International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(1): 577-579

Received: 01-11-2018
Accepted: 03-12-2018

NT Hirgal
M.Sc. Student, Pulses Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar, Maharashtra, India

SB Latake
Assistant Professor, Pulses Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar, Maharashtra, India

AP Chavan
Assistant Professor, Pulses Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar, Maharashtra, India

Correspondence
SB Latake
Assistant Professor, Pulses Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar, Maharashtra, India

Studies on biochemical changes in cowpea in response to virus infection

NT Hirgal, SB Latake and AP Chavan

Abstract
Infection due to any pathogen triggers a series of reaction in plant which alters the levels of various pathogenesis related (PR) proteins governing the degree of resistance of that plant. Amongst the various plant viruses infecting cowpea crop, Blackeye cowpea mosaic virus (BICMV) is one of the important and widespread one responsible for reduction in yield of the crop. Changes in the PR proteins *viz*. β-1, 3 glucanase, Phenylalanine ammonia lyase (PAL), peroxidase and polyphenol oxidase were estimated from leaves of cowpea genotypes inoculated with BICMV. The results indicated that resistant varieties had higher levels of PR proteins than moderately resistant. In susceptible variety decreasing trend in PR proteins over the period was recorded indicating that more induction of PR proteins in cowpea is positively correlated with resistance to BICMV.

Keywords: Cowpea, BICMV, PR proteins, reiststance

1. Introduction
Diseases are prominent biotic stresses known to affect productivity of pulses and particularly that of cowpea crop. Amongst various biotic stresses of the crop, viruses constitute the major group of pathogens (Mali and Thottapilly, 1986) [11]. In fact, viral diseases are significantly contributing to the reduced yield of cowpea in Asia, Africa and Latin America. The effect of viruses could be devastating and a major constraint to the production of cowpea. The crop is infected by about 40 viruses worldwide (Hughes and Shoyinka, 2003) [5]. Blackeye cowpea mosaic virus (BICMV) belonging to potyvirus group is the dominant and important one infecting cowpea crop in Maharashtra state (Mali and Kulthe, 1980) [10]. The virus is reported to cause varying degrees of symptoms ranging from mild mosaic, mottling, yellowing, leaf distortion to stunting in cowpea.
Infection by pathogen triggers a defense mechanism in the plant due to which the levels of various biochemical parameters either increases or decreases. These biochemical synthesized in plant after pathogen infection plays a crucial role in governing resistance of plant to the pathogen. In fact, these are important markers in deciding resistance or susceptibility of the plant. Therefore, during present investigation, levels of various pathogenesis related (PR) proteins in cowpea cultivars in response to BICMV infection were estimated.

2. Material and Methods
The virus infected cowpea leaves typical symptoms of BICMV were collected from farmer's field and experimental farm of Pulses Improvement Project, MPKV, Rahuri. The virus was maintained under glasshouse conditions on susceptible cowpea variety VCM-8 as a systemic host. The identity of the virus was confirmed as BICMV by electron microscopy and host range studies. Twenty five (25) promising cowpea genotypes obtained from Pulses Improvement Project, MPKV, Rahuri were screened under glasshouse conditions against BICMV by artificial sap inoculation method. The seedlings were raised in earthen pots filled with sterilized soil and were inoculated with the virus sap at vegetative growth stage. The plants were observed regularly for virus symptoms and per cent disease incidence was worked out. Based on disease incidence, genotypes were categorized as resistant (< 10%), moderately resistant (10-30%) and susceptible (> 30%). Genotypes showing resistant, moderately resistant and susceptible reaction were selected for biochemical analysis. For this, seeds of theses genotypes were sown under glasshouse conditions and the plants were inoculated with the virus sap. The changes in the levels of various PR proteins *viz*., β-1, 3 glucanase, Phenylalanine ammonia lyase (PAL), peroxidase and polyphenol oxidase from the leaves of the plants by adopting standard procedure 15, 30, 45 and 60 days after sowing of the seeds.

Firstly, soluble protein content was determined by method of Lowry *et al*. (1951) [9] while reducing sugar by Nelson Somogyi's method (Somogyi, 1952) [16]. The assay of β-1, 3 glucanase was carried out as per method described by Rakshit *et al*. (2000) [14], PAL activity by method of Campos *et al*. (2004) [4] and peroxidase and polyphenol oxidase was determined by method given by Kumar and Khan (1982) [8].

3. Results and Discussion

Upon artificial inoculation, the virus produced symptoms viz., chlorosis, mosaic, mottling and stunting etc. on the susceptible cultivar VCM-8 within 20-22 days under glasshouse conditions. The electron microscopy of the virus was carried out using Transmission Electron Microscope (TEM) available with Department of Plant Pathology and Agril. Microbiology, MPKV, Rahuri. The microphotographs revealed the presence of flexous rod shaped particles of the virus typical to potyvirus group. The host range was typical to that of BICMV. Thus based on symptoms, particle morphology and host range the virus was identified as BICMV which is regarded as strain of Bean common mosaic virus.

4. Biochemical changes in cowpea genotypes due to BICMV

On the basis of disease reaction data of the 25 genotypes screened against BICMV by mechanical sap inoculation, two resistant (Phule vithai and PCP-090210), two moderately resistant (PCP-09024 and Phule Pandhari) and one susceptible (PCP-09037) genotype were analyzed for levels of various biochemical parameters.

5. β-1, 3 glucanase

The data obtained on activity of β-1, 3 glucanase from inoculated leaves of cowpea cultivars is presented in Table 1. It was observed that the β-1, 3 glucanase activity was markedly increased in resistant varieties *viz.*, Phule Vithai and PCP-090210 (1.95 to 8.41, 1.94 to 9.05 mg of glucose released mg-1 protein hr-1, respectively) than moderately resistant varieties *viz.*, PCP-09024 and Phule Pandhari (1.49 to 5.60 and 1.95 to 6.09 mg of glucose released mg-1 protein hr-1, respectively) from15 to 60 DAS. However the susceptible variety (PCP-09037) showed lowest activity of β-1, 3 glucanase. Thus, comparatively higher β-1, 3 glucanase activity was recorded in resistant cultivars.

These results are in agreement with Rakshit *et al*. (2000) [14] who reported that β-1, 3 glucanase activity in powdery mildew resistant lines (1.87 ± 0.20 µ mole glucose eq min-1mg-1 protein) was 2.03 times more than powdery mildew susceptible lines (0.92 ± 0.20 µ mole glucose eq min-1 mg-1 protein). Similarly, maximum enhancement of β-1, 3 glucanase activity in pea cultivars resistant to *Erysiphi polygoni* than susceptible cultivars was observed by Katoch *et al*. (2004) [7].

Table 1: β-1, 3 glucanase (mg of glucose released mg^{-1} protein hr^{-1}) activity from the leaves of cowpea genotypes

Genotype	15 Das	30 Das	45 Das	60 Das
PCP 09024 (MR)	1.49	2.15	4.80	5.60
Phule Pandhari (MR)	1.95	2.20	4.20	6.09
PCP 09037 (S)	1.10	1.34	1.52	1.29
Phule Vithai (R)	1.95	4.97	7.51	8.41
PCP 090210 (R)	1.94	4.70	8.13	9.05
Mean	1.68	3.07	5.23	6.09
SE (±)	0.011	0.010	0.010	0.012
CD at 5%	0.033	0.032	0.031	0.038

MR-Moderately resistant, S-Susceptible, R-Resistant

6. Phenylalanine ammonia lyase (PAL)

A significant increase in PAL activity was observed in resistant variety than susceptible. The increasing trend of PAL was noticed in varieties viz., Phule Vithai and PCP-090210 at 15, 30, 45 and 60 DAS than moderately resistant varieties *viz.*, PCP-09024 and Phule Pandhari. The susceptible variety PCP-09037 had lowest PAL activity.

Similar results were reported by many researchers. Kale and Choudhary (2001) [6] investigated expression of PAL activity in groundnut cultivar in response to biotic stress *Cercoporidum personatum.* Maximum activity was observed in resistant cultivars as compared to susceptible cultivar. However, increased level of PAL enzyme in resistant and susceptible cotton plants after inoculation with *Verticillium dahlia* were recorded by Xu *et al.* (2011) [17], but the increase was greater in the resistant lines as compared to control. Patel *et al*. (2013) [13] investigated the biochemical changes in mungbean induced by MYMV and reported that PAL was found in decreasing trend in the susceptible leaves as compared to resistant.

Table 2: Phenylanine ammonia lyase (µ moles of cinnamic acid mg^{-1} protein hr^{-1}) activity from the leaves of cowpea genotypes

Genotype	15 Das	30 Das	45 Das	60 Das
PCP 09024 (MR)	0.98	1.54	2.75	3.20
Phule Pandhari (MR)	0.95	1.95	3.04	3.43
PCP 09037 (S)	0.64	0.81	0.90	0.73
Phule Vithai (R)	1.40	3.23	5.88	7.14
PCP 090210 (R)	1.47	4.05	6.63	7.71
Mean	1.09	2.32	3.84	4.44
SE (±)	0.099	0.010	0.011	0.010
CD at 5%	0.260	0.031	0.032	0.030

7. Peroxidase and polyphenol oxidase

Similar trend as that β-1, 3 glucanase activity was also observed in case of peroxidase and polyphenol oxidase activity. Higher peroxidase and polypenol oxidase activity was recorded in resistant cultivars than susceptible. The two resistance varieties *viz*., Phule Vithai and PCP-090210 showed increasing peroxidase and ploy phenol oxidase activity at 15, 30, 45 and 60 DAS. However, lowest activity was recorded from the susceptible variety (PCP-09037) indicating its susceptibility to the virus.

Marked increase in the peroxidase activity in resistant genotypes had been reported earlier by various workers *viz*., infection of sterility mosaic in pigeonpea (Bhite *et al.* 1997) [3], wilt in chickpea (Singh *et al*. 2003) and *Rhizoctonia* in Norway sprus (Nagy *et al.* 2004) [12]. Similarly, Anuradha *et al*. (2015) [1] studied the biochemical changes in Banana due to Banana Bunchy Top Virus and found that the amount of peroxidase was significantly higher in resistant plant. As regards polyphenol oxidase activity Arora *et al*. (2009) [2] inoculated resistant and susceptible genotypes of mothbean with yellow mosaic virus and found that the activity of polyphenol oxidase showed an increasing trend in the inoculated leaves of resistant genotypes.

Thus, in general, it was noticed that the levels of various PR proteins showed an increasing trend in resistant and moderately resistant cultivars and the increase was more in resistant cultivars than the moderately resistant. In case of the susceptible cultivar the PR proteins increased upto 45 DAS and thereafter decreased at 60 DAS. This clearly indicated that more induction of PR proteins in cowpea plants is positively related with resistance to Blackeye cowpea mosaic virus suggesting that various PR proteins plays a role in

governing resistant/susceptibility of cowpea genotypes against BICMV. The findings of present investigation will be helpful in breeding programme for evolving disease resistant varieties as well as for management of the virus.

Table 3: Peroxidase (ΔA 340 min^{-1} mg^{-1} protein) activity from the leaves of cowpea genotypes

Genotype	15 Das	30 Das	45 Das	60 Das
PCP 09024 (MR)	0.80	1.95	2.63	3.41
Phule Pandhari (MR)	0.84	2.08	2.70	3.53
PCP 09037 (S)	0.30	0.41	0.55	0.37
Phule Vithai (R)	1.38	3.22	4.58	5.99
PCP 090210 (R)	1.41	3.32	4.85	6.81
Mean	0.95	2.19	3.06	4.02
SE (±)	0.009	0.010	0.01	0.07
CD at 5%	0.026	0.027	0.025	0.020

Table 4: Polyphenol oxidase (ΔA 340 min^{-1} mg^{-1} protein) activity from the leaves of cowpea genotypes

Genotype	15 Das	30 Das	45 Das	60 Das
PCP 09024 (MR)	0.24	0.55	0.95	1.21
Phule Pandhari (MR)	0.32	0.62	1.05	1.24
PCP 09037 (S)	0.16	0.25	0.27	0.20
Phule Vithai (R)	0.43	1.55	2.65	3.85
PCP 090210 (R)	0.49	1.92	2.71	4.11
Mean	0.32	0.98	1.53	2.12
SE (±)	0.062	0.008	0.067	0.08
CD at 5%	0.152	0.023	0.193	0.024

8. References

1. Anuradha C, Selvarajan R, Vasantha S, Suresha GS. Biochemical characterization of compatible plant virus interaction: A case study with bunchy top virus host pathosystem. J Plant. Pathol. 2015; 14(4):212-222.
2. Arora R, Joshi U, Gupta P, Singh J. Effect of yellow mosaic virus on pathogenesis related enzymes and chlorophyll content in mothbean. Acta. Phytopathol. Entomol. Hungarica. 2009; 44(1):49-60.
3. Bhite BR, Chavan JK, Kachare DP. A biochemical marker for resistance to sterility mosaic in pigeonpea. J Mah. Agric. Univ. 1997; 22(3):340-344.
4. Campos R, Nonogaki H, Suslow T, Saltveit MS. Isolation and characterization of a wound inducible phenylalanine ammonia lyase gene from romaine lettuce leaves. Physiologia Plantarum. 2004; 121(3):429-438.
5. Hughes JA. Shoyinka SA. Overview of viruses if legumes other than groundnut in Africa. In: Plant virology in sub-saharn Africa. Hughes JA, Odu J. (Eds.). International Institute of tropical Agriculture, Ibadan, Nigeria, 2003, 553-568.
6. Kale MC, Choudhary AD. Induction of phenylalanine ammonia lyase in groundnut cultivars in response to biotic and abiotic stress. Indain Phytopath. 2001; 54(3):288-292.
7. Katoch R, Mann ADS, Sohal BS, Munshi GD. Effect of elicitor spray and *Erysiphe polygoni* inoculation on β-1,3 glucanase activity in pea cultivars resistant and susceptible to powdery mildew. Indian J Plant Physiol. 2004; 9(3):316-319.
8. Kumar KB, Khan PA. Peroxidase and polyphenol oxidase in excised ragi (*Eleusine corocana* cv. PR-202) leaves during senescence. Indian J Exp. Biol. 1982; 20:412-416.
9. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin Phenol reagent. J Biol. Chem. 1951; 193(1):265-275.
10. Mali VR, Kulthe KS. A seedborne potyvirus causing mosaic disease of cowpea in India. Plant Dis. 1980; 64(10):925-928.
11. Mali VR, Thottappilly G. Virus diseases of cowpea in the tropics. Review of Tropical Plant Pathology. (Edited by Raychaudhari SP, Verma JP.) New Delhi, India; Today and Tomorrow's printer and Publishers, 1986, 3.
12. Nagy NE, Fossdal CG, Dalen LS, Lonneborg A, Heldal I, Johsen O. Effect of Rhizoctonia infection and drought on peroxidase and chitinase activity in Norway spruce (*Picea abies*). Physiologia Plantarum. 2004; 120(3):465-473.
13. Patel H, Kalaria R, Mahatma M, Chauhan DA. Physiological and biochemical changes induced by Mungbean yellow mosaic virus (MYMV) in mungbean (*Vigna radiate* L.). J Cell tissue Res. 2013; 1:34.
14. Rakshit S, Mishra SK, Dasgupta SK, Sharma B. Dynamics of β-1, 3 glucanase activity in powdery mildew resistant and susceptible lines of pea. J Pl. Biochem. Biotech. 2000; 9(2):95-98.
15. Singh G, Singh K, Kapoor SS, Verma MM, Bhan LK, Sidhu PS. Pigeonpea lines resistant to sterility mosaic. Indian Phytopath. 1987; 40:18-21.
16. Somogyi M. Notes on sugarcane determination. J Biol. Chem. 1952; 195(1):19-23.
17. Xu L, Zhu L, Tu L, Liu L, Yuan D, Jin L *et al*. Lignin metabolism has a central role in the resistance of cotton to wilt fungus *Verticillium dahlia* as revealed RNA-seq-dependent transcriptional analysis and biochemistry. J. Exp. Bot. 2011; 62(15):5607-5621.