almagro L., L.V. Gomez Ros., S.B. Navarro., R. Bru., A. R. Barcelo and M.A.Pedreno. 2009. *J. Experimental Botany*, Vol. 60 (2) 377-390

[3] Arus, P. 1983. Genetic purity of commercial seed lot. In Isozymes in plant genetics and breeding Part-A by S.D. Tanksley and T.J. Orton (Eds)., 415 – 423 Elsevier Sci. Publishers B.V. Amsterdam.

[4] Asada K (1992). Ascorbate peroxidase: a hydrogen peroxidescavenging enzyme in plants. *Plant Physiol*. 85:235-241.

[5] Borsani O, Valpuesta V, Botella MA (2001). Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. *Plant Physiol.* 126:1024-1030

[6] Bradford, M.M. 1976. Quantification of microgram a rapid and sensation method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Annals of Biochem.* 72. : 248 – 254.

[7] Carvalho D, Anastacio Q, Luciana M. 2006. Proteins and isozymes electrophoresis in seeds of Desti (Leguminosae caesalpinioidea) artificially aged. Rev Arv. 30:19–21.

[8] Caseteng, M.Y. and C. Y. Lee. 1987. Changes in apple polyphenol oxidase and polyphenol concentrations in relation to degree of browning. *J. Food Sci.*,52 : 985 – 989.

[9] Chance, B. and M. Mechly. 1955. Methods in Enzymology Vol. 2 : 764 S.P. Colowick and N. O. Kaplan (Eds), Acad. Press. New York.

[10] Chandrashekhar. P. 2001. Studies on molecular markers in cotton, Ph.D.(Agri) Thesis (unpubl.) submitted to Dr. PDKV, Akola.

[11] Cherry, J.P.; F.R.H. Katterman and J.E.Endrizzi. 1970. Comparative studies of seed proteins of species of *Gossypium* by gel electrophoresis. *Evolution*, 24 : 431 – 447.

[12] Cherry, J.P.; J.G. Simmons and J.D.Tallant. 1979. Cotton seed protein composition and quality of *Gossypium* species and cultivars. Beltwide Cotton Production Research Conference Proceedings. 123 – 127.

[13] Chittoor, JM., Leach JE, White FF (1999). Induction of peroxidase during defense against pathogens. in: Pathogenesis Related Proteins in Plants. S. K. Datta and S. Muthukrishnan, eds. CRC Press. Boca Raton, FL, U.S.A. pp.171-193

[14] Dionisio-Sese ML, Tobita S (1998). Antioxidant responses of rice seedlings to salinity stress. *Plant Sci.* 135:1-9

[15] Dongre, A.B.; S.B. Nandeshwar.; V.V. Singh.; J. Amndha and G. Balasubramani. 2001. Molecular Evaluation of cotton germplasm., In *CICR Annual Report 2000-2001*., CICR, Nagpur:28.

[16] Foyer CH, Noctor G (2000). Oxygen processing in photosynthesis: regulation and signaling. *New Phytol.* 146: 359-388

[17] Giber Vila.; Dinora M. Leon.; Hugo S. Garcia and Javier De La Cruz. 2003. Polyphenol oxidase actvity during ripening and chilling stress in 'Manila' mangoes.

ISSN: 2583-4053

Volume-1 Issue-3 || August 2022 || PP. 229-237

https://doi.org/10.55544/jrasb.1.3.30

J. Horticultural Sci .and Biotechnology., 78 (1) : 104 – 107.

[18] Hammond-Kosack KE, Jones JDG. 1996. Resistance gene dependent plant defense responses. *Plant Cell.* 8:1773–1791.

[19] Hernandez JA, Ferrer MA, Jimenez A, Barcelo AR, Sevilla F (2001). Antioxidant systems and O_2^{-}/H_2O_2 production in the apoplast of pea leaves. Its relation with saltinduced necrotic lesions in minor veins. *Plant Physiol.* 127:827-831.

[20] Hu, G., N.L.Houstan, D. Pathak, Linnea Schmidt, J.J.Thelen, Jonathan F. Wendel 2011. Genomically biased accumulation of seed storage proteins in allopolyploid cotton. *Genetics* 189, 1103–1115

[21] Jetiyanon K (2007). Defensive-related enzyme response in plants treated with a mixture of Bacillus strains (IN937a and IN937b) against different pathogens. *Biol. Control* 42:178-185

[22] Jockusch H. 1966. The role of host genes, temperature and polyphenol oxidase in the necrotization of TMV infected tobacco tissue. *Phytopathol.* 55:185–192.

[23] Juan Zhao, Yuguo Wang, Jishu Zhang, Yuanhuai Han, Zhifen Yang, and Wenxin Feng. 2012. Induction of defensive enzymes (isozymes) during defense against two different fungal pathogens in pear calli., *African J of Biotechnology* Vol. 11(72), pp. 13670-13677

[24] Krishna, T.G. and N.Jawali. 1997. DNA isolation from single or half seeds suitable for Random Amplified Polymorphic DNA analysis. *Analytical Biochemistry,* 250 : 125-127.

[25] Kulkarni, M.M. and R.A.Patil. 2003. Electrophoretic protein profiles of cotton cultivars for varietal identification. *J.Indian Soc.Cotton Improv.*, 145: 150.

[26] Liu, Q., Llewellyn, D. J., Singh, S. P. & Green, A. G. 2012. In Flowering And Fruiting. (eds Oosterhuis, D. M. & Cothran, J. T.) 133–162

[27] Lowry, O.H.; N.H.Rosebrough; A.L.O.Far and R.J.Randel. 1951. Protein measurement with Folin Phenol reagent *J. Biol. chem.*, 193: 265 –275.

[28] Maksimov I, Troshina N, Surina O, Cherepanova E. 2014. Salicylic acid increases the defense reaction against bunt and smut pathogens in wheat calli. J Plant Interact. 9:306–314.

[29] Markert, C.L. and F. Moller. 1959. Multiple forms of enzymes: Tissue, ontogenetic and species - specific patterns. *Proc. Natl. Acad. Sci.*, USA., 45 : 756 – 763.

[30] Marshal D. R. and A.H.D. Brown (1975) The charge state model of protein polymorphism in natural population. *J. Mol. Ecol.* Vol. 6 149-163

[31] Metlitskiy L.V. , O. L. Ozertskovskaya .1985. How plants defense themselves against diseases. Mir Publishers.

[32] Mohamed H, EL-Hady AA, Mansour M, El-Rheem, ElSamawaty A. 2012. Association of oxidative stress components with resistance to flax powdery mildew. *Trop Plant Pathol.* 37:386–392.

[33] Namrata Idnani. 2002. Electrophoretic analysis of Protein and Isozyme in cotton – A Review. *J. Cotton. Res. Dev.*,16 (1): 69 – 77.

[34] Nijenhuis, B.T. 1971. Estimation of the proportion of inbred seed in Brussels sprouts hybrid seed by acid phosphate isozyme analysis. *Euphytica.*, 20 : 498 – 507.

[35] Noctor G, Foyer CH (1998). Ascorbate and glutathione: keeping active oxygen under control. *Ann. Rev. Plant Biol*. 49:249-279.

[36] Novacky, M.M. and R.E. Hampton. 1968. Peroxidase isozyme in virus infected plants. *Phytopathology.* 58 : 301 – 305.

[37] Park, V. K.; H.H. Salo.; T.D.Almeida and R.H. Moratti. 1980. Polyphenol oxidase of mango var Eaden. *Food Sci*., 45: 1619 – 1621.

[38] Prasad, R.; D. Prasad.; A.D.Taneja and V.P. Kudesia. 2001. SDS – PAGE of soluble seed proteins for identification of cotton cultivars. *J. Indian. Soc. Cotton. Improv.*, 26 (2) : 89 – 91.

[39] Roy, M.; N. Mandal and P.K.Das. 2001. Seed protein characterization and isozyme diversity for cultvar identification in grasspea (*Lathyrus sativus* L.). *Indian J. Genet.*, 61 (3) : 246 – 249.

[40] Ryan S.A. and W.R.Scowcroft. 1987. A Somaclonal variant of wheat with additional Beta-amylase isozyme. *Theor Appl Genet.*, 73 (3): 459 -464

[41] Salisbury, Frank B. and Cleon W. Ross. 1978. Plant Physiology., Second Edition., Wadsworth Publishing Company, Inc., Belmont, California.

[42] Sapna Joshi. 2005. Genetic Diversity analysis in cotton using morphological and biochemical markers. M.Sc. Thesis (Unpubl.) submitted to Dr. PDKV, Akola.

[43] Satija, D.R.; Adarsh Bala and S.K.Gupta. 2002. Genetic Diversity in relation to protein and protein fraction in chickpea (*Cicer arietinum*), *Crop Improv.*, 29 (2) : 122 –135.

[44] Scandalios, J.G. 1974. Isozymes in development and differentiation. *Ann. Rev. Plant Physiol.* 25: 225-258

[45] Seevers P.M., J.M.Daly and F.F.Catedral. 1971. The Role of Peroxidase Isozymes in Resistance to Wheat Stem Rust Disease. *Plant Physiol.*, 48, 353-360

[46] Shadmanov R. K., A.R.Shadmanova and J.A. Scheer. 2018. Biotechnology of Accelerated Breeding and Improvement of Cotton Varieties. *J Biotech Res Biochem* 1: 001.

[47] Shutov, A. D., Kakhovskaya, I. A., Braun, H., Baumlein, H. & Muntz, K. 1995. Legumin-like and vicilin-like seed storage proteins: Evidence for a common single-domain ancestral gene. *J. Mol. Evolu.* 41, 1057–1069

[48] Silva HSA, Romeiro RS, Macagnan D, Halfeld-Vieira BA, Pereira MCB, Mounteer A (2004). Rhizobacterial induction of systemic resistance in tomato plants non-specific protection and increase in enzyme activities. *Biol. Control* 29:288-295.

[49] Sulman M, Fox G, Osman A, Inkerman A, Williams P, Michalowitz M. 2001. Relationship between total peroxidase activity and susceptibility to black point in

ISSN: 2583-4053

Volume-1 Issue-3 || August 2022 || PP. 229-237

https://doi.org/10.55544/jrasb.1.3.30

mature grain of some barley cultivars. Proceeding of the 10th Australian Barley Technical Symposium. Canberra, ACT, Australia, 16–20 Sep. 2001.

[50] Thipyapong P, Steffens JC (1997). Tomato polyphenol oxidase (differential response of the polyphenol oxidase F promoter to injuries and wound signals). *Plant Physiol.* 115: 409-418

[51] Tornero P, Chao R, Luthin W, Goff S, Dangl J. 2002. Largescale structure, function, analysis, of Arabidopsis RPM1 disease resistance protein. *Plant Cell.* 14:435–450

[52] Ujjainkar V.V. and V.D. Patil (2020). Marker-assisted selection in American cotton genotypes using biochemical and molecular profiling techniques. *International J Advance Res. Idea Innovations in Technology*, Vol. 6 (3) 989-993.

[53] Ujjainkar V.V., V.D. Patil and M.P.Moharil (2020). Diversity assessment of parental linies through biochemical and molecular markers in upland cotton (*Gossypium hirsutum* L.) *International J of Genetics.*, Vol 12 (6) 740 – 744

[54] Vallejos, C.E. 1983. Enzyme activity staining. In : Isozymes in Plant genetics and breeding., Part A., Tanksley S.D. and T.J. Ortan (Eds): 469 – 515. Elsevier Science Acad. Publishers, Amsterdam.

[55] Wise B. and M. Morrison. 1971. Localization of isozyme form of peroxidase in the cotton plant, *Phytochemistry.*, Vol. 10 (10)., 2355-2359

[56] Yoshida K, Kaothien P, Matsui T, Kawaoka A, Shinmyo A. 2003. Molecular biology and application of plant peroxidase genes. *Appl Microbiol Biotechnol.* 60:665–670.

[57] Zeeshan Siddique, Khalid Pervaiz Akhtar, Amjad Hameed, Nighat Sarwar, Imran-Ul-Haq & Sajid Aleem Khan. 2014. Biochemical alterations in leaves of resistant and susceptible cotton genotypes infected systemically by cotton leaf curl Burewala virus, *Journal of Plant Interactions* 9:1, 702-711.

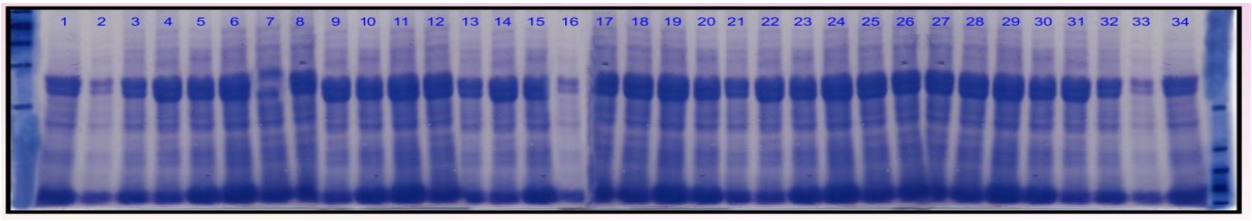

Plate 1: Protein analysis of thirty-four cotton genotypes (SDS-PAGE)

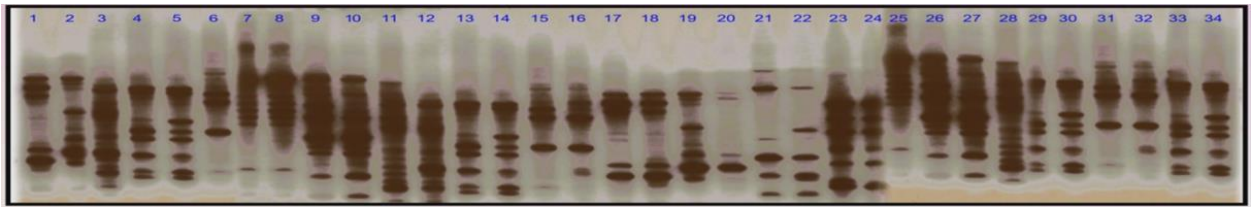

Plate 2: Esterase analysis of thirty-four cotton genotypes (PAGE)

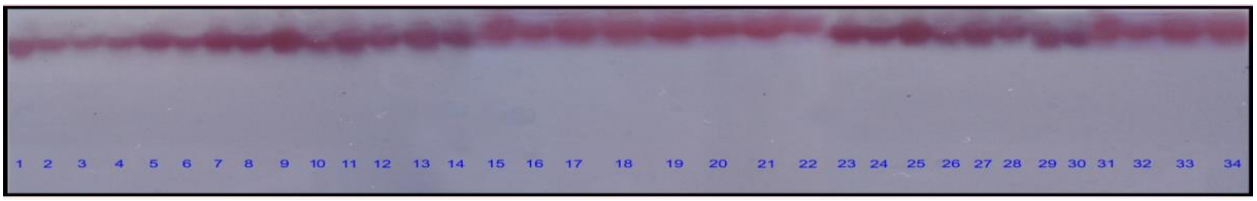

Plate 3: Peroxidase analysis of thirty-four cotton genotypes (PAGE)

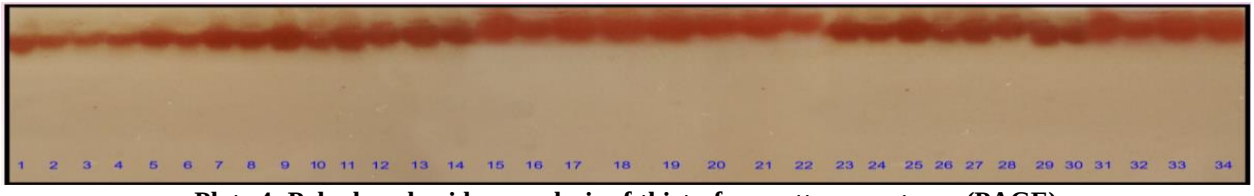

Plate 4: Polyphenol oxidase analysis of thirty-four cotton genotypes (PAGE)