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EFFECT OF SOME ROD SHAPED CUCURBIT VIRUSES ON CHLOROPHYLL *A* AND *B* CONTENTS IN *CUCURBITA MOSCHATA* POIR PLANTS AT DIFFERENT STAGES OF PATHOGENESIS

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The chlorophyll *a* content was reduced with respect to all isolates within 10 days after inoculation. At 40 days after inoculations, this component was found to be increased from that found at 10 days of inoculation with respect to $BGMV_1$, VYV, CLV_1, CLV_2 and WMV-1. But it was found to be reduced with BMG_2, CuMV and PEMV. No change of this component was however observed with PMMV. At 70 days after inoculation, the chlorophyll *a* content was reduced with $BGMV_1$, CLV_1, CLV_2, WMV-1, PEMV aud PMMV whereas with $BGMV_2$ and VYV it was found to increase from that found at 40 days after inoculation. At this stage of pathogenesis, no change of chlorophyll *a* content was observed with CuMV. Chlorophyll *b* content was also reduced with respect to all isolates at 10 days after inoculation. $BGMV_1$, CLV_1, CLV_2 and WMV-1 increased this component from that found at 10 days after inoculation and $BGMV_2$, VYV, CuMV, PEMV and PMMV decreased it as compared to 10 days after inoculation. At 70 days after inoculation $BGMV_1$, CLV_1, CuMV, CLV_2, WMV-1 PEMV and PMMV decreased this component from that found at 40 days after inoculation. But $BGMV_2$ and VYV increased it from that at 40 days after inoculation.

INTRODUCTION

Recently Ghosh and Mukhopadhyay isolated nine different rod-shaped viruses from the diseased plants collected from the fields of cucurbit growing

areas of W. Bengal. These isolates were identified by them as strains (Isolate A_1 and A_2) of Bottlegourd mosaic virus (Vasudeva and Lal, 1943), strains (Isolate A_4 and A_6) of cucurbit latent virus (Webb and Bohn, 1961), vein yellowing (Isolate A_3) virus (Harpaz and Cohen, 1965), cucurbit mosaic (Isolate A_5) virus (Hariharasubramanian and Badami, 1964), watermelon mosaic (Isolate A_7) virus-1 (Webb and Scott, 1965), pumpkin enation mosaic (Isolate A_8) virus (Ghosh and Mukhopadhyay, 1977) and pumpkin mild mosaic (Isolate A_9) virus (Ghosh and Mukhopadhyay, 1977.). All these viruses were found to differ from each other in their phenotypic characters such as physical properties host range, transmissibility and antigenic properties. The isolate A_1 resembled the isolate A_2 and the isolate A_4 resembled the isolate A_6 in their phenotypic properties to a large extent. With a view to find out whether the similar viruses induced similar biochemical alterations at least with respect to chlorophyll *a* and chlorophyll *b* contents, the present investigation has been undertaken.

MATERIAL AND METHODS

Healthy *C. moschata* seedling were raised in sufficient number in insect proof condition. The seedlings were separately inoculated at the age of 10 days with nine isolates, namely isolate A_1 or strain 1 of bottlegourd mosaic virus ($BGMV_1$), A_2 or strain 2 of bottlegourd mosaic virus ($BGMV_2$), A_3 or vein yellowing virus (VYV). A_4 or strain 1 of cucurbit latent virus (CLV_1), A_5 or cucurbit mosaic virus (CuMV), A_6 or strain 2 of cucurbit latent virus (CLV_2), A_7 or watermelon mosaic virus-1 (WMV-1), A_8 or pumpkin enation mosaic virus (PEMV) and A_9 or pumpkin mild mosaic virus (PMV), maintained in *C. moschata* plants following usual sap inoculation technique. The plants thus inoculated were kept under insect proof condition for the development of symptoms.

Leaf samples (apical three leaves) were collected from the inoculated plants and the healthy plants as well at three different stages of pathogenesis. The first collection was made at the time of symptom expression or in other words 10 days after inoculation (stage 1). The second collection was made at 1 month

after symptom expression that means 40 days after inoculation (stage II) and third collection was made at 2 months after symptom expression or in other words 70 days after inoculation (stage III).

To estimate the chlorophyll content (a and b) of the samples of all the collections, the samples were first thoroughly washed with glass distilled water. The method described by Mackinney (1941) and Yoshida *et al* (1971) were utilized for determination of chlorophyll contents. 2/g of each sample were cut into small pieces. The tissues were then grinded and homogenised in a glass morter and pestle, adding requisite quantity of 80 per cent acetone. The homogenisation was continued till tissues became completely chlorophll free. The supernatant was then decanted through filter paper (Whatman No. 42). The extraction process was repeated thrice following the similar procedure. Total volume of the supernatant was made upto 100 ml in a volumetric flask with 80 per cent acetone. Then 5 ml of this solution was transferred into a 50 ml volumetric flask and the volume was made up with 80 per cent acetone. Then samples were utilised for estimating chlorophyll content by spectrophotometric technique. 3 ml of the prepared solution was taken in each cuvette and the absorbancy of this solution was determined at two different wave lengths namely 663 mμ (for chlorophyll *a*) and 645 mμ (for chlorophyll *b*) in a Beckman Model DU Spectrophotometer. The absorbancy recorded in this way was converted to obtain the quantity of chlorophyll *a* and *b* present in the test solution by following the method of Yoshida *et al* (1971).

RESULTS

The results obtained on the effect of nine isolates on chlorophyll *a* and chlorophyll *b*, contents of *C. moschata* leaves at three different stages of pathogenesis have been presented in the Tables 1 and 2 respectively.

Table : 1. *Chlorophyll a content of the leaves of pumpkin* (C. moschata Poir) *plants at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates*

Isolates	Chlorophyll *a* content (mgm/gm of fresh weight			Average	'F' test	C. D. at 5%
	Stage I	Stage II	Stage III			
Bottlegourd mosaic virus ($BGMV_1$) (Isolate A_1)	0.15	0.25	0.06	0.15		
Bottlegourd mosaic virus ($BGMV_2$) (Isolate A_2)	0.12	0.05	0.09	0.08		
Vein yellowing virus (VYV) (Isolate A_3)	0.06	0.08	0.17	0.10		
Cucurbit latent virus (CLV_1) (Isolate A_4)	0.04	0.30	0.08	0.14		
Cucurbit mosaic virus CuMV) (Isolate A_5)	0.17	0.16	0.16	0.16		
Cucurbit latent virus (CLV_2) (Isolate A_6)	0.14	0.33	0.05	0.17	*	0.02
Watermelon mosaic virus-1 (WMV-1) (Isolate A_7)	0.12	0.27	0.05	0.14		
Pumpkin enation mosaic virus (PEMV) (Isolate A_8)	0.20	0.13	0.05	0.12		
Pumpkin mild mosaic virus (PMMV) (Isotate A_9)	0.15	0.15	0.06	0.12		
Healthy	0.24	0.35	0.24	0.27		
Average	0.13	0.20	0.10			
'F' test	*					
C. D. at 5%	0.01					

Stage x Isolate 'F' test = *
C. D. at 5% = 0.05

In case of chlorophll *a* content (Table 1), it was observed that all the isolates after being inoculated to healthy pumpkin plants, significantly reduced this component. When the first stage of pathogenesis was considered, it was observed that maximum reduction took place with isolates A_3 (VYV) and A_4 (CLV_1) where the percentages of reduction were found to be 75.0 and 83.3

respectively. Minimum reduction of chlorophyll *a* content was observed with the isolate A_8 (PEMV) where the percentage of reduction was found to be 16.6. In case of the other isolates namely A_1 ($BGMV_1$), A_2 ($BGMV_2$), A_5 (CuMV), A_6 (CLV_2), A_7 (WMV-1) and A_9 (PMMV), the respective percentages of reduction were found to be 37.6, 50. 0, 29.1, 41. 6, 50.0 and 37. 4.

Table 2. *Chlorophyll b content of the leaves of pumpkin* (C. moschata poir) *plants at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates.*

Isolate	Chlorophyll *b* content (mgm/gm of fresh weight)			Average	'F' test	C. D. at 5%
	Stage I	Stage II	Stage III			
Bottlegourd mosaic virus ($BGMV_1$) (Isolate A_1)	0.46	0.56	0.11	0.36		
Bottlegourd mosaic virus ($BGMV_2$ (Isolate A_2)	0.42	0.14	0.22	0.26		
Vein yellowing virus (VYV) (Isolate A_3)	0.25	0.22	0.41	0.29		
Cucurbit latent virus (CLV_1) (Isoate A_4)	0.18	0.70	0.23	0.37		
Cucurbit mosaic virus (CuMV) (Isolate A_5)	0.55	0.48	0.25	0.43		
Cucurbit latent virus (CLV_2) (Isolate A_6)	0.50	0.62	0.09	0.40	*	0.11
(Watermelon mosaic virus-1 (WMV-1) (Isolate A_7)	0.45	0.61	0.14	0.40		
Pumpkin enation mosaic virus (PEMV) (Isolate A_8)	0.70	0.33	0.08	0.37		
Pumpkin mild mosaic virus (PMMV) (Isolate A_9)	0.54	0.33	0.17	0.35		
Healthy	0.76	0.72	0.41	0.63		
Average	0.48	0.47	0.21			
'F' test	*					
C. D. at 5%	0.06					

Stage x isolate 'F' test = *
C. D. at 5% = 0.20

When the second stage of pathogenesis was considered, it was observed that the chlorophyll *a* contents of healthy leaves were found to increase. The percentage of increase in this case was found to be 45.8. Increase in chlorophyll *a* content was also observed at this stage with the isolates A_1 ($BGMV_1$), A_3 (VYV), A_4 (CLV_1), A_6 (CLV_2) and A_7 (WMV-1). The percentages of increase of the chlorophyll *a* content with respect to these isolates were 66.6, 33.3, 650.0, 135.7 and 125.0 respectively. But the chlorophyll *a* contents of the leaves with respect to isolates A_2($BGMV_2$), A_2 ($BGMV_2$), A_5 (CuMB) and A_8 (PEMV) were found to further decrease at this stage pathogenesis from that observed at the first stage. The respective percentages of decrease were 58.3, 5.8 and 35.0. With the isolate A_9 (PMMV), however, the chlorophyll *a* content remained more or less same at both the stages.

When the third stage of pathogenesis was considered, it was observed that the chlorophyll *a* content of healthy leaves was found to decrease. The percentage of decrease was found to be 31.4. At this stage of pathogenesis, reduction of chlorophyll *a* content was found with all the isolates except A_2($BGMV_2$) and A_3(VYV). The percentages of reduction with respect to the second stage for the isolates A_1 ($BGMV_1$), A_4 (CLV_1), A_6 (CLV_2), A_7 (WMV-1), A_8 (PEMV) and A_9 (PMMV) were 76.0, 73.3, 84.8, 81.4, 61.5 and 60.0 respectively. With the isolates A_2 ($BGMV_2$) and A_3 (VYV) the chlorophyll *a* content was found to increase further and the percentage of increase in them were found to be 80.0 and 112.5 respectively. With the isolate A'_5 (CuMV) on the other hand, the chorophyll *a* content did not alter from that found at the second stage.

In case of chlorophyll *b* (Table 2) content also it was observed that all the isolates, after being inoculated to healthy pumpkin plants significantly reduced this component. first stage of When the pathogenesis was considered, it was observed that as in case of chlorophyll *a*, maximum reduction of chlorophyll *b* content was observed with isolates A_4 (CLV_1) and A_3 (VYV), where the percentage of reduction were found to be 76.3 and 67.1 respectively. Minimum reduction of chlorophyll *b* content was observed with the isolate A_8 (PEMV), where the percentage of reduction was found to be 7.8. In case of

the other isolates namely A_1(BGMV$_1$), A_2(BGMV$_2$), A_5 (CuMV), A_6(CLV$_2$), A_7 (WMV-1) and A_9 (PMMV), the respective percentages of reduction were found to be 39.4, 44.7, 27.6, 34.2, 30.7 and 28.9.

When the second stage of pathogenesis was considered, it was observed that in contrast to chlorophyll *a*, the chlorophyll *b* content of healthy leaves were found to decrease. The percentage of decrease in this case was found to be 5.2. But this component was found to increase at this stage with the isolates A_1 (BGMV$_1$), A_4 (CLV$_1$), A_6 (CLV$_2$), and A_7 (WMV-1). The percentages of increase of the chlorophyll *b* content with respect to these isolates were 21.7, 288. 8, 24.0 and 35.5 respectively. But the chlorophyll *b* contents of the leaves with respect to isolates A_2 (BGMV$_2$), A_3 (VYV), A_5(CuMV), A_8 (PEMV) and A_9 (PMMV) were found to decrease at this stage of pathogenesis from that found at the first stage. The respective percentages of decrease were 66.6, 12.0, 12.7, 52.8 and 38.8.

When the third stage of pathogenesis was considered, it was observed that the chlorophyll *b* content of healthy leaves was found to decrease further. The percentage of decrease was found to be 4.3.0. At this stage of pathogenesis reduction of chlorophyll *b* content was found with all the isolates except A_2 (BGMV$_2$) and A_3(VYV). The percentages of reduction with respect to the second stage for the isolates A_1(BGMV$_1$), A_4(CLV$_1$), A_5(CuMV), A_6(CLV$_2$), A_7(WMV-1), A_8(PEMV) and A_9(PMMV) were 80.3, 67.1, 47.9, 85.4, 77.0, 75.7 and 48.4 respectively. With the isolates A_2 (BGMV$_2$) and A_3 (VYV), the chlorophyll *b* contents, however, were found to increase. The percentage of increase at this stage with respect to the second stage were 57.1 and 86.3 respectively.

DISCUSSION

It is apparent that the isolates altered the chlorodhyll *a* content in the inoculated leaves, but the pattern of alteration differed with the isolates, Within 10 days after inoculation, the chlorophhyll *a* content was found to decrease with all the isolates but with the advance of infection, however, the level of alterations

differed. After 40 days of inoculation, the chlorophyll *a* content was found to increase to large extentfrom that found after 10 days of inoculation with A_4 (CLV_1), A_6(CLV_2) and A_7 (WMV-1). High increse was found with A_1($BGMV_1$), moderate increase was found with A_3(VYV). On the other hand, high decrease was found with A_2($BGMV_2$), moderate decrease was found with A_8(PEMV), very slight decrease was found with A_5(CuMV) and no change was found with A_9 (PMMV). With the further advance of infection, or in otherwards, 70 days after inoculation, the chlorophyll *a* content was found to decrease to large extent from that found after 40 days of inoculation in A_1($BGMV_1$), A_4(CLV_1), A_6(CLV_2) A_7(WMV-1), A_8(PEMV) and A_9(PMMV). In A_3(VYV) it was found to increase maximum and moderate increase was found with A_2($BGMV_2$), whereas with A_5(CuMV) the chlorophyll *a* content remained more or less the same.

It is apparent that the isolates altered the chlorophyll *b* content in the inoculated leaves, but the pattern of alteration differed with the isolates. Within 10 days after inoculation, the chlorophyll *b* content was found to decrease with all the isolates but with the advance of infection, however, the level of alterations differed with the isolates. After 40 days of inoculation, the chlorophyll *b* content was found to increase to a large cxtent from that found after 10 days of inoculation with A_4(CLV_1) and moderate increase was observed with A_1($BGMV_1$), A_6(CLV_2) and A_7(WMV-1). On the other hand. High decrease was found with A_2($BGMV_2$) moderate decrease was found with A_8(PEMV) and A_9(PMMV) and very slight decrease was found with A_3(VYV) and A_5(CuMV). With the further advance of infection, or in otherwords 70 days after inoculation, the chlorophyll *b* content was found to decrease to a large extent from that found after 40 days of inoculation in A_1($BGMV_1$), A_4(CLV_1), A_5(CuMV), A_6(CLV_2), A_7(WMV-1) A_8(PEMV) and A_9(PMMV). But with A_3(VYV) it was found to increase in large extent and with A_2($BGMV_2$) the chlorophyll *b* content was found to moderately increased.

The pettern of alterations of both Chlorophyll *a* and *b* appears to be similar with respect to the different isolates. In case of the isolates $BGMV_1$, CLV_1, CLV_2 and WMV-1 both chlorophyll *a* and *b* first increased and then reduced at the third stage of pathogenesis. In case of the isolate $BGMV_2$, there was first

reduction of chlorophyll *a* and *b* content then there was an increase of bothof them at the third stage. In case of isolate VYV, the chlorophyll *a* content slightly increased at the second stage but greatly increased at the third stage. With the same isolate, the ehlorophyll *b* content first reduced then greatly increased. The chlorophyll *a* content with CuMV, no remarkable alterations of the chlorophyll *a* content were observed. The chlorophyll *b* content with this isolate and also with PEMV and PMMV, slightly decreased at the second stage and greatly decreased at the third stage. The same situation has also been observed with chlorophyll *a* content with PEMV. The chlorophyll *a* content with PMMV, however, did not alter very much.

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