

## Changes in Nitrogen and Protein Content of Cowpea (*Vigna unguiculata*) due to Geminivirus Infection

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**Abstract:** Cowpea, *Vigna unguiculata* is an important legume crop cultivated worldwide as vegetable and pulse crop. Cowpea is susceptible to many virus diseases amongst which geminivirus infection is more prevalent in northern India. Yellow mosaic caused by geminivirus for which whitefly acts as a vector reduces yield from 40-80% area. Virus infection generally results in the drastic bio-chemical and physiological changes in the host plants. For the estimation of protein and nitrogen, stem and leaf samples were collected separately from healthy and infected cowpea plants at various intervals. In the present study it was observed that the infection had increased the total nitrogen and protein content of the host plant. The higher nitrogen content in the infected plant nitrogen content was found to be maximum in leaves. Infected leaves and stem had higher protein and nitrogen contents than their healthy counterparts although protein and nitrogen contents of the plants also increased with time.

**Keywords:** Cowpea, Geminivirus, Nitrogen, Protein.

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### I. Introduction

Cowpea, *Vigna unguiculata* is an important legume crop cultivated worldwide as vegetable and pulse crop. In India cowpea is grown as sole intercrop, mixed and in agroforestry combination in the area of western, central and peninsular region including Maharashtra. Cowpea is susceptible to many virus diseases amongst which geminivirus infection is more prevalent in study area.[1] Almost every part of cowpea plant is infested by one or another insect species. The viral disease of cowpea have devastating effect on the production of crop, amongst viral disease Yellow mosaic caused by geminivirus for which whitefly acts as a vector reduces yield from 40-80%. Whiteflies are prevalent in cowpea growing area, insect feeds on plant and transmit geminivirus particle. Virus infection reduces the yield of cowpea resulting in economic loss to the cultivator. Virus disease provide a system to study host pathogen interaction as isolated virus particle do not possess any metabolic system, Virus infection generally results in the drastic bio-chemical and physiological changes in the host plants. These changes might be used in setting up the characteristic of the disease as a supplement to the symptomatology.[2]

Cowpea plant infected with geminivirus leaves are characterized by the appearance of yellowing followed by curling, puckering and rugosity of leaves, stunting of plants and malformation of floral organs. The disease is commonly, widespread, destructive and inflicts heavy losses annually.[3] Present study deals with the nature of change in nitrogen and protein content of geminivirus infected cowpea plants.

### II. Materials & Method

For the estimation of protein and nitrogen, stem and leaf samples were collected separately from healthy and infected cowpea plants at various intervals. Soil temperature and light conditions were similar for control and virus treated plants for one set of experiments. The first harvesting was done after 12 days after inoculation (initial appearance of symptoms) followed by 20, 28 and 36 days (with gradual increase in appearance of symptoms) 44 and 52 days (with no further apparent increase in symptoms).

#### 2.1 Protein Estimation:

For estimation of protein in leaf and stem samples, the 100g samples were oven dried. These samples were crushed with 10ml of trichloroacetic acid and centrifuged at 1600 rpm. The supernatant was discarded. The residue was well mixed with one ml. of digestion mixture (32g Salicylic acid/litre of Conc. H<sub>2</sub>SO<sub>4</sub>) and allowed to stand for 30 minutes. To this 4 drops of 50% sodium-thiosulphate followed by 5ml of another digestion mixture (2.4g Selenium / litre conc. H<sub>2</sub>SO<sub>4</sub>) were added. The contents were boiled till the digestion of material was complete. Five drops of 10% perchloric acid were added and content were heated gently till the solution became clear. The solution was made upto 100ml with distilled water. One ml. of this solution, 8.5ml. Nessler's

reagent and 0.5ml of gum-ghatti solution were added in a colorimetric tube and mixed thoroughly. The ammonia thus evolved was measured by spectrophotometer at 440nm. A standard solution of ammonium sulphate was prepared. A portion of this solution was taken and treated with Nessler's reagent and gum-ghatti solution similarly. Observations were taken in the same manner, non-soluble nitrogen was calculated by the following formula using ammonium sulphate as standard.

The amount of nitrogen was calculated with the help of a factor derived from standard curve obtained from different concentration of ammonium sulphate plotted against their respective O.D.

$$\text{Factor} = \frac{\text{Concentration of Standard}}{\text{Optical density of Standard}}$$

The above formula gives the amount of non-soluble nitrogen. This was multiplied by a factor 6.25 to obtain the total protein [4]. The same procedure was followed with each sample. The observations are presented in table 1.

### 2.2-Preparation of Nessler's Reagent:

50g of potassium iodide (K.I.) was dissolved in 50ml of distilled water and then 25g mercuric chloride  $\text{HgCl}_2$  was dissolved in 450ml distilled water. In KI solution,  $\text{HgCl}_2$  solution was added drops by drop until a slight red precipitate appeared. Then 5N sodium hydroxide (NaOH) solution was prepared by dissolving 50g NaOH in 250ml distilled water. In 200ml of NaOH solution (5N) mixture of KI and  $\text{HgCl}_2$  was introduced. The volume of the solution was made upto one litre by adding distilled water and then filtered.

### 2.3-Estimation of Total Nitrogen:

For estimation of total nitrogen in leaf and stem samples one hundred mg dried material was transferred to a micro-Kjeldhal flask to which 3ml of digestion mixture (32g. of Salicylic acid/liter of concentrate and nitrogen free  $\text{H}_2\text{SO}_4$ ) was added. It was mixed well and allowed to stand for 30 minutes. Then 4 drops of 50%. Sodium thiosulphate solution followed by another 5ml of digestion mixture (2.5g. of selenium/liter concentrate nitrogen free  $\text{H}_2\text{SO}_4$ ) were added. The content was heated slowly on a hot plate till the digestion of material was completed. After the digestion was completed, about 5 drops of 10% perchloric acid were added and the contents were gently heated till the solution became clear. The entire solution was then made upto 100ml. with addition of distilled water. In a colorimetric tube 1ml. of this solution , 8.5ml. of Nessler's reagent and 0.5ml. of gum-ghatti solution were added. The content was mixed thoroughly and thus ammonia evolved during nesslerization was estimated colorimetrically at 400nm with spectrophotometer. A standard solution of ammonium sulphate (AR quality) was prepared. A portion of this solution was taken and treated with Nessler's reagent and gum-ghatti solution. Total nitrogen was calculated by the following formula using  $(\text{NH}_4)_2\text{SO}_4$  as standard.

$$\text{Factor} = \frac{\text{Concentration of Nitrogen in the standard}}{\text{O.D. of same solution}}$$

The observations are presented in Table1.

**TABLE – 1**  
Effect of Geminivirus infection on Protein and Nitrogen content of leaf and stem

Days after Inoculation	Protein content of Leaf		Protein content of Stem		Nitrogen content of leaf		Nitrogen content of stem	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
12	13.56	13.93	7.92	8.46	2.17	2.26	1.29	1.37
20	13.79	14.16	8.75	9.21	2.21	2.29	1.41	1.49
28	14.31	15.82	9.43	10.11	2.33	2.54	1.53	1.67
36	15.03	17.31	11.13	12.03	2.45	2.81	1.80	1.96
44	15.81	18.07	12.19	13.09	2.53	2.93	1.97	2.09
52	16.57	19.56	13.07	14.17	2.67	3.17	2.10	2.31

### III. Conclusion

In the present study it was observed that the infection had increased the total protein and nitrogen of the host plant in healthy leaves as compared to diseased leaves and later protein and nitrogen content increases with time in older infected tissues. In the present study, protein and nitrogen content was found to be maximum in leaves. The results from table-1 revealed that geminivirus infected plant parts leaves and stem had higher protein contents than their healthy counterparts. These results are in agreement with the reports of many other researchers and the increased protein and nitrogen content in the present study corresponds to the findings of others. Similar observations were found by many other workers in different plants infected with viruses. The proteins are complex polymer of amino acid with high molecular weight. They are most important chemical constituents of living organisms. "virus infection of plants should be regarded as change in the protein

metabolism of the host cells" [5] In mungbean plant infected with mungbean yellow mosaic virus total nitrogen and protein content was increased in diseased leaf as compared to healthy leaf [6]. Imbalances in total leaf proteins were noticed in the mosaic infected citrus species. Citrus plant infected with yellow mosaic has more protein content in infected leaves compared to healthy. The main component of total nitrogen may be insoluble protein, free amino acid and nucleic acid and amides.[7]

Mixed infection further deteriorates the quality and quantity of protein in seed. Taiwo et al[8] studied, the effect of inoculating two commercial cultivars of cowpea (*Vigna unguiculata*) (cvs) ("Oloyin" and "Olo II") with the , *Cowpea aphid-borne mosaic* (CABMV), *Cowpea mottle* (CMeV) and *Southern bean mosaic* (SBMV) viruses individually as well as in mixtures. (CABMV+ CMeV, CABMV+ SBMV, CMeV+SBMV, and CABMV+ CMeV+SBMV) at 10 and 28 days after planting on the growth, yield and nutritive content of seeds from infected plants were evaluated. Viral treatments resulted in the production of seeds with a lower protein content of 24.8-28.9% compared with the 28.5-30.4% protein in seeds from the control plants. Plants inoculated 10 Day after planting with the triple viruses produced the seeds with the least protein content (24.8-27.1%).

The biochemical changes in Geminivirus infected, diseased leaf of *Capsicum annum* were observed and estimated by Meena *et al*,[9] protein content were significantly increased in diseased leaf in comparison to healthy leaf and reached up to 3.90 mg/g, 1.8mg/g and 1.20 mg/g of fresh weight of tissue, respectively, while in healthy leaf these were 3.60 mg/g, 1.3 mg/g and 0.90 mg/g of fresh weight of tissue, respectively.

Urdbean leaf crinkle virus (ULCV) infection in black gram (*Vigna mungo*) ULCV infection resulted in significant increase in total soluble protein contents of the leaves in both genotypes. In a study Ashfaq et al[10] 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 Shukla & Rao, 1994; Langhams & Glover, 2005 [11], [12] Changes in nitrogen metabolism generally alters the protein level of the host which is regulated by an enzyme nitrate reductase which reduces exogenous nitrates to nitrites which after reducing to ammonium ions is used for protein synthesis[13]. Any change in the activity of nitrate reductase enzyme have an effect on the production of reduced nitrogen and consequently on the nitrogen and protein content, Nitrogen content Increased in alfalfa leaves as compared to the healthy plant samples,[14]. In Lettuce plant infected with Lettuce mosaic virus increased nitrate content was observed after 15 days of inoculation [15]. In papaya leaves, Papaya leaf reduction virus infection greatly increased total nitrogen content, this increase in total nitrogen is higher after the 10th day of inoculation. It was also observed that concentration of the virus in the leaves of inoculated plants increased upto the 15th day, then after it remained stationary upto the 30<sup>th</sup> day after inoculation[2]

The effects of papaya ringspot virus (PRSV) infection, on protein and non-protein nitrogen contents in the leaves of the host, *Carica papaya* L. were examined. After 12 weeks of inoculation, a threefold increase in non-protein and a corresponding decrease in protein nitrogen contents were detected in the systemically infected leaves, when compared to those of healthy leaves. Similar effects of PRSV infection were observed in naturally infected field plants. The increase in the non-protein nitrogen which accompanied the decrease of protein nitrogen content in leaves, may be probably due to the accumulation of free amino acids as a result of the hydrolysis of host proteins. As the total nitrogen content remained significantly unchanged it appears that the virus had not directly induced the synthesis of amino acids that were required to build its capsid protein, but has had obtained by the hydrolysis of the protein already present in the leaf tissue.[16] Nitrogen plays an essential role in the nutrient relationship between plants and pathogens. The development of a pathogen which is a stress causes induction of some physiological process related to nitrogen remobilization via the induction of enzymes involved in amino acid metabolism, especially Glutamine Synthetase and Glutamine synthase which are the key enzymes in nitrogen assimilation [17]. Studies by Ali et al [18] showed that GS activity interaction in Tomato Bushy Stunt Virus-infected *L. esculentum* as well as in *C. pepo* displayed similar response, GS activity was decreased to ~ 42 % in infected *L. esculentum* leaves and to ~ 46% in infected *C. pepo* leaves of its activity in corresponding control leaves. while, Glutamine synthase activity showed different trend in interaction with TBSV infection in both host plants since, Glutamine synthase activity was reduced in infected *C. pepo* leaves to about 38% of its activity in healthy control leaves; it increased in infected *L. esculentum* to ~13 fold its activity in healthy control leaves. However, viruses have differential effects on both Glutamine dehydrogenase and Glutamine Synthetase activities, in a similar manner as during senescence, virus infection cause a decrease in the total Glutamine Synthetase activity and an increase in Glutamine dehydrogenase activity[19]. Such findings were suggested that N-remobilization was favored in virus infected leaves[20]

## References

- [1] Malathi, V.G., Usharani, J.S., Sivalingam, P.N., Rouhibaksh, A., Padma Latha, K.V. and Periasamy, M., Diversity and Complexity of Begomoviruses. *Annu. Rev. Plant Pathol.* 3, 2004, 225-270
- [2] Singh, A.B. The Effect of Infection with Papaya Leaf Reduction Virus on the Total Nitrogen and carbohydrate Content of Papaya Leaves. *Phyton (Austria)* Vol. 15 Fasc. 1 — 2, 1973, 37-43,
- [3] Pande Shail Effect of Cowpea Golden Mosaic Virus Infection on Chlorophyll Content in *Vigna Unguiculata* Leaf. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 8(2), 2017, 2480-2486
- [4] Plummer, D.T. *An Introduction to practical Biochemistry.* (Tata Mc Graw Hill, Publishing Co. Ltd., Bombay, 1971)

## *Changes in Nitrogen and Protein Content of Cowpea (Vigna unguiculata) due to Geminivirus*

- [5] Bawden, F.C. and Pirie, N.W. (1952). Physiology of virus diseases. Ann. Rev. Pl. Physiol., 3, 1952, 177 - 188.
- [6] A Sinha & M Srivastava, Biochemical changes in Mungbean Plants infected by Mungbean Yellow Mosaic Virus. Int. J of Virology 6(3) , 2010,150-157
- [7] Gaddam, Susmila Aparna, Kotakadi, Venkata Subbaiah, Sai Gopal DVR, Reddy, MN, Biochemical Variability among Isolates of Citrus Yellow Mosaic Virus Infecting Different Species of Citrus in India. Research Journal of Pharmaceutical, Biological and Chemical Sciences 3 (3), 2012, 1243-59
- [8] Taiwo, M.A., Akinjogunla O.J., Cowpea viruses: Quantitative and qualitative effects of single and mixed viral infections. African Journal of Biotechnology 5 (19), 2006, 1749-1756
- [9] Meena, Rishi Kesh, Sankhla, Indu Singh and Patni, Vidya Biochemical Changes to Capsicum Annuum Leaves Infected with Gemini Virus. Plant Archives 16(1), 2016, 257-260
- [10] M. Ashfaq, M. Aslam Khan, M. Javed, N., Mughal, S.M., .Shahid, M and Sahi, S.T. Effect of Urdbean Leaf Crinkle Virus Infection on Total Soluble Protein and Antioxidant Enzymes in Blackgram Plants. Pak. J. Bot., 42(1), 2010, 447-454.
- [11] Shukla, K. and G.P. Rao. 1994. Effect of Cucumber mosaic virus infection on growth and nitrogen metabolism of pea plants. Virology in the Tropics. (Eds.): Narayan Rishi, K. L. Ahuja & B.P. Singh. Malhotra Publishing House, New Delhi, India pp: 11-164.
- [12] Langham, M. and K. Glover. 2005. Effects of Wheat streak mosaic virus (genus: Tritimovirus, family: Potyviridae) on spring wheat. Phytopathology, 95: 556.
- [13] Beevers L and Hageman R H. Nitrate reduction in higher plants. Annu. Rev. Plant Physiol. 20, 1969, 495-522
- [14] Yardımcı, Nejla, Eryiğit, Handan and Erdal, Ibrahim. Effect of Alfalfa Mosaic Virus (AMV) on the Content of Some Macro- and Micronutrients in Alfalfa. Journal Of Culture Collections Volume 5, 2006-2007, 90-93
- [15] El-Fahaam, Y.M., Fegla G.I., Wagih, E.E., El-Karyoni, H.A. Biochemical Changes in Lettuce Plant Infected with Lettuce Mosaic Virus. J. King. Saud. Univ. 2 Agric. Sci. (2), 1990, 271-277
- [16] Wijendra, W.A., Ranaweera S.S. and Salim, N. The Effects of Papaya Ringspot Virus Infection on the Nitrogen Metabolism of Carica Papaya L.: Part I. Protein and Non-Protein Nitrogen Contents in Leaves. Vidyodaya J. of Sci., Vol. 5, No.1, 1995 pp. 123-130
- [17] Solomon P.S., Tan K.C., Oliver RP. The nutrient supply of pathogenic fungi; a fertile field for study. Mol Plant Pathol. 4, 2003, 203-210.
- [18] Ali, Awatif S., Fattouh, Faiza A., Fathy, Radwa M., 438-448, Physiological Responses to Infection by Tomato bushy stunt virus in Different Host Plants. Intl J Agri Crop Sci. Vol., 8 (3) 2015, 438-448.
- [19] Pageau K, le Reisdorf-Cren M, Morot-Gaudry JF, Masclaux-Daubresse C.. The two senescence-related markers, GS1 (cytosolic glutamine synthetase) and GDH (glutamate dehydrogenase), involved in nitrogen mobilization, are differentially regulated during pathogen attack and by stress hormones and reactive oxygen species in Nicotiana tabacum L. leaves. J Exp Bot. 57, 2006, 547-557.
- [20] Tavernier V, Cadiou S, Pageau K, Lauge R, Reisdorf-Cren M, Langin T, Masclaux-Daubresse C. The plant nitrogen mobilization promoted by Colletotrichum lindemuthianum in Phaseolus leaves depend on fungus pathogenicity. J Exp. Bot. 58, 2007, 3351-3360.

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