J. Mycopathol, Res, **51(1)** : 81-87, 2013; (ISSN 0971-3719)

Influence of bean common mosaic virus infection on protein, nitrogen, amino acid and leghaemoglobin content in hyacinth bean

MANISHA SRIVASTAVA[1], U.P.GUPTA[1] AND ASHA SINHA[2]
[1]*Department of Botany, Harish Chandra P.G. College, Varanasi-221001, U. P.*
[2]*Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, B.H.U., Varanasi-221005. U. P., India.*

Received : 10.09.2012 Accepted : 21.02.2013 Published : 29.04.2013

Hyacinth bean, *Dolichos lablab* L. is a common bean. It is a summer growing annual or short-lived perennial fodder legume. Experiment conducted to find out the influence of Bean common mosaic virus on the protein, nitrogen, amino acid content in healthy and diseased hyacinth bean leaves, stem and roots and Leghaemoglobin content in nodules at different intervals 15, 30, 45, 60 and 75th days after inoculation. Total protein, nitrogen and amino acid content was found to be high in virus infected *Dolichos lablab* L. crop at 60th days interval. Nodules of healthy plants have higher leghaemoglobin content than those of virus infected hyacinth bean.

Key words: Bean Common Mosaic Virus, protein, nitrogen, amino acid and Leghaemoglobin.

INTRODUCTION

Virus multiplication in plants generally cause synthesis of abnormal proteins resulting in an alteration in type and the amount of total proteins. Gradual increase in the protein content is observed by Shukla *et al.* (1984) throughout the experimental period in leaf, stem and root of bean common mosaic virus infected French bean plants. Gupta (1990) notices increase protein content in root nodules of soybean mosaic virus infected soybean (*Glycine max* L. Merr.). Singh (1995) observes increased protein content in leaves, stems and roots of pea plants infected with bean yellow mosaic virus. Singh and Shukla (2009) report nitrogen and protein contents are higher in infected tissue of *Carica papaya* L. infected by Papaya Ring Spot Virus (PRSV) compared to healthy counterparts. Sinha and Srivastava (2010) report protein content increased in virus infected mungbean leaves. Ashfaq *et al.* (2010) have studied ULCV-infected plants, both susceptible and resistant, appeared to have increased total soluble protein contents at 15 and 30 days after inoculation.

There are reports of both increased as well as decreased nitrogen content due to virus infections. John (1963) in Dolichos enation mosaic virus infected *Dolichos lablab* L. plants observes increase in nitrogen level due to infection. Gupta (1990) has reported more nitrogen content in nodule of soybean (*Glycine max* L. Merr.), infected with soybean mosaic virus. Singh (1995) has observed higher nitrogen content in leaves, stems and roots of pea plants infected with bean yellow mosaic potyvirus. Muqit *et al.* (2007) have conducted an experiment to determine the changes in ash gourd (*Benincasa hispida*) due to infection of *Bottlegourd mosaic virus* (BgMV), *Watermelon mosaic virus 2* (WMV2) and *Papaya ringspot virus* (PRSV). Total nitrogen and protein are decreased due to BgMV and WMV2 infection while they increase in case of PRSV. Verma and Gupta (2008) have reported that the total nitrogen content in leaf, stem and root of virus-infected samples is higher than that of healthy ones. The gradual increase in total nitrogen content is observed throughout the experimental period in healthy and diseased plants. Sinha and Srivastava (2010) have reported total nitrogen (N) content are higher in the virus-infected plants in all 3 varieties viz., HUM-2 (Malviya Jagriti), ML-192

Email: sri.manisha.25@gmail.com

and Pusa Baisakhi infected by Mungbean yellow mosaic virus.

The synthesis of various virus proteins by plant tissues almost certainly involves changes in their free amino acid pool, although such changes may be small and transitory (Selman *et al.* 1961). Many workers have reported an increase in number and concentration of free amino acids in virus infected plants while a few have observed decrease also. John (1963) has found that the disease development in lablab minor by dolichos enation mosaic virus was accompanied by an increase in free amino acids, reaching a peak coinciding with full symptom expression. Singh (1995) has pointed out that the total free amino acids are higher in leaves, stems and roots of pea plants infected with bean yellow mosaic potyvirus than in the healthy plant parts. Hemida (2005) has estimated total free amino acids in leaves of two host plants (*Vicia faba* and *Phaseolus vulgaris*) inoculated with BYMV for 4, 12 and 20 days. In *Phaseolus vulgaris,* pigment contents, carbohydrates and free amino acids are decreased with time. Strategy of defense mechanism in each host plant against BYMV infection has varied. According to Bhagat and Yadav (2005), free amino acid decreased in the infected leaves of 'Pusa Sawani' as compared to Parbhani Kranti', susceptible 'Vaishali Vadhu' by Bhindi yellow vein mosaic virus (BYVMV). Verma and Gupta (2007) have reported that the Bean common mosaic virus infection influenced the concentration of free amino acid in French bean plant. The disease plant has higher concentration of free amino acid than the healthy plant. In general, there is an increase in the concentration of different amino acid with the age of plant. The levels of reducing sugars, free amino acids and proteins are significantly higher in CMV infected tomato plants compared to the control (Maria, 2012).

The leghaemoglobin pigment present in nodules plays an important role in the process of nitrogen fixation. Nutman (1958) has believed that the pigment leghaemoglobin is responsible for the pink color of nodules and is related to the activity of nitrogen fixation in nodules. According to Virtanen and Laine (1964) the essentiality of the leghaemoglobin for nitrogen fixation is confirmed by the fact that a cessation of fixation is attended by a change in colour of the pigment from red to green. Rajgopalan and Raju (1972) have found that the formation of leghaemoglobin in nodules of *Dolichos lablab* L. is not affected by Dolichos enation mosaic virus. The peak concentration of the pigment, however, attained earlier in nodules of infected plants than those of virus free ones. Rao and Shukla (1988) have reported lesser leghaemoglobin content in pea root nodules infected with cucumber mosaic virus and *Sesbania* mosaic virus, respectively. Patil and Sayyad (1991) have undertaken the study of leghaemoglobin content in cowpea nodules as influenced by virus *Rhizobium* interactions. They have reported that virus infection reduced the leghaemoglobin content substantially. In a similar study on *Vigna sinensis*, grown in three different culture media, Upadhyaya *et al.* (1991) have found that cowpea vein banding mosaic virus affected the leghaemoglobin content adversely. Gross reduction of nodulation is achieved by virus inoculation on broad bean plants cv. Giza 402. It produces smaller, fewer nodules and reduces its leghaemoglobin content. This difference is accompanied with the presence of BBSV particles in the root nodule cells (Mamdouh *et al.,* 2011).

MATERIALS AND METHODS

Virus identification

The virus isolate under study was found to be easily transmitted by sap and identified through Electron Microscopy (a uniform decoration was obtained, the viruses having filamentous particle morphology of 750 nm (l.) and 15 nm (w)) at Plant Virology Unit, Division of Plant Pathology, IARI, New Delhi.

The experiment was carried out during year 2009 to 2010. Leaves, stem and roots of Hyacinth bean plant were separated carefully from healthy and virus infected plants at various days after inoculation. The first harvesting was carried out after 15th days of inoculation followed by 30, 45, 60 and 75th days after inoculation to study effect of Bean common mosaic virus on the protein, nitrogen, amino acid and leghaemoglobin content in healthy and diseased nodules of Hyacinth bean plant.

Estimation of protein content

Protein content has been estimated by Lowry's method using bovine serum albumin (BSA) as a standard (Lowry *et al.*, 1951). Extraction of leaves, stem and roots of Hyacinth bean plant were usually carried out with Phosphate buffer used for the enzyme assay. 500 mg of the leaves, stem and roots samples were weighed and grounded with a pestle and mortar in 5-10 ml of the phosphate buffer. Centrifuged and supernatant was taken for protein estimation. 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard were pipetted out in to a series of test tubes. 0.2 ml of the leaves, stem and roots samples were pipetted out extract in other test tubes. The volume was maintained to 1 ml in all test tubes. A tube with 1 ml of distilled water was served as the blank. Added 5 ml of Alkaline copper solution to each tube including the blank, mixed well and allowed standing for 10 minutes. Added 0.5 ml of Folin – Ciocalteau reagent mixed well and incubated at room temperature in the dark for 30 min. Blue colour was developed. The reading was taken at 660 nm. A standard graph was drawn and calculated the amount of protein in the leaves, stem and roots samples.

Estimation of nitrogen content

Nitrogen content has been estimated by Microkjeldahl method (Jackson, 1973). The leaves, stem and roots samples of different days intervals were taken separately and powdered in a mechanical grinder using fine mesh, and the powdered (0.5 g) were mixed with a little (5 g) catalyst mixture (Na_2SO_4, $CuSO_4$ and Selenium powder) and 10 ml. concentrated H_2SO_4. This mixture was digested in a microkjeldahl flask. The volume of digest was made to 100 ml. Ten ml aliquot of the above solution was taken and distilled with 40% NaOH in a distillation plant and the distillate was collected in a flask containing 10 ml of 2% boric acid with 2 drops of mixed indicator. This distillate was titrated against N/28 hydrochloric acid to a sharp pink end point. 1 ml N/28 hydrochloric acid is equal to 0.5 mg of nitrogen, from which total nitrogen was calculated.

Estimation of amino acid content

Amino acid content was estimated by method of Yemm and Cocking (1955). 0.5g of the leaves, stem and root samples were taken separately and grounded in a pestle and mortar with a small quantity of acid washed sand. In this homogenate, 5 to 10 ml. of 80% ethanol was added and filtered. The filtrate was stored. The extraction was repeated twice with the residue and pooled all the supernatants. The volume was reduced, if needed by evaporation and the extract was used for the quantitative estimation of total free amino acids. 0.1-ml-plant extract was taken and to it added 1 ml. of ninhydrin solution. The volume was maintained to 2 ml with distilled water. The tubes were heated in a boiling water bath for 20 minutes. 5 ml of the diluent solution were added and mixed the contents. The intensity of the purple colour against a reagent blank was read after 15 minutes in a colorimeter at 570 nm. Blank were prepared by taking 0.1 ml 80% ethanol. The amount of free amino acids was calculated from a standard curve drawn with l-leucine. The amount of free amino acids has been expressed as mg/100 mg dry weight of the sample.

Estimation of leghaemoglobin content

Leghaemoglobin content was estimated by method of Appleby and Bergesen (1980). The fresh nodules were mixed with 1-3 ml of phosphate buffer and crushed. Filtered through two layers of cheesecloth. Discarded the nodule debris. Clarified the turbid reddish brown filtrate by centrifugation at 10,000g for 10-30 min. The samples were diluted. The 2 ml of the extract added in an equal volume of alkaline pyridine reagent and mixed. The solution becomes greenish- yellow due to the formation of ferric hemochrome and to it added a few crystals of sodium dithionite to reduce the hemochrome. The samples were stirred without aeration. The absorbance was recorded at 556 nm after 2-5 minutes against a reagent blank. Leghaemoglobin concentration was calculated by following formula:

$$\text{Lh concentration} = \frac{A_{556} \times 2D}{33.9}$$

where, D is the initial dilution.

The data collected in the present work were summarized in an analysis of variance (ANOVA) table. F – values have been calculated by the method of Bailey (1959).

RESULTS AND DISCUSSION

The results presented in Table 1 show that protein content of leaves, stem and roots taken from healthy samples increased gradually up to 75th days. Changes in the protein content of roots were similar to leaves and stem Per cent increase in protein content was maximum in leaves followed by stem and roots. These observations are statistically significant.

Table 1: Effect of Bean common mosaic virus infection on protein content in leaves, stem and roots of *Dolichos lablab* L. plant

Days after inoculation	Protein content (mg/100mg dry weight)					
	Leaves		Stem		Roots	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
15	1.27	1.343	1.283	1.352	0.066	0.675
30	1.314	1.406	1.306	1.423	0.730	0.756
45	1.365	1.476	1.365	1.499	0.780	0.817
60	1:466	1.636	1.483	1.583	0.941	1.012
75	1.434	1.567	1.417	1.533	0.850	0.899
Average	1.369	1.486	1.370	1.478	0.673	0.832
F- calculated	Treatment	68*	Treatment	40*	Treatment	13.333*
Value	Interval	40*	Interval	21.429*	Interval	96.667*

* Significant at 5 % level of significance

The results presented in Table 2 show that nitrogen content of leaves, stem and roots taken from healthy samples increased gradually up to 60th days. Changes in the total nitrogen content of roots were similar to leaves and stem. These observations are statistically significant.

The results presented in Table 3 show that amino acid content of leaves, stem and roots taken from healthy samples increased gradually up to 60th days. The amino acid content in the diseased leaves was more than its healthy counterparts and it increased up to 60th days after inoculation. Changes in the amino acid content of roots were similar to leaves and stem. These observations are statistically significant.

The results presented in Table 4 show a gradual increase in the leghaemoglobin content in both the healthy and virus infected nodules up to 60th days after inoculation and thereafter it decreases. The maximum concentration of leghaemoglobin content was 13.168 mg/g in healthy nodules, whereas in virus infected ones it was reduced to 12.424 mg/g on the same day of harvesting (60th days after inoculation). Nodules of healthy plants always had higher leghaemoglobin content than those of virus infected ones throughout the experimental period. These observations are statistically significant.

The present studies reveal that BCMV infected plants (leaf, stem and roots) had higher protein content than their healthy counter parts (Table 1). The results are in agreement with Gupta (1990); Singh (1995); Bhagat and Yadav (2005); Yardimci *et al.* (2007); Singh and Shukla (2009); Ashfaq *et al.* (2010); Sinha and Srivastava (2010) and Maria (2012). The increased protein content in the present case corresponds to findings of Hills and Mckinney (1942) who pointed out that the protein content increases or decreases with increasing or decreasing nitrogen content in susceptible and resistant strains of tobacco. An increase in the nitrogen content (Table 2) recorded in the present is in agreement with this view. Chinnandurai and Nair (1971) interpreted increase in cytoplasmic protein due to utilization of chloroplastic protein for virus protein synthesis. Singh and Mall. (1976) found that synthesis of virus protein was due to qualitative and quantitative increase in amino acids by virus infection. The increase in protein content with age of the plants may be due to high requirement of enzymes with age of the plants and other proteinaceous substances for senescence. The higher percentage seems due to virus multiplication which entails the synthesis of virus specific abnormal protein that accumulates and ultimately raises the percentage over healthy. Increase in protein contents observed in infected mungbean plant leaves may also be correlated with respiration. Increased nitrogen uptake by diseased plants associated with rapid respiration probably helps in the synthesis of more amino acids (Szczepanski and Redolfi, 2008). Ashfaq *et al.* (2010) observed higher total protein content that was more likely to be due to the increased level of viral proteins in the plant and this is in agreement with previous findings of other research workers (Langhams and Glover, 2005; Yardimci *et al.* 2007).

It is almost generally agreed that virus infection of plants should be regarded as a change in the nucleoprotein metabolism of the host (Bawden, 1959). Thus, the most remarkable changes in a virus- infected plant are in the nitrogen metabolism. The present studies have revealed a striking disparity in total nitrogen found in healthy and virus-infected plants. In leaf, stem and root of diseased plant, it showed an increase (Table 2). Similar or even more increase in total nitrogen has been reported by many workers i.e. John (1963); Selman and Grant (2008); Verma and Gupta, (2008) and Sinha and Narayanswamy and Ramakrishnan (1966); Singh and Mall (1978) and also in the present study (Table 1). Selman *et al.*, (1961) reported increase in amino acid contents while Ford and Tu (1969) and Tu *et al.*, (1970) noticed increased ammonium and amides in virus infected plants. Hayashi (1962) claimed accumulation of nitrogen due to increase in activity of amino activating enzymes. Maximum increase in nitrogen content was noticed in case of leaves and minimum in roots (Table 2). The increase in roots may be due to accumulation of nitrogen, which is not being metabolized efficiently or by the usual pathways of the healthy plant (Tu *et al.* 1970). Bollard (1956) reported that translocation of nitrogen from leaves to other parts occurred in the form of amides *viz.,* glutamine and if that transfer was retarded by a damaged translocation facility (due to virus synthesis), the glutamine

Table 2: Effect of Bean common mosaic virus infection on total nitrogen content in leaves, stem and roots of *Dolichos lablab* L. plant

Days after inoculation	Total Nitrogen content (mg/100mg dry weight)					
	Leaves		Stem		Roots	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
15	1.88	2.01	0.672	0.896	0.496	0.783
30	2.30	2.58	0.784	0.952	0.576	0.873
45	2.95	3.15	1.316	1.428	1.067	1.39
60	3.66	4.14	1.764	1.988	1.614	1.845
75	3.48	3.82	1.401	1.848	1.461	1.587
Average	2.854	3.14	1.184	1.422	1.043	1.296
F- calculated	Treatment	25.5*	Treatment	17.25*	Treatment	50.794*
Value	Interval	163.5*	Interval	56*	Interval	153.667*

* Significant at 5 % level of significance

Srivastava (2010). The main components of nitrogenous materials of plants are probably insoluble proteins, free amino acids, nucleic acids, amines, amides and inorganic nitrogen. The increase in any of these may cause increased level of total nitrogen. Alteration in any one of the components may disturb the nitrogen metabolism. A few viruses such as tobacco mosaic virus and potato virus protein contribute significantly to the total protein of the host and as a consequence there is an increase in total N. This might be one of the possibilities in the present case too. Accumulation of protein in virus infected plants were reported by John (1963); would accumulate and account for the rapid rise in total nitrogen content of diseased leaves. According to Jensen (1969) it may lead to depletion of nitrogen at the growing points resulting in stunting and chlorosis because of nitrogen deficiency.

There are numerous reports on the effect of virus infection on the concentration of free amino acids in virus-infected plants (Singh 1995; Hemida 2005; Bhagat and Yadav, 2005,Verma and Gupta, 2007 and Maria, 2012). In present study also increase in concentrations of amino acids in leaves, stems and roots of the Bean common mosaic virus in-

Table 3: Effect of Bean common mosaic virus infection on amino acid content in leaves, stem and roots of *Dolichos lablab* L. plant

Days after inoculation	Amino acid content (mg/100mg dry weight)					
	Leaves		Stem		Roots	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
15	1.650	1.745	1.076	1.250	0.42	0.467
30	1.841	1.999	1.242	1.483	0.767	1.013
45	2.022	2.345	1.325	1.805	1.027	1.52
60	2.628	2.835	1.879	1.989	1.24	1.973
75	2.235	2.356	1.656	1.799	0.9	1.333
Average	2.075	2.256	1.436	1.665	0.870	1.261
F- calculated	Treatment	20.25*	Treatment	13.2*	Treatment	11.578*
Value	Interval	77*	Interval	18.2*	Interval	11.455*

*Significant at 5% level of significance

fected Hyacinth bean plants have been observed (Table 3). The accumulation of amino acids in virus affected plants is considered by Selman *et al.* (1961) to be indicative of a block in the protein synthesis of plants or of an enhanced amino acid activating system . Hayashi (1962) and Ford and Tu (1969) reported that changes in concentration of free amino acids in virus infected maize were a result of alteration in metabolism resulting from or associated with virus multiplication. The increased concentration of amino acid contents in infected plant samples observed in the present study can be explained in agreement with these views. More plausible explanation has been proposed by Selman *et al.* (1961) and Bozarth and Diener (1963) that the accumulation of free amino acids in the possible cause for such an increase in total nitrogen content. In the present investigation total amino acid concentrations of infected leaf showed an increase (Table 3), although this increase was also noticed in stem and root as well. These findings indicated the probable role of amino acids in the partial increase of total nitrogen.

The leghaemoglobin content was found to be higher in healthy nodules than the virus infected ones (Table 4). Leghaemoglobin is an important factor for pink colouration in nodules (Nutman, 1958) and it is reported to be a prerequisite for nitrogen fixation. The decrease in leghaemoglobin content in nodules of virus infected Hyacinth bean plant is probably due to metabolic disturbance in the host plant caused by BCMV infection. It is also possible that interaction between the virus and *Rhizobium* might have lowered the leghaemoglobin content. Patil and Sayyad (1991) reported that virus-*Rhizobium* interactions have reduced the leghaemoglobin content in the nodules of cowpea plant. Joshi and Carr (1967) found in white clover that the healthy plants with effective rhizobia produced large, mostly elongated, pink nodules while diseased plants with ineffective *Rhizobium* produced small, white nodules. An increased concentration of nitrogenous fractions was observed in BCMV infected Hyacinth bean (Table 2). Unless, the nitrogen previously fixed was effectively utilized and / or transported elsewhere, the presence of an excess nitrogenous compounds could alter the C: N ratio, which might inhibit the normal rate of nitrogen fixation. This could also result in decreased leghaemoglobin synthesis. Application of nitrogen fertilizer has been found to inhibit nodulation and nodule weight Tu *et al.* (1970). Thus, it seems that an increase in total nitrogen content in the virus-infected pant (Table 2) may be the fundamental reason for the decrease in leghaemoglobin content.

ACKNOWEDGEMENTS

We are immensely thankful to Dr. R. K. Jain Head, V. G. Malathi Principal Scientist, In-charge, Dr. Jasvir Singh and Baljeet Kaur Technical officer, Plant Virology Unit, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi for Electron microscopy.

REFERENCES

Appleby, C.A. and Bergersen, F.J. 1980. In: methods for evaluating biological nitrogen fixation (Ed. Bergersen F.J.) *John Wiley and Sons*, NewYork p. 315.

Ashfaq, M.; Khan, Aslam; Javed, N.; Mughali, S.M.; Shahid, M. and Sahi, S.T. 2010. Effect of Urdbean leaf crinkle virus infection on total soluble protein and antioxidant enzymes in Blackgram plants. *Pak. J. Bot.*, **42:** 447-454.

Bailey, N.T.J. 1959. "Statistical methods in Biology." 5th Imp. Univ. books, English Univ. Press Ltd., London.

Bawden, F.C. 1959. Physiology of virus diseases. *Ann. Rev. Pl. Physiol.*, **10:** 239-253.

Bhagat, A.P. and Yadav B.P. 2005. Biochemical and enzymatic activity changes induced by Bhindi yellow vein mosaic virus in bhindi. *Indian Jouranl of Virology*, **16:** (1-2).

Bollard, E.G. 1956. Nitrogenous compounds in plant xylem sap. *Nature*, 178: 1189-1190.

Bozarth, R.F. and Diener, T.O. 1963. Changes in concentration of free amino acids and amides induced in tobacco plants by potato virus and potato virus Y. *Virology*, **21**: 188-193.

Chinnadurai, G. and Nair, M.C. 1971. Effect of Dolichos yellow mosaic on chlorophyll and leaf proteins. *Curr. Sci.*, **40:** 18-19.

Ford, R.E. and Tu, J.C. 1969. Free amino acid contents in corn infected with maize dwarf mosaic and sugarcane mosaic virus. *Phytopathology,* **59:** 179-182.

Gupta, U.P. 1990. The effect of Soybean mosaic virus infection on protein content in nodules of Soybean *(Glycine max* (L.) *Merr.).* Abstr., 6th Ann. Conv. Of Indian virological society, Pune, India. Pp 77.

Hayashi, Y. 1962. Amino acid activation in tobacco leaves infected with tobacco mosaic virus. *Virology,* **18:** 140-141.

Hemida, Sameh Kamal, 2005. Effect of Bean Yellow Mosaic Virus on Physiological Parameters of *Vicia faba* and *Phaseolus vulgaris. Int. J. Agri. Biol.,* **7** : 154 – 157.

Hills, C.H. and McKinney, H.H. 1942. The effect of mosaic virus infection on the protein content of susceptible and resistant strains of tobacco. *Phytopathology,* **32:** 857-866.

Jackson, M.L. 1973. Soil chemical analysis. Prentice Hall, New Delhi, India.

Jensen, S.C. 1969. Composition and metabolism of Barely leaves infected with Barley yellow dwarf virus. *Phytopathology,* **59:** 1694-1699.

John, V.T. 1963 . Some aspects of nitrogen metabolism in virus induced mosaic diseases. *Proc. Indian Acad. Sci.,* **57**: 307-325.

Joshi, H.U. and Carr, A.J.H. 1967. Effect of clover phyllody virus on nodulation of white clover (*Trifolium repeans*) by *Rhizobium trifolii* in soil. *J. Gen. Microbial.,* **49:** 385-392.

Langham, M. and K. Glover 2005. Effects of *Wheat streak mosaic* virus (genus: Tritimovirus, family: *Potyviridae*) on spring wheat. *Phytopathology,* **95:** 556.

Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and R.J., Randall 1951. Protein measurement with Folin phenol reagent. *J Biol Chem,* **193:** 265-275.

Mamdouh, Abd El-Ghaffar,Sabah, Abo-El Maaty and Sabry, Mahmoud. 2011. Identification and detectability of broad bean stain virus in broad bean seeds and effects on nodulation. Archives of Phytopathology and Plant Protection **44** : 390-403.

Maria Kalogirou, 2012. Aantiviral and quality effects of chemical elicitors and cucumber mosaic virus (cmv) infection on tomato plants and fruits. Ph.D thesis, Cranfield university Cranfield health Plant science laboratory.

Muqit, A.; Akanda, M. and Kader, K.A. 2007. Biochemical alteration of cellular components of ash gourd due to infection of three different viruses. *Int. J. Sustain. Crop Prod.,* **2:** 40-42.

Narayanswamy, P. and Ramakrishnan, K. 1966. Studies on the sterility mosaic disease of Pigeon Pea III. Nitrogen metabolism in infected plants. *Proc. Ind. Aca. Sci.,* **63:** 288-296.

Nutman, P.S. 1958. "The Physiology of nodule formation" pp. 87-107. In: E.G. Hallsworth (ed.). Nutrition of legumes. Butterworths Sci. Publ., London.

Patil, P.L. and Sayyad, A.N. 1991. A study on cowpea mosaic virus (CPMVS). *Rhizobim* interaction in cowpea. *Journal of Maharastra Agricultural University,* **16:** 332-334.

Rajagopalan, N. and Raju, R.N. 1972. The influence of infection by Dolichos enation mosaic virus on nodulation and nitrogen fixation by field bean. *Phytopath Z.,* **73:** 285-309.

Rao, G.P. and Shukla, K. 1988. Influence of Sesbania mosaic virus infection on nodulation and nitrogen fixation in Pea (*Pisum sativum* L.). *J. Agric. Sci.,* 110: 391-394.

Selman, I.W. and Grant, Sheila A. 2008. Some effects of nitrogen supply on the infection of tomato plants with tomato spotted wilt virus. *Annals of Applied Biology,* **45:** 448-455.

Selman, I.W.; Brierley, M.R.; Pegg, G.F. and Hill, T.A. 1961. Changes in the free amino acids in tomato plants inoculated with tomato spotted wilt virus. *Ann. Appl. Biol.,* **49**: 601-615.

Shukla, K.; Gupta, A.K. and Joshi R.D. 1984. Nitrogen and protein content of French bean (*Phaseolus vulgaris* L.) plants experimentally infected with seed borne viruses. *Indian Journal of Plant Pathology,* **2**: 161-163.

Singh, Anand, 1995. Effect of bean yellow mosaic virus on nitrogenous and protein content of pea. *Plant Disease Research.,* 10: 80-82.

Singh, Vimla and Shukla, K. 2009. Effect of PRSV Infection on Pigment Content and Assimilation of Carbohydrate in *Carica papaya* L. *Annals of Plant Protection Sciences,* **17**: 1.

Sinha, A. and Srivastava, M. 2010. Biochemical changes in Mungbean plants infected by Mungbean Yellow Mosaic Virus. *International Journal of Virology,* **6**: 150-157.

Singh, R. and Mall, T.P. 1976. Effect of infection by arhar mosaic virus on the Hill reaction and primary productivity of pigeon pea leaves. *Ind. J. Exp.,* **14**: 376-377.

Singh, R. and Mall, T.P. 1978. Changes in chemical composition of pigeon pea fruits due to arhar (pigeon pea) mosaic virus infection. *Acta Phytopathology. Acad. Sci. Hungaricae,* **13:** 41- 44.

Szczepanski, M. and Redolfi, P. 2008. Changes in the Proteins of Bean Leaves Infected With Tobacco Necrosis or Alfalfa Mosaic Viruses. *Journal of Phytopathology,* **113**: 57-65.

Tu, J.C.; Ford, R.E. and Grau, C.R. 1970. Some factor affecting nodulation and nodular efficiency in soybean mosaic virus. *Phytopathology,* 60: 1653-1656.

Upadhyaya, P.P.; Sharma, A. and Tripathi, O.P. 1991. Effect of cowpea vein banding mosaic virus infection on nodular physiology and nitrogen fixing efficiency in *Vigna sinensis* Linn. International Conference on Virology in the Tropics, Lucknow, India. Dec. 2-6, pp. 177.

Vanderveken, J. 1964. Influence dum virus sur la nodulation chez. *Trifolium repens. Ann. Inst. Pasteur,* **107:** 143-148.

Verma, Poonam and Gupta, U.P. 2007. Influence of Bean common mosaic virus infection on free amino acid content in French bean (*Phaseolus vulgaris* L.). *Res. On Crops,* **8** : 695 – 699.

Verma, Poonam and Gupta, U.P. 2008. Influence of Bean Common Mosaic Virus Infection on Total Nitrogen Content in French Bean (*Phaseolus vulgaris* L.). *Advances in Plant Sciences,* **21**: 517 – 518.

Virtanen, A.I. and Laine, T. 1964. Red, brown and green pigments in leguminous root nodules. *Nature,* **157**: 25-26.

Yardimci, N.; Eryigit, H. and Erdal, I. 2007. Effect of Alfalfa mosaic virus (AMV) on the content of some macro-and micronutrients in alfalfa. *Journal of Culture Collections,* **5:** 90-93.

Yemm, E.W. and Cocking, E.C. 1955. The determination of amino acid with ninhydrin. *Analyst,* **80:** 209-213.