INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2005/07–2–154–157
http://www.ijab.org

Effect of Bean Yellow Mosaic Virus on Physiological Parameters of *Vicia faba* and *Phaseolus vulgaris*

SAMEH KAMAL HEMIDA
Department of Botany, Faculty of Science, University of Assiut, Assiut, Egypt
Corresponding e-mail: samehhemida@yahoo.com

ABSTRACT

A virus causing mosaic, mottling, malformation and distortion in faba bean (*Vicia faba*) was found in various fields in Assiut Governorate, Egypt. The virus isolate was detected and identified as bean yellow mosaic virus (BYMV) using DAS-ELISA technique and by symptoms produced in some host plants. Pigment content (chlorophylls a, b and carotenoids), water soluble carbohydrates, total soluble proteins and total free amino acids were estimated in leaves of two host plants (*Vicia faba* and *Phaseolus vulgaris*) inoculated with BYMV for 4, 12 and 20 days. In *Vicia faba*, the virus isolate induced a highly gradual decline in photosynthetic pigments and an increase in carbohydrates and soluble proteins with age. However, free amino acids remained more or less constant. In *Phaseolus vulgaris,* pigment contents, carbohydrates and free amino acids were decreased with time. However, soluble proteins remained constant. Strategy of defense mechanism in each host plant against BYMV infection was varied.

Key Words: BYMV; Physiological effects; *Vicia faba; Phaseolus vulgaris*

INTRODUCTION

Faba bean is subjected to infection with different viruses all over the world such as broad bean wilt (Taylor & Stubbs, 1972), bean leaf roll (Ashby, 1984), echtes ackerbohnemosaik (Gibbs & Paul, 1970), faba bean necrotic yellow (Babin *et al*., 2000), bean yellow mosaic (Bos, 1969).

Faba bean is an important food legume crop in Egypt. Many viruses have been isolated and identified from faba bean plants on basis of host range, symptoms, negative staining and serology. The most important virus on faba bean is bean yellow mosaic which reported as a serious viral disease attacking a wide range of cultivated legumes plants (Allam & El-Kady, 1966; Makkouk, 1993; Rizkalla *et al*., 1995). In the survey that has been carried in Assiut governorate for virus infections in faba bean fields (2003/2004), different viral symptoms (e.g. mosaic, mottling, malformation and vein banding) were observed. These symptoms causing qualitative and quantitative deleterious effects on the plant yield. The observed symptoms are closely resembled to those of bean yellow mosaic virus (Bos, 1970). Various metabolites of host tissue were altered due to viral infection as reported by Naidu *et al*. (1986), Mohanty and Sridhar (1989), Srinivasulu and Jeyarajan (1990), Chakraborty *et al*. (1994), Clover *et al*. (1999), and Hemida and Abdel-Razik (2002). The objective of this investigation was, therefore, to isolate, to identify the causal virus and study its effects on some physiological parameters of the two host plants (*Vicia faba, Phaseolus vulgaris*).

MATERIALS AND METHODS

Virus isolation and identification. Leaf samples of naturally infected faba bean plants (*Vicia faba* L.) showing mosaic and malformation were selected during a survey in Assiut governorate. These samples were used as a virus source for mechanical inoculation of healthy faba bean seedlings kept in insect-proof glasshouse. The inoculated plants showing symptoms were used for further inoculations. Virus inoculum was prepared either from single local lesion developed on the inoculated leaves of *Chenopodium amaranticolor* or from systemically infected leaves of faba bean plants.

The virus isolate was identified according to: (i) differential hosts and symptomatology: seedlings of nine plant species belonging to four families were mechanically inoculated by virus sap prepared in phosphate buffer pH 7.0. Seven replicates were used for each plant species. The inoculated plants were kept in the glasshouse under observation for visual virus symptoms, (ii) by serological test: The presence of virus was determined by double antibody sandwich-enzyme linked immuno-sorbent assay (DAS-ELISA) as described by Clark and Adams (1977) using the bean yellow mosaic virus (BYMV) ELISA kit obtained from Agricultural Genetic Engineering Institute (AGERI), Agriculture Research Center (ARC), Giza, Egypt.

Effect of BYMV infection on different physiological parameters of *Vicia faba and Phaseolus vulgaris.* Pigment contents (chlorophyll a, b and carotenoids), water soluble carbohydrates, total water soluble proteins and total free amino acids were determined in the leaves of the two host

plants at 4, 12 and 20 days after inoculation with bean yellow mosaic virus (BYMV). The plants of both species were grown in 15 cm diameter pots under natural conditions in a glasshouse at 15-25°C. Seedlings of 4-5 leaf stage were selected for different analysis. Mechanical inoculation was done by a juice prepared from virus infected faba bean leaves ground in phosphate buffer, pH 7.0 using carhorundum (600 mesh) as an abrasive. Control plants were treated only by buffer in presence of dusted carborundum. Three replicates of each crop species either for infected or control treatment were used per period for each test. Leaves collected were at the same stage of each plant. The photosynthetic pigments, chlorophylls a, b and carotenoids were determined using the spectrophotometric method according to Metzner *et al*. (1965). Total soluble carbohydrates were determined by the anthrone-sulphuric acid method (Fales, 1951) as detailed by Badour (1959). The method of Lowry *et al*. (1951) was used for soluble protein determination. Free amino acids were determined according to Moore and Stein (1948).

Statistical Analysis. Experimental data were subjected to one way analysis of variance (ANOVA) and the differences between means were separated by the least significant difference (L.S.D.; Gomez & Gomez, 1984).

RESULTS

Symptoms expressed in some host plants. The characteristic symptoms induced by the virus in some test plants are listed in Table I. The virus produced a mild mosaic locally and systemically in *Vicia faba* accompanied with twisting and distortion of the leaves (Fig. 1a & b). Necrotic local lesions appeared in the inoculated leaves of *Chenopodium amaranticolor* and *Phaseolus vulgaris*. Systemic infection in these two hosts was varied; vein banding in the first and clear mosaic and crinkling in the second (Fig. 1c). No symptoms were recorded after virus-inoculation of *Beta vulgaris*, *Cucurbita pepo, Pisum sativum, Datura metal, D. stramonium* and *Nicotiana glutinosa*. Using DAS-ELISA, Table I also shows that bean yellow mosaic virus was found and confirmed to be the causal virus of the above mentioned symptoms.

Table I. Symptoms of the virus in some host plants and virus detection by DAS-ELISA test

Host	Symptoms		DAS-ELISA
	local	systemic	test
Chenopodiaceae			
Beta vulgaris	-	-	-
Chenopodium amaranticolor	NLL	VB	+
Cucurbitaceae			
Cucurbita pepo	-	-	-
Leguminosae			
Phaseolus vulgaris	NLL	M	+
Pisum sativum	-	-	+
Vicia faba	M	M	+
Solanaceae			
Datura metal	-	-	-
D. stramonium	-	-	-
Nicotiana glutinosa	-	-	+

- = negative reaction; + = positive reaction; NLL = necrotic Local Lesions; M = mosaic; VB = vein banding

Effect of the virus on physiology of the two host plants. In *Vicia faba* plants infected with BYMV, there was a highly gradual decline in photosynthetic pigments with age (Table II). This reduction was much more pronounced in chlorophyll a especially at the end of the experimental period in comparable with that of four days after infection. Accumulation of the carbohydrates was opposite to the

Fig. 1. *Vicia faba* plants: (a) Healthy plant, (b) Bean yellow mosaic virus – infected leaves showing mottling, malformation and mosaic and (c) *Phaseolus vulgaris* leaves infected systemically with BYMV and showing a clear mosaic and crinkling

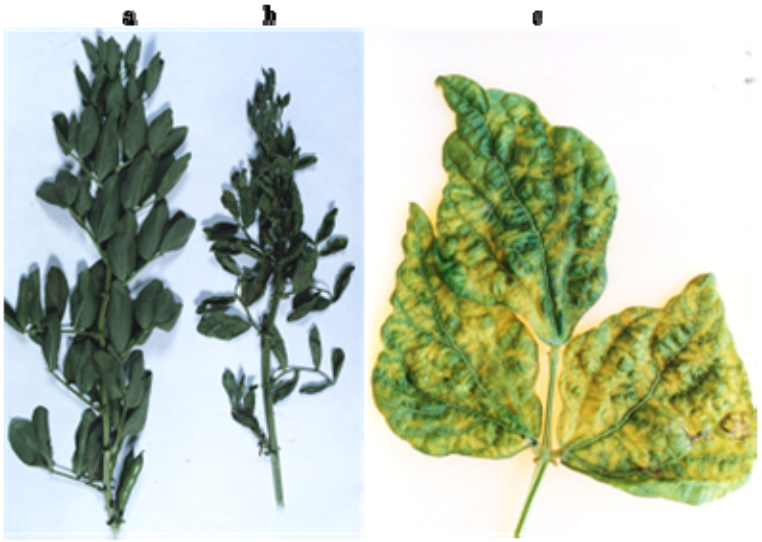

Table II. Pigment content, soluble carbohydrates, soluble proteins and total free amino acids in leaves of *Vicia faba* infected with bean yellow mosaic virus. Values are calculated as mg/g fresh weight and they are means of three replications

Infection period	4 days			12 days			20 days		
Test	H	I	L.S.D.	H	I	L.S.D.	H	I	L.S.D.
Pigment content									
Chlorophyll a	1.71	1.43	0.98	1.61	0.50*	0.91	1.61	0.42*	0.80
Chlorophyll b	0.70	0.63	0.31	0.60	0.25*	0.20	0.60	0.25	0.36
Carotenoids	0.51	0.48	0.16	0.26	0.19*	0.06	0.25	0.17*	0.06
Soluble carbohydrates	18.8	10.4*	2.35	24.7	14.9*	8.46	19.3	16.60	5.18
Total soluble proteins	0.37	0.19*	0.09	0.38	0.24*	0.12	0.32	0.56*	0.11
Total free amino acids	256.3	135.2*	48.56	240.0	137.2	9.58	250.0	140.80*	73.99

Table III. Pigment content, soluble carbohydrates, soluble proteins and total free amino acids in leaves of *Phaseolus vulgaris* infected with bean yellow mosaic virus. Values are calculated as mg/g fresh weight and they are means of three replications

Infection period	4 days			12 days			20 days		
Test	H	I	L.S.D.	H	I	L.S.D.	H	I	L.S.D.
Pigment content									
Chlorophyll a	2.19	2.08	0.17	1.05	0.85	0.30	1.23	0.43	0.13
Chlorophyll b	1.16	1.58*	0.18	0.48	0.35	0.20	0.46	0.17	0.10
Carotenoids	0.58	0.57	0.06	0.28	0.23	0.08	0.31	0.14	0.13
Soluble carbohydrates	32.4	39.2	10.02	25.80	22.10	14.0	15.1	8.40	3.57
Total soluble proteins	0.34	0.24	0.11	0.33	0.24	0.13	0.29	0.24	0.10
Total free amino acids	86.4	166.7*	35.90	72.00	86.40	33.23	73.50	39.20*	16.80

H = Healthy plant; I = Infected plant. * Values are significantly different at 5% probability

pigment contents, where the carbohydrates were increased with time (Table II). Similarly, proteins exhibited the same trend as in case of carbohydrates. On the percentage basis, the protein content of *Vicia faba* at 20 days from infection was about 195% over that measured four days after infection. It is likely that this host plant vigorously increased its proteins as a chemical response to the viral infection. Total free amino acids remained more or less constant over time, although their content was decreased by infection (Table II).

Table III shows that the concentrations of chlorophyll a, b and carotenoids in leaves of virus-infected *Phaseolus vulgaris* decreased slightly up to 12 days from treatment, then a sharp reduction was obtained at 20 days comparing with their corresponding healthy plants. At 20 days of infection, the reduction was about 50, 50 and 40% in chlorophylls a, b and carotenoids, respectively, comparing with values of infected plants after 12 days. After 20 days of infection, the reduction in the carbohydrate level was 79% of four days infected plants (Table III). Soluble proteins remained constant over the experimental period. However, total free amino acids were considerably decreased with time. The reduction in amino acid content was 50 and 77% at 12 and 20 days after infection, respectively, as compared with those of four days samples.

DISCUSSION

Nine plant species belonging to four families were tested for their sensitivity to infection by virus isolate. Described symptoms in leaves of *Chenopodium amaranticolor*, *Phaseolus vulgaris* and *Vicia faba* are nearly the same as described by Bos (1970) for bean yellow mosaic virus. Inoculations were further confirmed by positive reactions to the antiserum against BYMV in DAS-ELISA test. Although no visible symptoms were observed with *Pisum sativum* and *Nicotiana glutinosa*, a positive reaction was recorded by ELISA test. Therefore, it seems that BYMV infected these two species symptomlessly. Although, Bos (1970) ranked the two plant species among BYMV-susceptible host species, it is likely that the BYMV isolate dealt with may be slightly different.

Although in virus-infected *Vicia faba* plants pigmentation was decreased with time, carbohydrates increased progressively. The reduction in photosynthetic pigments may reflect a disturbance in photosynthetic apparatus. But, this disturbance in photosynthetic apparatus may not be a limiting factor in the biosynthesis of saccharides. Similar conclusion has been reached by Farghly (2001) under salinity and drought in some wheat cultivars. This conclusion is very important especially when taken into consideration the elevated accumulation of proteins in virus-infected *Vicia faba* plants.

Virus infection unexpectedly increased the incorporation of amino acids into proteins, which was consistent with the reduced amino acid accumulation. It is probably that virus infection increased protein accumulation

via the incorporation of amino acids rather than via the carbohydrates translocation. This can not confirm the explanation of Prasad *et al*. (1985) in which they attributed increase protein content partly due to proteinous nature of virus itself. In fact this was accompanied by a slight reduction in growth of the infected plants (Fig. 1b). Therefore, it can be proposed that the infected *Vicia faba* plants modified its strategy from state of growth to state of survival by using carbohydrates and proteins as self-defense components in response to the viral infection. However, this defense strategy might be different with plant age and species or with viruses. In support to the previous proposal is that when *Phaseolus vulgaris* inoculated with the same virus, there was a different response in the studied parameters and consequently the defense mechanism as follows: i) the pigmentation and carbohydrates went parallel to each other, where they were decreased with time. Thus, in *Phaseolus vulgaris* synthesis of carbohydrates was completely associated with photosynthetic apparatus, ii) chlorophyll b, carbohydrates and free amino acids were increased while protein content was maintained constant over the experimental time. Therefore, the defense mechanism of the plant against virus-infection seems to depend upon proteins and to some extend upon amino acids.

In agreement with our results is the finding of Redolfi (1983) who recorded that the hypersensitive viral infection (Tobacco necrosis virus) of bean (*Phaseolus vulgaris*) leaves resulted in the accumulation of at least three soluble protein components in leaf tissue, which was virus stimulated but host-dependent.

The two tested plants tolerated the viral infection up to 20 days. However, the defense mechanism was varied in each. The *Vicia faba* used carbohydrates and proteins while the *Phaseolus vulgaris* made its self-defense mainly through maintenance of proteins. The effect of the viral infection was more obvious in *Phaseolus vulgaris* than in *Vicia faba*, especially at the end of the experimental period. It is likely that these two plant genera are among the main host plants of bean yellow mosaic virus as reported by Matthews (1993).

Acknowledgments. I would like to thank Prof. Dr.M.A.K. Shaddad for his kind and most valuable remarks and advises in preparing this manuscript.

REFERENCES

Allam, E.K. and E.A. El-Kady, 1966. A virus causing a mosaic disease of broad bean and its vector *Aphis craccivora* in Egypt. *Entomol. Exp. Appl.,* 9: 413–8

Ashby, J.W., 1984. Bean leaf roll virus. C.M.I./A.A.B. Descriptions of plant viruses, No. 286.

Babin, M., V. Ortiz, S. Castro and J. Romero, 2000. First detection of faba bean necrotic virus in Spain. *Plant Dis*., 84: 707–2

Badour, S.S.A., 1959. Analytisch-Chemische untersuchung des Klaiummangles kei *Chlorella* in Vergleich mit anderen Mangelzustanden. *Ph.D. Dissertation,* Gllingen. Goettingen Univ., Germany

Bos, L., 1969. Inclusion bodies of bean yellow mosaic virus, some less known closely related viruses and beet mosaic virus. *Neth. J. Plant Path.,* 75: 137–43

Bos, L., 1970. Bean Yellow Mosaic Virus. C.M.I./A.A.B. *Descriptions of Plant Viruses*, 40: 4

Chakraborty, S., A. Sinha and B.V. Reddy, 1994. Effect of cucurbit mosaic viruses on chlorophyll and total phenol content of cucurbits. *Crop Res. (Hisar),* 7: 461-465.

Clark, M.F. and A.M. Adams, 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.,* 34: 475–83

Clover, G.R.G., S.N. Azam-Ali, K.W. Jaggard and H.G. Smith, 1999. The effects of beet yellows virus on the growth and physiology of sugar beet (*Beta vulgaris*). *Plant Path.,* 48: 129–38

Fales, F.W., 1951. The assimilation and degradation of carbohydrates by yeast cells. *J. Biol. Chem.,* 193: 113–24

Farghly, F.A., 2001. Physiological response of some wheat cultivars to salinity stress. *M.Sc. Thesis,* Bot. Dept., Faculty of Sci., Assiut Univ., Egypt

Gibbs, A.J. and H.L. Paul, 1970. Echtes ackerboh-nemosaik-virus. C.M.I./A.A.B. *Descriptions of Plant Viruses*, 20: 4

Gomez, K.A. and A.A. Gomez, 1984. Statistical procedures for agricultural research. John Wiley & Sons. Inc. New York

Hemida, S.K. and N.A. Abdel-Razik, 2002. Studies on mosaic virus of cucumber in Assiut. *Bull. Fac. Sci., Assiut Univ.,* 31: 113–21

Lowery, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.,* 193: 291–7

Makkouk, K.M., 1993. Survey for faba bean diseases in Egypt with special emphasis on viruses. *Annual Report ICARDA,* pp. 1–6

Matthews, R.E.F., 1993. *Diagnosis of Plant Virus Diseases.* p. 374. CRC Press, Boca Raton, Florida

Metzner, H., H. Rau and H. Senger, 1965. Uhtersuchungen zur synchronisierbar keit enzelner pigmentmangel- Mutanten von *Chlorella*. *Planta*, 65: 186–94

Mohanty, S.K. and R. Sridhar, 1989. Physiology of rice tungro virus disease: changes in leaf pigments due to infection. *Acta Phytopathol. Entomol. (Hung.),* 24: 375–86

Moore, S. and W.W. Stein, 1948. Amino acid free photometric ninhydrin method for use in the chromatography of amino acids. *J. Biol. Chem.,* 176: 367–88

Naidu, R.A., M. Krishnan, M.V. Nayudu and A. Gnanam, 1986. Studies on peanut green mosaic virus infected peanut (*Arachis hypogaea* L.) leaves. III- Changes in the poly peptides of photosystem II particles. *Physiol. and Mol. Plant Path.*, 29: 53–8

Prasad, B., O.P. Verma and L.N. Daftari, 1985. Effect of leaf curl virus infection on metabolism of sesame leaves. *Ind. Phytopath.,* 38: 343–5

Rizkalla, L.R., K.M. Makkouk, M.A. Madkour and M.B. Solf, 1995. Isolation and identification of major legume viruses and development of ELISA kits for their diagnosis. *In: Proc. 7th. Annual Coordination Meeting (ICARDA).* Cairo 10-14 Sep. 1995, ARC, Egypt

Srinivasulu, B. and R. Jeyarajan, 1990. Biochemical factors in *Oryzae sativa* L. in relation to rice tungro virus disease resistance. *Ind. J. Virol.,* 6: 46-49

Taylor, R.H. and L.L. Stubbs, 1972. Broad bean wilt virus. C.M.I./A.A.B. *Descriptions of Plant Viruses*, 81: 4

(Received 23 December 2004; Accepted 10 January 2005)