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ISOZYME MARKERS IN COTTON BREEDING-III. VARIATION IN THE INTENSITY OF ISOZYMES OF ENZYME PEROXIDASE EXHIBITED BY DIFFERENT LOCI OF DIFFERENT COTTON VARIETIES AND THERE RELATIONSHIP WITH COTTON LEAF VIRUS DISEASE

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Abstract

Isozymes of the enzyme peroxidase was studied in different resistant, tolerant and susceptible varieties of cotton growing at a location known as a hot spot for cotton leaf curl virus disease. Plant material used for isozyme extraction was collected from the field in the month of September when leaf curl virus disease reached its peak and the results were compared with those obtained in July when disease intensity stays in between 50 to 80%. Significant differences were observed in the Pox. activity (as measured by the intensity of bands) exhibited by different loci in different varieties while among different varieties, variation in mean Pox. activity of 7 loci was generally not significant. In most of the cases, Pox. activity increased with increase in virus intensity from July to September. The differences due to locations in Pox. activity exhibited by a specific locus in a variety were mostly non-significant in September compared to July when they were significant. When differences in mean Pox. activities of 3 varieties growing simultaneously at three locations in the month of July and September were compared, significant increase in the activity was observed at locus 1 in varieties growing at location-1 while locus 7 exhibited progressive decrease in the activity at location-111. The mean varietal Pox. activity increased in all the varieties and at all the 3 locations but the differences were not significant. Prevalence of cotton leaf curl virus at location-111 appeared to be positively correlated with locus 4a and 6 under low to medium virus intensity while under high virus intensity, locus 2 appeared to be correlated with increased Pox. activity. Possibilities of using Pox. activity as marker for selection of varieties to be cultivated under the areas of high disease intensity are discussed.

Introduction

The isozyme have been and are still being used in plants as genetic and biochemical tools for studying varietal identification in general and environmental stability in particular (Bailey, 1983; (Kvarstskhelia *et al.*, 1997; Tanksley & Orton, 1983). The most common among them are isozymes of peroxidases. The activity and different forms of peroxidases can be correlated with any number of growth, development and defense processes (Bowles, 1990). However, its level of expression in plant systems can be altered by stress, chemical application and disease infection (Gasper *et al.*, 1982). Peroxidases in plants oxidizes a wide range of organic substrates and through the use of these oxidation products, are involved in important biosynthetic processes like lignification of the cell wall, degradation of IAA, biosynthesis of ethylene (Gazaryan *et al.*, 1996; Kobayashi *et al.*, 1996), wound healing (Birecka &

Miller, 1974; Parent *et al.*, 1985), tissue specificity (Bassri & Carlson, 1979), developmental regulation (Thorpe *et al.*, 1978), and defense against pathogens or disease resistance (Nessel & Mader, 1977). Most higher plants possess different isoforms of peroxidases (Van Huystee, 1987) of which some are capable of H_2O_2 formations (Mader *et al.*, 1980) and are reported to be triggered in plants by wounding while others have high affinity for phenolic substrates and are triggered in plants upon infection with viruses (Lagrimini *et al.*, 1987). In our earlier efforts to use different isozyme systems in cotton breeding (Farooq, 1999), we found significant differences in the activity of isozyme peroxidase in varieties of cotton growing at location-111 which is a hot spot for cotton leaf curl virus disease (Anon., 1995a) compared to the varieties growing at location-1 and 11 where disease intensity was comparatively less severe. To understand the possible role of disease prevalence at location-111, Pox. activity in varieties growing at one location (location 111) and 3 varieties growing simultaneously at all the three locations was determined in the month of September when disease intensity reached its peak (100%) and the results were compared with those obtained in July. The objectives were i) to observe comparative differences occurring in Pox. activity due to increase in disease intensity, ii) identification of a locus or loci contributing significantly towards such differences and iii) to determine (if possible) the significance and relationship of the Pox. activity specific to the identified locus/loci with intensity of the virus disease.

Materials and Methods

Material used in this study comprised Cotton cultivars CIM-1100 CIM-435, CIM 448, CIM-443 (resistant)., FH-682, BH-36, NIAB-78 and CIM-240 (tolerant) and S-14 and CIM-109 (susceptible, Anon., 1995a) and were growing at Punjab Seed Corporation, Khanewal (location 111). CIM-1100, CIM-240 and S-14 growing at two different experimental farms (location-1 and 11) of Central Cotton Research Institute (CCRI), Multan, were also used in this study.

Material was collected in the month of September, 1996 when cotton leaf curl virus disease reached its peak (Anon., 1995b). Isozyme extraction and staining was performed according to the methods of Davis (1964) and Vallejose (1983) while photo-documentation and statistical evaluation was according to Farooq *et al.*, (1997). Within the lines variation were removed as reported earlier (Farooq *et al.*, 1997; Farooq, 1999). In the first experiment, Pox. activity (in the form of banding intensity) was measured in cotton varieties growing at location-111, and the mean Pox. activities of different varieties exhibited by different loci were compared with the similar observation taken at location-1 and-11. In experiment 2, mean Pox. activity of individual locus in a resistant, a tolerant and a susceptible variety growing simultaneously at the three locations was studied. Comparisons were made among the mean Pox. activities exhibited by all the loci in the months of July and September in cotton leaf curl virus resistant and susceptible varieties growing at the three locations. Simple coefficient of correlations were calculated between % virus infection and Pox. activity exhibited by a specific locus of tolerant and susceptible varieties and a group of resistant, a group of tolerant and one susceptible variety growing at locations-11 and 111.

Result and Discussion

Table 1 showed variations in the intensity of bands exhibited by different loci of varieties growing at location-111 in the month of September. Among different loci, variations in Pox. intensities due to varieties were significant with locus 3b exhibited maximum intensity. Among different varieties however, variation in Pox. activity exhibited by 7 loci were not considerably different as it ranges between 2.45-2.83 (Table 1). Compared to the study made in July (Farooq, 1999), significant changes were observed in the study made in September (present study) as locus 4b exhibited banding intensities ranging between 2.0-3.6 while there was absolutely no activity at this locus in the month of July. Also, locus 3a presently exhibited banding intensities ranging between 1-3.6 showed negligible activity at this locus in the month of July (Farooq, 1999).

In order to see the differences of locations, mean Pox. activity (data not reported) of a specific locus in varieties growing at all the three locations was compared. It was interesting to note that there was generally no difference in the mean Pox. activity except for loci 4b and 7 which exhibited significantly lower and locus 6 which exhibited higher activity respectively at location-11 compared to locations-1 and-111 (Fig.1). This is contrary to the findings of July where differences in mean Pox. activity of a specific locus was significantly different in varieties growing under different locations.

For further clarification, Pox. activity in three varieties: CIM-1100 (resistant), CIM-240 (tolerant) and S-14 (susceptible) growing at three locations was studied. Contrary to the study made in July, in the present study, differences due to locations in

Table 1. Variation in the intensity of isozyme bands exhibited by different loci in different varieties of cotton growing at location-111.

	Intensities of isozyumic bands exhibited by different loci in cotton variety										
Isozyme loci	CIM 1100	CIM 435	CIM 443	CIM 448	FH 682	NIAB -78	BH-36	S-14	CIM-109	CIM-240	Mean
1	2.2	1.8	1.2	1.6	1.6	1.0	1.6	1.8	1.0	2.0	1.5d
2	1.0	2.0	1.8	2.4	2.0	1.2	1.8	2.0	1.8	1.8	1.8cd
3a	1.0	3.0	3.0	3.6	3.0	1.0	2.8	1.0	1.0	3.0	2.2c
3b	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0a
4a	3.5	4.0	4.0	3.6	3.2	3.4	3.2	4.0	3.6	3.6	3.64b
4b	3.2	3.0	2.8	2.5	3.2	3.6	3.6	2.0	3.0	3.6	3.05b
5	1.8	1.8	1.8	2.4	1.4	2.4	1.8	2.2	2.4	2.0	2.0c
6	1.6	1.0	1.0	1.0	1.8	2.0	1.8	1.8	1.8	1.2	1.5d
7	2.8	3.0	4.2	3.4	2.6	3.4	2.2	4.4	2.6	3.0	3.16b
Mean	2.45a	2.73a	2.75a	2.83a	2.64a	2.5a	2.64a	2.69a	2.47a	2.8a	

Figure followed by the same letters are not significantly different from each other at 5% level of significance according to Duncan's Multiple Range Test.

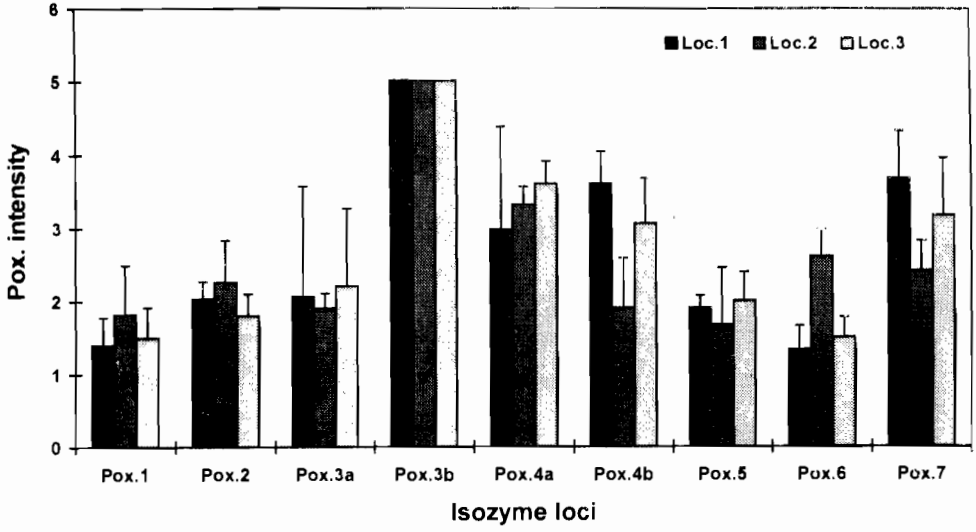

Fig.1. Variation in the intensity of isozymes of the enzyme peroxidase (Pox.) as exhibited by a specific locus in cotton varieties growing under three different locations (Loc. 1,2,3) in the month of September.

mean peroxidase intensity exhibited by 7 loci in a variety and a specific locus in three varieties were negligible (Table 2). Comparison of studies made in July and September to detect differences in mean Pox. activity exhibited by 7 loci in a variety however, revealed significant differences (Fig. 2). At locus 1, Pox. activity of all the three varieties increased in September but it is significant only at location-111 (60% increase) while activity at locus 7 decreased progressively with a maximum decrease at location-11 (-95%) and minimum at location-111 (7.60%). Activities at loci 3b, 4a (negligible to no activity in July) and loci 5 and 6 (negligible to very low activity in July) increased in September at all the three locations. The activity at loci 2 and 4b increased only at location-111. The mean varietal Pox. activity exhibited by all the 7 loci increased in September at all the three locations and in all the three varieties (Fig. 3) but differences were non-significant.

To see the relationship of Pox. activity of different loci with the intensity of virus disease, simple correlations were calculated between disease intensity and Pox. activity of cotton varieties growing at location-11 and 111. While no correlation was observed at location-11 (data not reported) for location-111, it was interesting to note that a locus positively correlated with % virus infection in the month of July will not show any correlation in the month of September and *vice versa* (i.e., a locus showing positive correlation with low to medium disease intensity will not show correlation with high disease intensity). When tolerant and susceptible varieties growing at location-111 were studied in the month of July, locus 4a and 6 appeared positively and significantly correlated with disease intensity (Table 3) however, the same varieties showed significant correlation with locus 2 when studied in September. Similarly, when a group of resistant, a group of tolerant with one susceptible variety were studied in July, locus

Table 2. Comparative variation in the intensity of isozymic bands exhibited by different loci in three varieties of cotton growing simultaneously at three different locations.

	Intensity of isozyme bands exhibited by different loci in three cotton varieties growing at											
	Location-1				Location-11				Location-111			
Pox loci	CIM-110	CIM-240	S-14	Mean	CIM-1100	CIM-240	S-14	Mean	CIM-1100	CIM-240	S-14	Mean
1	1.5	1.4	1.8	1.6d	2.2	1.8	1.2	1.7d	1.0	2.0	1.8	2.0c
2	1.8	2.2	2.8	2.0d	1.8	2.0	3.0	2.27c	1.0	1.8	1.0	1.3d
3a	1.5	4.0	4.0	3.2c	1.0	1.0	1.8	1.3d	1.0	3.0	2.0	1.7cd
3b	5.0	5.0	5.0	5.0a	5.0	5.0	5.0	5.0a	5.0	5.0	5.0	5.0a
4a	4.0	3.8	4.2	4.0b	2.0	2.0	1.5	1.8d	3.5	3.6	4.0	3.7b
4b	4.5	3.4	3.6	3.8bc	3.8	4.4	2.8	3.7b	3.2	3.6	2.0	2.9bc
5	1.0	2.2	2.8	2.0d	2.6	1.8	1.8	2.06c	2.8	2.5	2.2	2.5c
6	1.5	1.6	1.8	1.8d	2.2	2.0	2.2	2.13c	1.6	1.2	1.8	1.53d
7	3.0	2.8	3.8	3.2c	2.8	3.6	2.4	2.9bc	2.8	3.0	4.4	3.4b
Mean	2.64a	2.93a	3.31a		2.6a	2.62a	2.32a		2.43a	2.80a	2.7a	

Figure followed by the same letters are not significantly different from each other at 5% level of significance according to Duncan's Multiple Range Test.

Table 3. Correlation between disease intensity (% virus infection) and intensity of isozyme peroxidase exhibited by different loci in cotton varieties growing at location-111.

Description of material	Month of study	Locus #	Coefficient of correlation
1. Tolerant and susceptible varieties	July	4a	0.957*
	6***	0.960*	
2. Tolerant and susceptible varieties	September	2	0.992**
3. A groups of resistant, a group of tolerant and a susceptible varieity.	July	7	0.986*
4. A groups of resistant, a group of tolerant and susceptible varieities	September	6***	0.974*

*Significant at 5% level of significance, **significant at 1% level of significance

***Data on virus infection used for these correlation was average of 1994-1997 and was taken from Anonymous, 1995 and Anonymous, 1998.

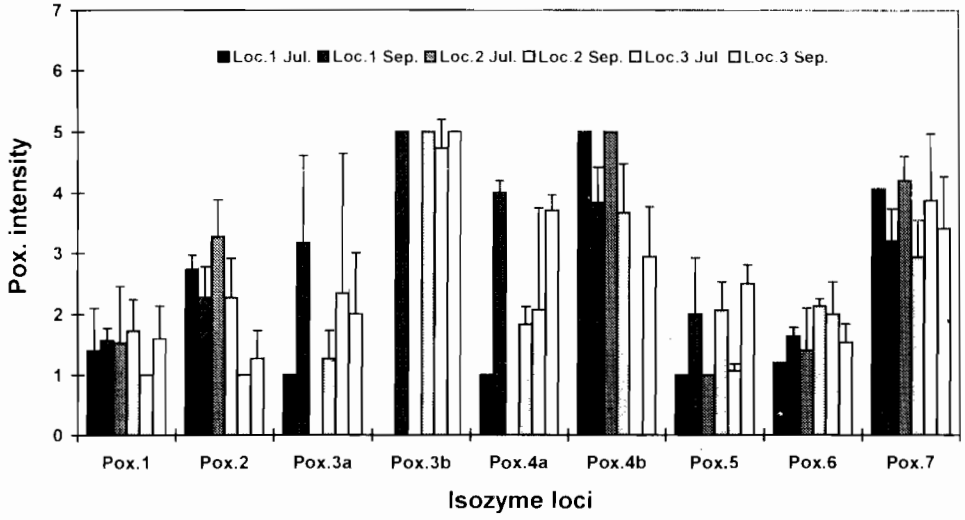

Fig.2. Comparative variation in the intensity of isozymes of the enzyme peroxidase (Pox.) as exhibited by a sepcific locus in different cotton varieties growing under three different locations (Loc. 1,2,3) in the month of July and September.

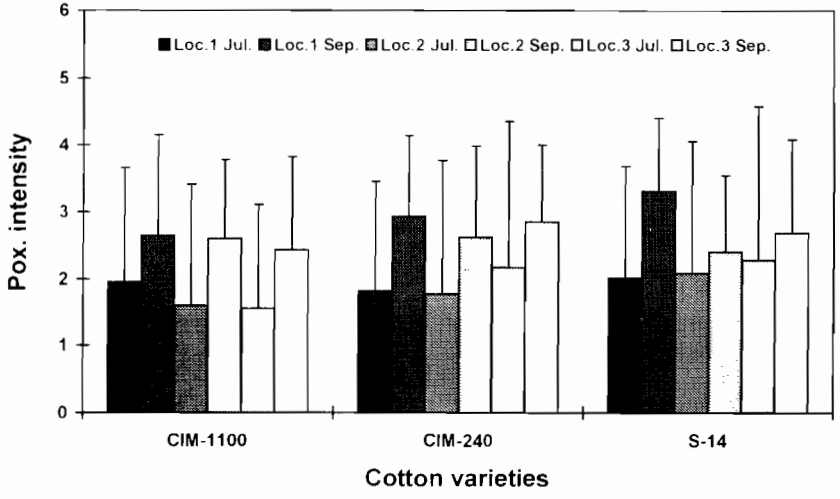

Fig.3. Comparative Variation in the intensity of isozyme of the enzyme peroxidase (Pox.) exhibited by all the 7 loci in a specific cotton variety growing under 3 different locations (Loc. 1,2,3) in the month of July and September.

7 appeared positively and significantly correlated with disease intensity while the same groups when studied in September showed correlation with locus 6.

Peroxidases in plants are often encoded by many different loci and show evidence of post translational modifications (Jaaska, 1983). Atleast 7 peroxidase bands are seen in rice (Ida *et al.*, 1972), 10 in tobacco (Sheen, 1970) and 13 in maize (Brewbaker & Hasegawa., 1975). Similarly, duplicated and/or multiple loci have also been observed for isozymes of esterases and peroxidases in tomato, rye and pea (Tanksley & Rick, 1980, Wehling & Schmidt-Stone, 1984; Weeden & Marx, 1987). The multiplicity of such duplicated loci have been explained as partly due to differences in their physiological functions however, no such divergence in functions has been demonstrated for peroxidases in plants (Weeden & Wendel, 1989). In the present study, we found 7 loci for peroxidase of which activity of the loci 3a, 3b, 4a and 4b varies generally with varieties and more specifically with the change in age of the plant and location of its cultivation. Age of the plant has been reported to have caused variation in the isozyme phenotype (Jones, 1984), there is hardly any report on the variation produced in isozyme phenotype due to variation in planting location. Since physiological functions of an individual isozyme depends upon its response to external stimuli (Sheen, 1983) which are different for location-111 being a hot spot for cotton leaf curl virus disease thus, it is quite possible that some of these loci exhibited variation due to ontogenetic changes occuring in the plants due to aging and some with the intensity of virus disease at this location. Among the above mentioned loci only locus 2 showed significant positive correlation with tolerant and susceptible varieties under the peak of virus attack while the same varieties showed positive correlation with locus 4a and 6 when virus disease was 50-80%. Thus if tolerant and susceptible varieties are to be planted at a virus hot spots, only the varieties with the ability to increase Pox. activity at locus 2, 4a and 6 will survive. Similarly, positive correlations at loci 6 and 7 with high and low to medium disease intensity respectively again indicated that if hot spots are to be cultivated with cotton, comparatively higher Pox. activity would be required for susceptible varieties to survive than tolerant varieties. The resistant varieties would possess minimum Pox. activity at these loci. Earlier investigation on such studies (Simons & Ross, 1970; Van Loon & Geelen, 1971· Weststeijn, 1976) showed an increase in peroxidase activity as well as qualitative changes in isozyme pattern as a consequence of infection of a hypersensitive plant with Tobacco Mosaic Virus (TMV). This increase in Pox. activity was believed to have deterring effects against any additional pathogen attack. In another study activity of two moderately anionic peroxidase isozyme increased in response to infection with TMV and thus supported the present findings of increased peroxidase activity in cotton in response of leaf curl virus attack (Lagrimini & Rothstein, 1987). It could be inferred from such studies that among the available virus susceptible but agronomically high standard varieties of cotton, selection can be made for plants possessing low Pox. activity at loci showing positive correlation with virus disease. Such plants would probably behave as resistant upon cultivation under the hot spot areas. The loci 3a, 3b and 4b showing variation in expression of Pox. due to location but could not be correlated with virus attack may have correlation with some other stresses (e.g., insect attack or nutrition deficiency etc.) which may prevail in the area of cultivation. Since

these parameters were not specifically considered in the present study therefore, any conclusive statement is difficult to make however, the present study did show the relationship of some of the loci with the disease intensity and to some extent indicated the function dependent multiplicity of different loci for isozyme peroxidase. There is a need that large number of varieties including resistant, tolerant and susceptible be studied at this location or any other location which is strongly under attack of virus disease and correlation of all the loci be studied with all the possible stresses prevalent under these areas. Results of such efforts would identify some of the loci which may help selection of varieties for cultivation of locations which are severely under the attack of virus.

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