Int.J.Curr.Microbiol.App.Sci (2017) *6*(2): 1540-1550

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 2 (2017) pp. 1540-1550**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2017.602.172

Antioxidant Enzymes in Leaves of Susceptible and Resistant Okra Genotypes against YVMV

Jalpesh S. Patel[1*], A. R. Japda[2], J.J. Dhruve[3] and N.J. Patel[3]

[1]Department of Biochemistry, College of Basic Science and Humanities, S. D. Agricultural University, Sardarkrushinagar, India
[2]Agriculture Research Station Amreli, Junagadh Agricultural University, Amreli-365601, India
[3]Department of Biochemistry, B.A. College of Agriculture, Anand Agricultural University, Anand, Gujarat–388 110, India
**Corresponding author*

Keywords

Isozyme, Okra, Peoxidase, Poly phenoloxidase, YVMV, Whitefly.

Article Info

Accepted:
24 January 2017
Available Online:
10 February 2017

A B S T R A C T

Yellow vein mosaic virus (YVMV), the most destructive viral disease of okra, has become a limiting factor in the successful cultivation of this crop. One of the major limiting factors of okra is the incidence of YVMV, its vector being whitefly. Infection of 100% plants in a field is very common and yield losses range from 50 to 94% depending on the stage of crop growth at which infection occurs. Emphasis is needed on breeding to develop yellow vein mosaic virus resistant variety. This study was undertaken to find out the potential source of resistance of okra to yellow vein mosaic virus under natural epiphytic condition. The experiment was conducted with ten genotypes/cultivars at Main Vegetable Research Station and Department of Biochemistry, B.A. College of Agriculture, Anand Agricultural University, Anand. Out of ten genotypes/cultivars GAO-5 was registered with the higher of peroxidase and polyphenol oxidase activities and minimum number of whitefly population at 35 and 60 days after germination (DAS) as compared to rest of all genotypes. Variation in total protein banding pattern was also obtained between resistant and susceptible genotypes. The number of bands was observed higher in resistant genotypes as compare to susceptible genotypes.

Introduction

Okra or lady's finger *Abelmoschus esculentus* (L.) Moench] is an annual, herbaceous plant with bisexual flower, which belongs to family *Malvaceae.* It is considered as an important vegetable crop of the tropical and subtropical regions of the world. Because of high consumer demand and thereby better price, farmers grow okra widely during the rainy and summer season. The crop is prone to damage by various insects, fungi, nematodes and viruses. Its cultivation in India is challenged by severe incidence of viral disease such as Yellow Vein Mosaic Virus (YVMV) disease spread by an insect vector, namely whitefly (*Bemisia tabaci* Gen). YVMV belongs to the genus *Begomo* virus, family *Geminiviridae*. Recently, it was found that at least 27 *begomo* viruses infect okra. *Begomo* viruses have high recombination rate and the emergence of 'B' biotype whiteflies is contributing to epidemics of *begomo* viruses in okra. The YVMV disease is characterized by a homogenous interwoven network of yellow vein enclosing islands of green tissues

within the leaf. In extreme cases, infected leaves become completely yellowish or creamy. If plants are infected within 20 days after germination, their growth is retarded with few leaves and malformed fruits resulting in loss ranging from 94% to 100%. YVMV cause mosaic, degradation of chlorophyll in vein, banding of vein and plant stunting.

The virus induces curved and irregular shape of leaves and also green to yellow colouration of leaves which affect plant growth, yield and quality of fruits (Ndunguru and Rajabu, 2004).The natural transmission of YVMV is through whitefly in a semi-persistent manner. The yield loss in okra due to infection of YVMV varies from 30-100% depending upon the age of plant (Sanwal *et al.,* 2014).

Biochemical markers like enzyme activities, isozymes, SDS-PAGE and molecular markers like RAPD, SSR are most widely used for characterization of any crop species at genetic level. Gandhi *et al.,* (2015) have reported that biochemical markers like isozyme of peroxidise (POX), polyphenoloxidase (PPO) and SDS PAGE are useful tools for screening of cotton genotypes. Keeping the above points in view, the present investigation was carried out on resistivity of different okra genotypes/ cultivars to whitefly and its correlation with oxidative enzyme activities, isozymes and SDS-PAGE of protein under field condition.

Materials and Methods

The experiment was conducted at the Main Vegetable Research Station, and Department of Biochemistry Anand Agricultural University, Anand. Seeds were sown in single 5m row with 20 cm plant and 45 cm row spacing. The experiment was carried out in randomized block design with three replications. All the recommended agronomic practices were adopted.

Ten genotypes/ cultivars AOL-09-2, AOL-09-17, AOL-10-22, AOL-11-34, AOL-11-37, AOL-11-39, AOL-11-49, GAO-5, Parbhani-krani, Pusa-sawani of okra were evaluated for their susceptibility to whitefly and were further studied to know the mechanism responsible for imparting the resistance in okra. The susceptibility of okra genotypes/cultivars to YVMV was evaluated on the basis of number of whitefly per plant at 35 and 60 days after sowing.

Isozyme analysis of POX and PPO was carried out as suggested by Guibault (1976) and Malik & Singh (1980), respectively with some modifications, while SDS-PAGE of okra genotypes was analyzed according to Laemmli (1970).

Results and Discussion

Peroxidase activity

Maximum peroxidase activity was recorded in resistant GAO-5 variety (4.96 Δ O.D. min^{-1} g^{-1}fw), which was at par with AOL 10-22. Minimum peroxidase activity was recorded in variety Pusasawani (1.92 Δ O.D. min^{-1} g^{-1}fw), which was at par with AOL 11-37 (Fig 1). Peroxidase does not play direct role in resistance against pathogen but plant disease resistance has often been correlated with elevated peroxidase activity and the oxidation of phenolics in diseased tissues. The role of peroxidase in plant defence mechanism has been attributed to its ability to oxidize key metabolites such asphenolics in plant or pathogen (Chittoor *et al.,* 1999). Ye and his co-workers (1990) have also recorded positive correlation between peroxidase activities of tobacco leaves to TMV. Peroxidase is also involved in lignin synthesis and degradation of cytotoxic levels of hydrogen peroxide entered in plant tissues as a result of pathogen attack (Van loon, 1997).

Polyphenol oxidase Activity

Maximum polyphenol oxidase activity was observed in resistant GAO-5 (3.17 Δ O.D. min^{-1} g^{-1} fw) which was at par with AOL 11-49. Minimum Polyphenol oxidase activity was recorded in genotype Pusasawani (1.23 Δ O.D. min^{-1} g^{-1}fw) (Fig 2). Khorsheduzzaman *et al.,* (2010) studied that from less susceptible genotypes of brinjal had the higher polyphenoloxidase activity. The PPO specific activity induced in resistant okra genotype could be a defensive response against YVMV infection and seems to be related to disease resistance. The polyphenol oxidase (PPO) seems to be compartmentalized in plant cells but as a result of pathogenic attack or during tissue senescence, membrane disruption may occur which can initiate formation of quinones following an increase in accessibility of PPO to its substrate (Mohammadi and Kazemi, 2002). The higher activities in resistant genotypes justify the role of antioxidant enzymes to maintain the nontoxic levels of H_2O_2 in cells or control the signal flux. The higher activity of peroxidase and PPO were observed in resistant pearl millet genotypes against downy mildew as compare to susceptible by Sapre *et al.,* (2014).

Whitefly

Ten genotypes of okra were evaluated against the population of whitefly at 35 and 60 DAS. The number of whitefly was recorded per 3 leaves/plant and data was presented in Table 1. Among all okra genotypes GAO-5 having minimum number (0.07) of whitefly as compared to rest of all genotypes, which was at par with AOL 10-22 (0.38) and AOL 11-49 (0.58).

Significantly maximum number (9.23) of whitefly was recorded in Pusasawani at 35 DAS. At 60 DAS Pusasawani showed the highest population(14.77), while GAO-5 (0.11) having the lowest population. Mean population of whitefly of both (35 DAS and 60 DAS) periods were checked and the results revealed that minimum whitefly population observed for genotype GAO-5 (0.09), which was at par with AOL 10-22 (0.49) and AOL 11-49 (0.76) as compared to all other genotypes. The susceptible variety Pusa-sawani recorded with maximum whitefly population (12.00). Thus from above results it can be proved that insects can damage more to susceptible varieties/genotypes as compared to resistant genotypes which was also reported by Ali *et al.,* (2005) in okra. The correlation between whitefly and biochemical constituents such as peroxides and polyphenol oxidase activities was (Table 2) found significant. The negative correlation was recorded between enzyme activities and population of whitefly. Thus with increasing activities of peroxidase or polyphenol oxidase the number of whitefly are decreased which indicate the defensive role of plants against whitefly.

Isozyme study

Peroxidase isozyme

The isozyme pattern of peroxidase was recorded among different okra genotypes. Total 6 types of bands were observed. The maximum numbers of bands four were recorded in GAO-5 and AOL 11-49. The genotypes GAO-5 and AOL 11-49 were differentiated by two unique bands having Rmvalue 0.454 and 0.471. Chawla *et al.,* (2014) have reported specific peroxidase band in two treatments which may give resistance to the penetration of pathogens.

Jaccard's similarity coefficient was calculated for all possible pairs of 10 genotypes of okra (Table 3).

Table.1 Number of whitefly in different okra genotypes (Pooled over periods)

Genotype	35 Days		60 Days	Pooled
GAO-5	0.75[a] (0.07)		0.78[a] (0.11)	0.77[a] (0.09)
AOL 10-22	0.94[ab] (0.38)		1.05[ab] (0.60)	0.99[ab] (0.49)
AOL 11-49	1.03[ab] (0.58)		1.18[b] (0.93)	1.10[ab] (0.76)
AOL 09-17	1.65[c] (2.28)		2.01[c] (3.65)	1.83[c] (2.96)
AOL 11-39	1.08[b] (0.66)		1.25[b] (1.06)	1.16[b] (0.86)
AOL 11-34	1.67[c] (2.35)		2.05[c] (3.77)	1.86[c] (3.06)
Parbhanikranti	1.42[c] (1.51)		1.71[b] (2.41)	1.56[c] (1.96)
Pusasawani	3.12[e] (9.23)		3.91[e] (14.77)	3.51[e] (12.00)
AOL 09-2	2.10[d] (3.29)		2.59[d] (6.22)	2.34[d] (5.06)
AOL 11-37	2.14[d] (4.06)		2.65[d] (6.50)	2.39[d] (5.28)
Mean	1.59 (2.50)		1.92 (4.00)	1.75 (3.25)
S.Em±	**T**	0.09	0.12	0.12
	P	-	-	0.04
	T X P	-	-	0.11
C. D. at 5%	**T**	0.28	0.37	0.37
	P	-	-	-
	T X P	-	-	0.31
C. V. %	10.162		11.124	10.79
Figures in parentheses are retransformed values; those outside are square root transformed values				
Means sharing similar letters are not significantly different by DMRT at P = 0.05				

Table.2 Correlation between enzyme activities and insect population

	Whitefly 35 days	Whitefly 60 days
Peroxidase	-0.776**	-0.776**
PPO	-0.776**	-0.776**

Table.3 Genetic similarity matrix of peroxidase data based on Jaccard's similarity coefficient

	AOL-09-2	AOL-09-17	AOL-10-22	AOL-11-34	AOL-11-37	AOL-11-39	AOL-11-49	GAO-5	Parbhani-kranti	Pusa-sawani
AOL-09-2	1.00									
AOL-09-17	1.00	1.00								
AOL-10-22	0.50	0.50	1.00							
AOL-11-34	0.33	0.33	0.50	1.00						
AOL-11-37	0.50	0.50	0.25	0.50	1.00					
AOL-11-39	1.00	1.00	0.50	0.33	0.50	1.00				
AOL-11-49	0.20	0.20	0.33	0.20	0.25	0.20	1.00			
GAO-5	0.20	0.20	0.33	0.20	0.25	0.20	1.00	1.00		
Parbhani-kranti	1.00	1.00	0.50	0.33	0.50	1.00	0.20	0.20	1.00	
Pusa-sawani	0.50	0.50	0.25	0.50	1.00	0.50	0.25	0.25	0.50	1.00

Table.4 Genetic similarity matrix of PPO data based on Jaccard's similarity coefficient

	AOL-09-2	AOL-09-17	AOL-10-22	AOL-11-34	AOL-11-37	AOL-11-39	AOL-11-49	GAO-5	Parbhani-kranti	Pusa-sawani
AOL-09-2	1.00									
AOL-09-17	0.75	1.00								
AOL-10-22	0.33	0.25	1.00							
AOL-11-34	0.40	0.60	0.25	1.00						
AOL-11-37	0.50	0.75	0.33	0.75	1.00					
AOL-11-39	0.60	0.80	0.20	0.80	0.60	1.00				
AOL-11-49	0.50	0.40	0.33	0.40	0.20	0.60	1.00			
GAO-5	0.50	0.40	0.33	0.40	0.20	0.60	1.00	1.00		
Parbhani-kranti	0.60	0.80	0.20	0.80	0.60	1.00	0.60	0.60	1.00	
Pusa-sawani	0.60	0.80	0.20	0.80	0.60	1.00	0.60	0.60	1.00	1.00

Table.5 Genetic similarity matrix of Total protein data based on Jaccard's similarity coefficient

	AOL-09-2	AOL-09-17	AOL-10-22	AOL-11-34	AOL-11-37	AOL-11-39	AOL-11-49	GAO-5	Parbhani-kranti	Pusa-sawani
AOL-09-2	1.00									
AOL-09-17	0.40	1.00								
AOL-10-22	0.36	0.31	1.00							
AOL-11-34	0.88	0.30	0.39	1.00						
AOL-11-37	0.67	0.44	0.39	0.75	1.00					
AOL-11-39	0.50	0.67	0.25	0.38	0.38	1.00				
AOL-11-49	0.39	0.33	0.91	0.42	0.42	0.27	1.00			
GAO-5	0.33	0.29	0.92	0.36	0.36	0.23	0.83	1.00		
Parbhani-kranti	0.63	0.57	0.23	0.50	0.33	0.80	0.25	0.21	1.00	
Pusa-sawani	0.67	0.63	0.29	0.56	0.75	0.57	0.31	0.27	0.50	1.00

Fig.1 Peroxidase activity of different okra genotypes

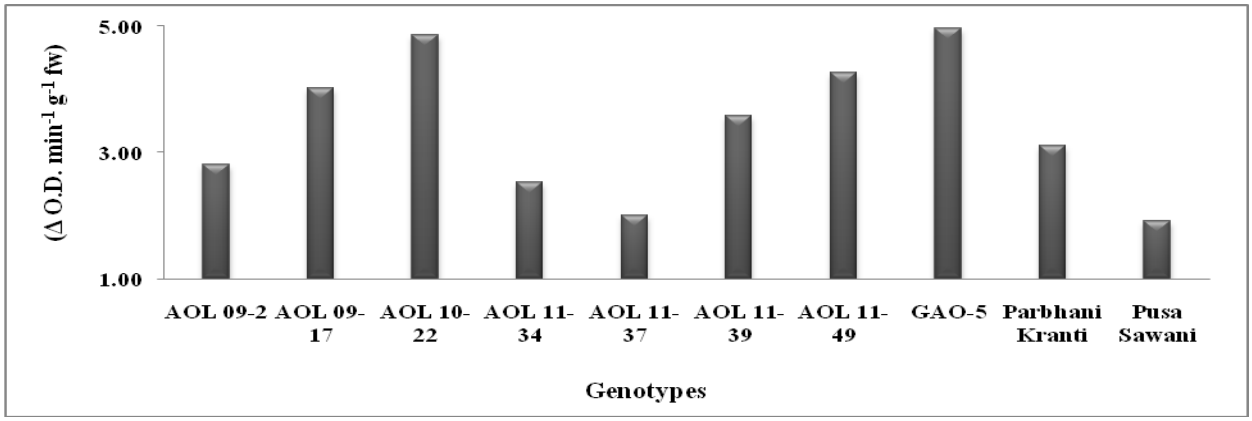


Fig.2 Polyphenol oxidase activity of different okra genotypes

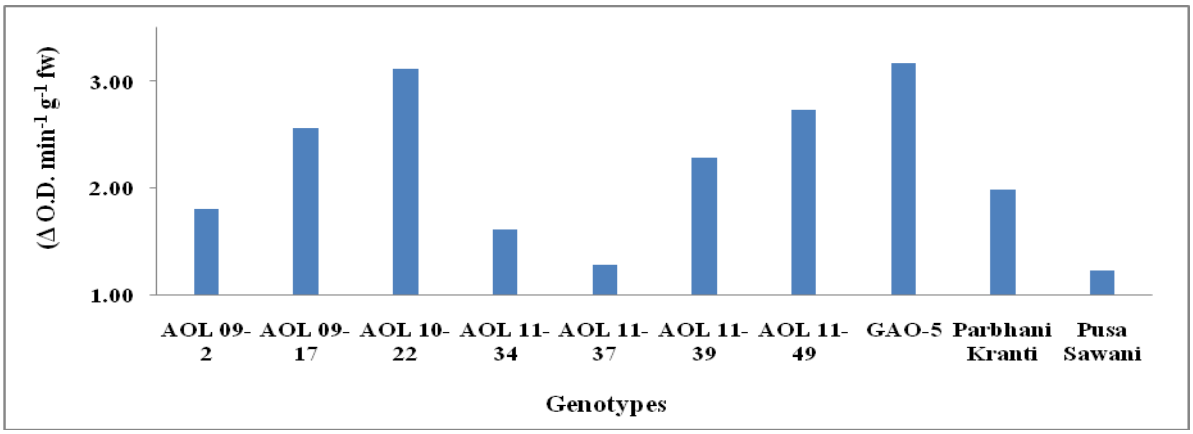


Fig.3 Dendogram showing clustering of peroxidase isozyme

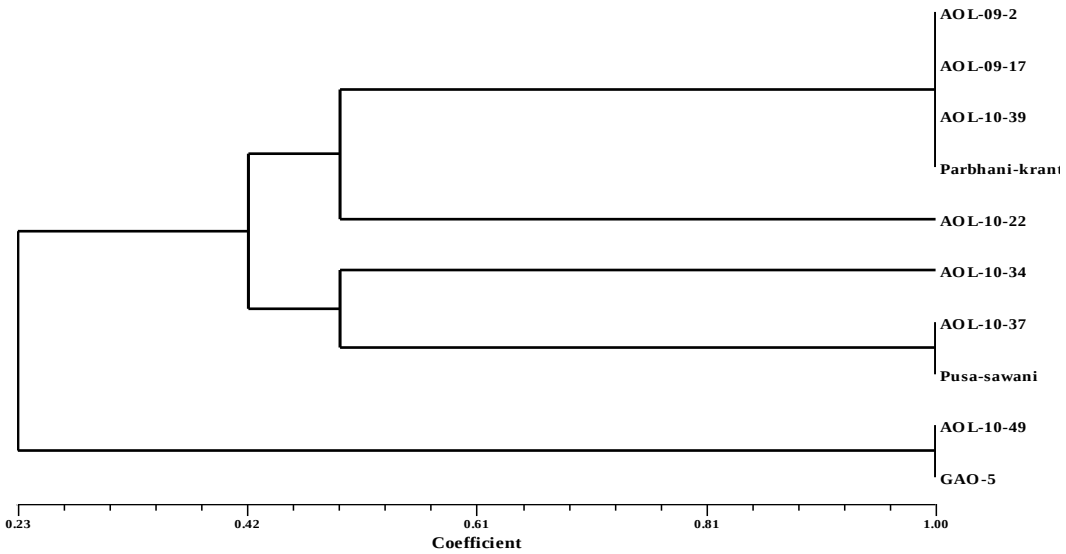


Fig.4 Dendogram showing clustering of PPO isozyme

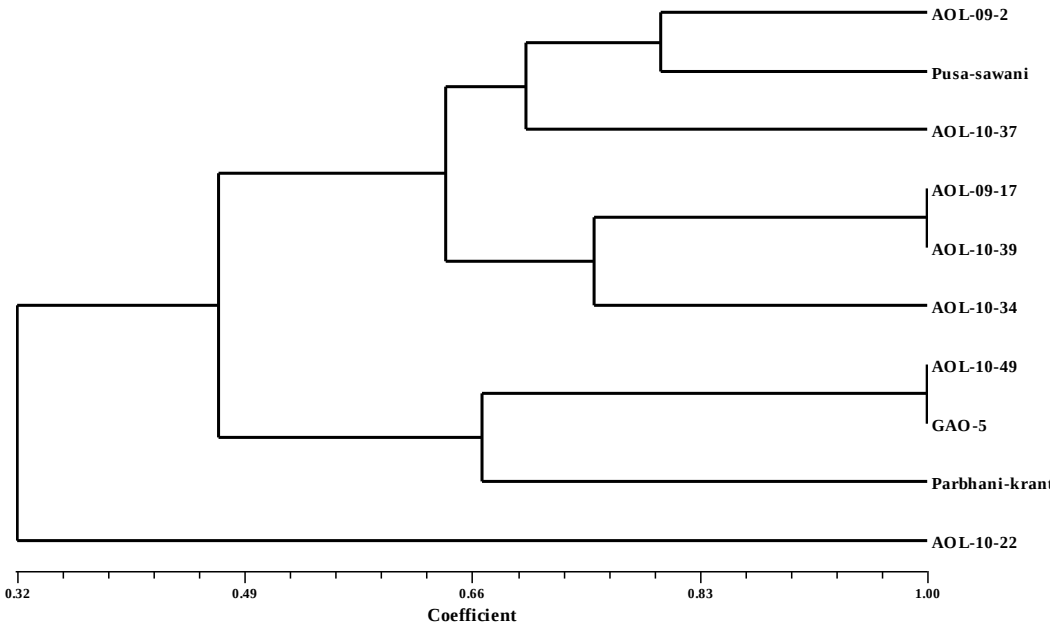
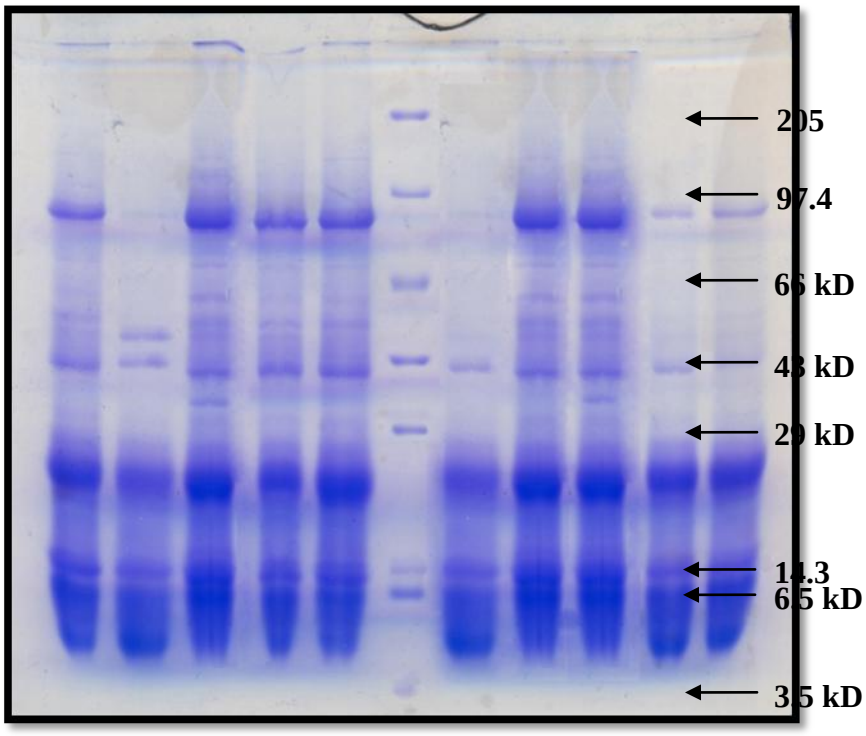
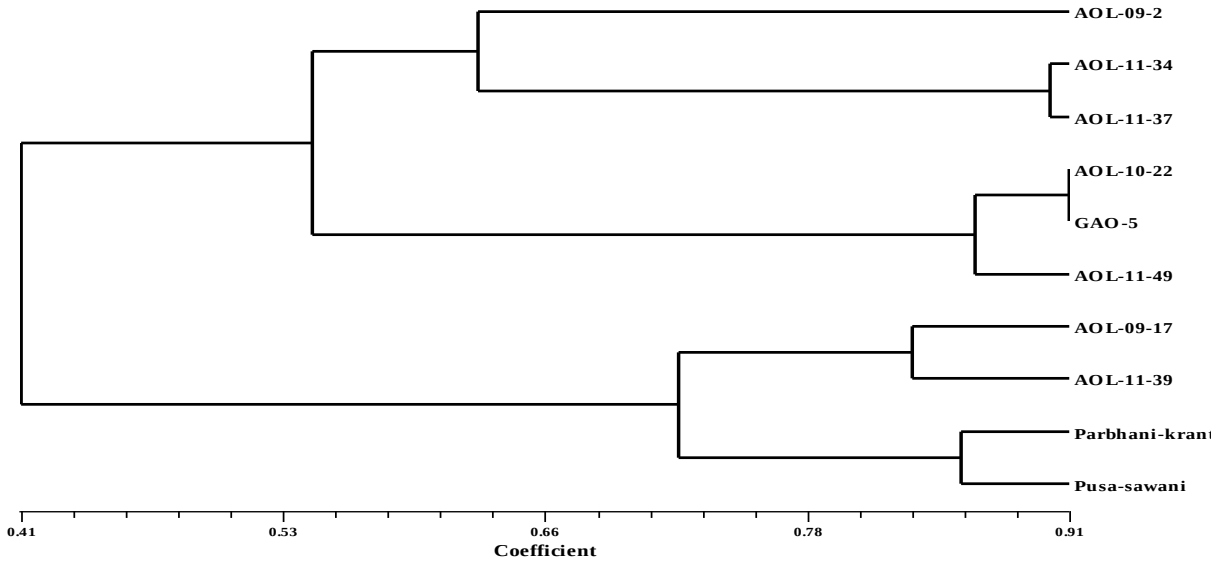


Fig.5 SDS page of leaf protein from different okra genotypes.



M = Protein marker

Fig.6 Dendogram showing clustering of 10 okra genotypes constructed obtained from Total protein analysis



The highest and the lowest similarity index value were found 0.5 and 0.2 respectively, whereas the average similarity coefficient among genotypes was 0.57 (Table 3). Based on this similarity index a dendogram was prepared and shown in Fig 3. Resistant genotypes included in Cluster B, GAO-5 and AOL 11-49, while cluster A was subclassified in two sub clusters which included susceptible Pusasawani, AOL 11-34 and AOL 11-37 and moderately resistant genotypes AOL 09-2, AOL 09-17, AOL 11-39 and Parbhanikranti.

Polyphenol oxidase isozyme

The polyphenol oxidase isozyme was observed among different okra genotypes. Polyphenol oxidase was observed with total five different types of bands. Pusasawani and AOL 11-39 genotypes observed with all five bands. The band having Rm value 0.744 was observed in all genotypes except resistant genotypes GAO-5, AOL 10-22 and AOL 11-49. Similar type of study carried out by Khorsheduzzaman *et al.*, (2010) in different brinjal genotypes.

Jaccard's similarity coefficient was calculated for all possible pairs of 10 genotypes of okra (Table 4). The lowest similarity index value 0.20 was found between GAO-5 and AOL 11-39, whereas the highest similarity index value 1.0 was found between GAO-5 and AOL 11-49 and the average similarity coefficient among genotypes was 0.64 (Table 4). Based on this similarity index a dendogram was prepared and shown in Fig 4.The dendogram contains 2 clusters in which one cluster contains only resistant genotype AOL 10-22. The other main cluster divided into 2 sub clusters A1 and A2. Cluster A2 contains resistant genotype GAO-5 and moderately resistant genotypes AOL 11-49 and Parbhanikranti.

SDS-PAGE of okra leaves protein

The total proteins were fractioned into fifteen bands among different genotypes (Fig 5). The maximum and minimum numbers of bands were observed in GAO-5(11) and AOL 11-39 (5). The resistant genotypes GAO-5 and AOL 10-22 were differentiated by one unique band having Rm value 0.529. Torkpo *et al.*, (2006)

analyzed 20 okra (*Abelmoschus esculentus* L.) accession diversity through total as well as seed storage proteins. A total of 34 reproducible and easily scorable bands were observed with the number of bands per accession ranging from 1 - 21.

Jaccard's similarity coefficient on the basis of presence and absence of bands was calculated for all possible pairs of 10 genotypes of okra (Table 5). The highest similarity index value 0.92 was found between GAO-5 and AOL 10-22, while the least similarity index value 0.21 was found between GAO-5 and Parbhanikranti. The average similarity coefficient among genotypes was 0.57 (Table 5). Based on this similarity index a dendogram was prepared and shown in Fig 6.

Clustering pattern of total protein

Two clusters namely A and B were formed at a similarity coefficient of 0.41 (Fig. 6). Cluster A consist genotypes AOL 09-2, AOL 11-34 and AOL 11-37 in one minor cluster, while genotypes GAO-5, AOL 10-22 and AOL 11-49 in another minor cluster. Cluster B consist two minor clusters. One minor cluster consist genotype AOL 09-17 and AOL 11-39, while other minor cluster consist Parbhanikranti and Pusasawani. As per above results SDS-PAGE is more useful for screening of okra genotypes as compare to isozymes.

Results of this study indicated that ten genotypes of okra can be divided into three groups: resistant to YVMV (GAO-5, AOL-10-22 and AOL-11-49), moderately resistant (AOL-09-17, AOL-11-39, AOL-11-34 and Parbhani-kranti), susceptible to YVMV (Pusa-sawani, AOL-09-2 and AOL-11-37).Resistant genotypes showed more number of bands then the susceptible, which may be a useful source of resistant genes to yellow vein mosaic virus. The GAO-5 was found to be most promising variety against yellow vein mosaic infestation in the field.

References

Ali, S., Khan, M.A., Habib, A., Rasheed SandIftikhar, Y. 2005. Correlation of environmental conditions with okra yellow vein mosaic virus and *Bemisiatabaci* population density. *Int. J. Agril. Bio.,* 7(1): 142-144.

Chawla, N., Kaur, H., Pathak, M. and Chawla, R. 2014. Effect of different seed priming treatments on activity and isozyme pattern of antioxidant enzymes in okra.*I nternational J. Adv. Res.,* 2(10):662-670.

Chittoor, J.M., Leach, J.E. and White, F.F. 1999.Induction of peroxidase during defense against pathogens, in: S.K. Datta, S. Muthukrishnan (Eds.), Pathogenesis: Related Proteins in Plants, CRC Press, Boca Raton, FL, pp 291.

Gandhi, K., Chaudhary, N., Litoriya, N., Patel, N.J. and Talati, J.G. 2015. Identification of cotton (*Gossypium herbaceum*L.) genotypes using electrophoretic techniques. *Indian J. Agric. Biochem.,* 28(2): 122-127.

Guibault, G.G. 1976. Handbook of enzymatic methods of analysis. Marcel Dekker, Inc. New York: 147.

Khorsheduzzaman, K.M., Alam, M.Z., Rahman, M., Mian, A., Kand Mian, I.H. 2010. Biochemical basis of resistance in eggplant (*Solanum melongena*L.) to *Leucino desorbonalis* Guenee and their correlation with shoot and fruit infestation. *Bangladesh J. Agri. Res.,* 35(1): 149-155.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* 227: 680-685.

Lagrimini, L.M. and Rothstein, S. 1987. Tissue specificity of tobacco peroxidase isozymes and their induction by wounding and tobacco mosaic virus infection. *Plant Physiol.,* 84: 438-442.

Lamont, Jr. W.J. 1999. Okra a versatile vegetable crop. *Horticultural Technol.*, 9(2): 179-184.

Malik, C.P. and Singh, M.B. 1980. Plant enzymology and Histoenzymology. Plant enzymology and Histoenzymology. Kalyani publishers, New Delhi 110 002.

Mohammadi Mand Kazemi, H. 2002.Changes in peroxidase and polyphenol oxidase activities in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. *Plant Sci.,* 162: 491-498.

Ndunguru, J. and Rajabu, A.C. 2004. Effect of okra mosaic virus disease on theabove-ground morphological yieldcomponents of okra in Tanzania. *Scientia Horticulturae,* 99: 225-235.

Sanwar, S.K., Singh, M., Singh Band Naik, P.S. 2014. Resistance to Yellow Vein Mosaic Virus and Okra Enation Leaf Curl Virus: challenges and future strategies. *Curr. Sci.,* 106(11): 1470-1471.

Sapre, S.S., Bosamia, T.C., Shah, A.K. and Talati, J.G. 2014. Enzymatic study and proximate composition of pearl millet genotypes in relation to downy mildew. *Indian J. Agric. Biochem.,* 27(1): 13-19.

Torkpo, S.K., Danquash, E.Y., Offei, S.K. and Blay, E.T. 2006. Esterase, Total Protein and Seed Storage Protein Diversity in Okra. *West African J. Appl. Ecol.,* 9: 1-7.

Van Loon, L.C. 1997. Induced resistance in plants and the role of pathogenesis-related proteins. *Eur. J. Plant Pathol.*, 103: 753–765.

Ye, X.S., Pan, S.Q. and Juc, J. 1990. Activity, Isozyme pattern and cellular localization of Peroxidase as related to Systemic Resistance of Tobaco to Blue Mold (*Peronosporatabacina*) and to Tobaco Mosaic Virus. *Phytopathol.,* 80: 1295-1299.

How to cite this article:

Jalpesh S. Patel, A. R. Japda, J.J. Dhruve and Patel, N.J. 2017. Antioxidant Enzymes in Leaves of Susceptible and Resistant Okra Genotypes against YVMV. *Int.J.Curr.Microbiol.App.Sci.* 6(2): 1540-1550. doi: http://dx.doi.org/10.20546/ijcmas.2017.602.172