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STUDY ON EFFECT OF CABMV INFECTION ON THE CHANGES IN CHEMICAL COMPOSITION OF *COWPEA* CV. *PUSA DOFASALI*

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ABSTRACT

The total nitrogen protein, nitrate nitrogen, nitrite nitrogen, and total free amino acids are higher in CABMV infected cowpea cv. Pusa Dofasali plant parts i.e. leaves, stems and roots than their healthy counterparts. However, the ammoniacal nitrogen was lower in infected plant parts than the healthy ones. The maximum amounts of total nitrogen, protein and total free amino acids was recorded in diseased leaves, nitrate nitrogen, and nitrite nitrogen in diseased roots and ammoniacal nitrogen in healthy leaves. The minimum amounts of total nitrogen, protein, nitrate nitrogen, nitrite nitrogen, ammoniacal nitrogen, and total free amino acids were present in the stem. Generally, nitrogenous fractions showed an increase up to 80th day of inoculation and afterwards, their concentration decreased. The concentration of total nitrogen, protein, nitrate nitrogen, nitrite nitrogen and total free amino acids was higher in diseased seed but ammoniacal nitrogen was higher in healthy seeds than diseased ones. An increased concentration of nitrogenous fraction in the virus infected plant parts of cowpea was observed. Unless previously fixed nitrogen was effectively used (or) transported elsewhere, the presence of excess nitrogenous compounds could alter the C/N ratio, which might inhibit the normal rate of nitrogen fixation. Virus infection usually brings about drastic changes in many physiological processes such changes were mainly studied with the vegetative parts of the host. Alteration due to virus infection in leguminous fruits received very little attention. Therefore, it was planned to study the effect of CABMV infection on the chemical composition of the cowpea cv. Pusa Dofasali fruits.

KEYWORDS: ***Cowpea Pusa Dofasali, CABMV, nitrogen, infections, virus***

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INTRODUCTION

Cowpea (Pusa Dofasali) is probably a native of central Africa where it grows wild. It is cultivated in the warm latitudes of the World especially within the tropics. It has now been introduced in many countries and suitable varieties are grown throughout the World during the warm season. It is placed in the family Leguminosae, In India; *cowpea* is cultivated for fodder, vegetables, or seeds.[1] (Figure-1) The dried seeds of *cowpea* are an important pulse in the tropics and sub-tropics, mostly in Africa. Fresh seeds and immature pods are eaten raw and they may be frozen or canned. The seeds are sometimes used as a coffee substitute. The young shoots and leaves are eaten as spinach and provide one of the most widely used pot-herbs in tropical Africa; they are often dried and stored for use in the dry season. In addition to these, *cowpea* also forms nutritive fodder singly and in mixture with other non-leguminous fodders. The crop also yields excellent forage both for cutting and stall feeding and for making into hay or silage. The stalks and leaves are said to be used in the preparation of green dye. In Northern Nigeria, a strong fiber is extracted from the peduncles of a variety of *cowpea*.[2] Virus infection usually brings about drastic changes in many physiological processes such changes were mainly studied with the vegetative parts of the host. Alteration due to virus infection in leguminous fruits received very little attention. Therefore, it was planned to study the effect of CABMV infection on the chemical composition of the cowpea cv. Pusa Dofasali fruits.

Figure 1
Cowpea (Pusa Dofasali)

MATERIALS AND METHODS

All the experiments during the present study were carried out in the insect-proof glasshouse, which was regularly fumigated to maintain insect free conditions. The conditions of the insectary and the methods of culturing and handling the insects were the same as described by Watson (1936, 1938 & 1972).[3-5] All the glassware required for determining the properties of virus isolates were sterilized before use. To characterize the causal agent of *cowpea* mosaic diseases, the culture of the virus was maintained on *cowpea* cv. *Pusa Dofasali.* Biological, physical and chemical methods were employed to gain a better understanding of the causal agent. Standard procedures and their modifications, if any, employed during the present investigation have been described below:

Maintenance and Raising of Test Plant

Potting media

The mixture of unsterilized sand, loam and compost (1:1:2) was prepared for raising the test plants. Soil mixtures, as well as the clay pots used, were sterilized by autoclaving them for one hour at 120^{o}c and 18 lbs pressure. Seedlings of different plants were raised in shallow clay pots of 25cm diameter and transplanted into pots of 15cm diameter at 2 to 4 leaf stage. Seeds of some species were sown directly into clay pots of 15 cm diameter depending upon the plant. Healthy seedlings, raised and maintained in an insect-proof chamber, received the uniform daylight and agro-requirements.

Virus culture

The culture of the virus isolates (*cowpea* virus isolates I and II) were obtained from naturally infected cowpea *Pusa Dofasali* plants growing in the field. Stock cultures of both the virus isolates were maintained on *cowpea* cv. *Pusa Dofasali* in insect-proof chamber where no other host susceptible to virus isolates was grown. The cultures were transferred to young and healthy *cowpea* cv. *Pusa Dofasali* at frequent intervals to have a ready stock of virus inoculum of each isolate.

Virus inoculum

The virus inoculum was prepared by macerating the leaf tissues showing distinct symptoms, in a sterilized mortar with 0.1 M phosphate buffer (pH 7.0) at the rate of one ml per g of tissue. The juice was passed through a muslin cloth and the filtrate was used as inoculum in all the experiments.

Hosts

Seeds of different legume species were obtained from the division of plant introduction, Indian agricultural research institute, New Delhi, Jhansi (U.P.), Economic botanists (Legumes), Chandra Shekhar Azad Agriculture University, Kanpur (U.P.) and Haryana Agriculture University, Hissar, while the seeds of other species were obtained from disease free plants. Before sowing seeds were treated with 0.1 percent mercuric chloride for 2 min, washed in running water till the washing had no trace of mercuric chloride and dried in the shade.

Inoculation

The inoculation was made by gently rubbing the upper surface of cotyledonary or primary leaves (as the case may be) of plants with the fore-finger dipped in infective sap. During inoculation care was taken to ensure uniform pressure and spread of inoculum. Leaves were lightly dusted with 600 mesh carborundum power, prior to inoculation. The inoculated leaves were washed immediately with a jet of distilled water. The control plants were treated similarly, using the neutral phosphate buffer solution only. The details of other methods used in further studies have been given along with the experiments.

RESULTS

Virus infection usually brings about drastic changes in many physiological processes such changes were mainly studied with the vegetative parts of the host. Alteration due to virus infection in leguminous fruits received very little attention.[1,6] Therefore, it was planned to study the effect of CABMV infection on the chemical composition of the *cowpea* cv. *Pusa Dofasali* fruits Two lots of 120 *cowpea cv. Pusa Dofasali* seedlings of uniform size in each lot were taken. Seven days old seedlings of the first lot were sap inoculated with CABMV and the second lot with 0.5 M phosphate buffer (pH 7.0) to serve as the healthy control. Fully mature pods of *cowpea* cv. *Pusa Dofasali* were collected at the 100th day after inoculation. Just after collection, the pods were kept in an oven at 80^{o}c for 24 h. Different fruit parts (fruit coat, seed coat and cotyledons) were separated and analyzed separately for different carbohydrates, nitrogenous and phosphorus fractions as described in materials and methods. The findings of the analysis are summarized in Tables 1 to 3.

Carbohydrate Contents

The findings of the Table 1 indicate that except starch, total sugars, reducing sugars and non-reducing sugars were higher in diseased fruit coat, seed coat and cotyledons than their healthy counterparts. Among the fruit parts, maximum accumulation of carbohydrate fraction (except starch) was in the cotyledons, followed by seed coat and fruit coat. Highest content of starch was noticed in the cotyledon of healthy *cowpea* cv. *Pusa Dofasali* by fruit coat and seed coat.

Table 1

Total, reducing, non-reducing sugars and starch content (mg/100 mg dry wt.) of cowpea cv. Pusa Dofasali fruit parts infected with CABMV

Carbohydrate Contents	Fruits Parts	Healthy	Diseased	% increase (+) or Decrease (-) over healthy
Total Sugars	Fruit coat	3.50	4.70	+34.28
	Seed Coat	5.25	6.50	+23.80
	Cotyledon	12.30	13.40	+8.94
Reducing Sugars	Fruit coat	1.80	2.20	+23.33
	Seed Coat	3.22	3.40	+5.59
	Cotyledon	7.92	8.08	+2.02
Non-Reducing Sugars	Fruit coat	1.70	2.48	+45.88
	Seed Coat	2.03	3.10	+52.03
	Cotyledon	4.38	5.32	+21.23
Starch	Fruit coat	10.30	8.80	-14.56
	Seed Coat	7.45	6.62	-10.60

	Cotyledon	13.35	11.25	-15.73

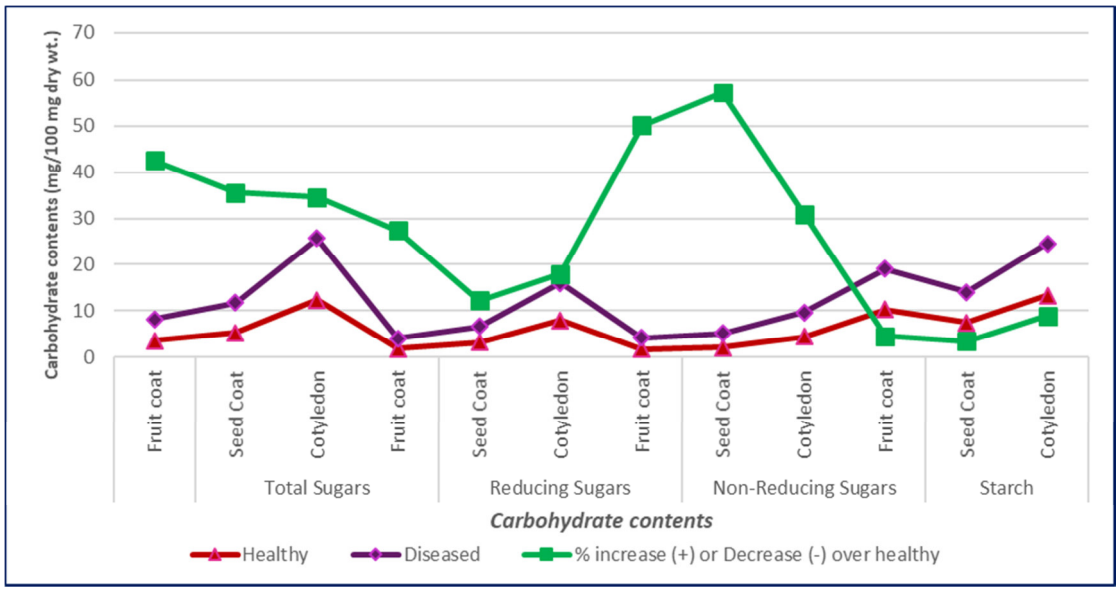

Figure 2

Carbohydrate contents of cowpea cv. Pusa Dofasali fruit parts infected with CABMV

Nitrogen Contents

A perusal of the Table 2 indicates that total nitrogen, protein, total free amino acids, nitrate nitrogen, and nitrite nitrogen were higher in different parts of the diseased fruits than their healthy counterparts. The maximum concentration of these nitrogenous fractions was in the diseased seed cotyledons followed by seed coat and fruit coat. The content of ammoniacal nitrogen was higher in the fruits of healthy plants than the diseased ones.

Table 2

Nitrogen contents (mg/100 mg dry wt.) of cowpea cv. Pusa Dofasali fruit parts infected with CABMV

Nitrogen Contents	Fruits Parts	Healthy	Diseased	% increase (+) or Decrease (-) over healthy
Total Nitrogen	Fruit coat	1.70	1.80	+5.88
	Seed Coat	1.80	2.00	+11.11
	Cotyledon	2.85	3.10	+8.77
Protein	Fruit coat	4.75	5.57	+17.26
	Seed Coat	12.50	15.00	+20.00
	Cotyledon	16.18	18.70	+16.81
Nitrate Nitrogen	Fruit coat	0.140	0.175	+25.00
	Seed Coat	0.145	0.178	+22.76
	Cotyledon	0.180	0.282	+56.66
Nitrite Nitrogen (µg/100 mg)	Fruit coat	12.00	13.15	+9.58
	Seed Coat	14.00	15.75	+12.50
	Cotyledon	15.25	19.30	+26.56
Ammoniacal nitrogen	Fruit coat	0.086	0.074	-13.95
	Seed Coat	0.090	0.080	-11.11
	Cotyledon	0.220	0.200	-9.09
Total free amino acids	Fruit coat	0.86	1.02	+18.32
	Seed Coat	0.94	1.20	+27.65
	Cotyledon	1.12	1.56	+39.28

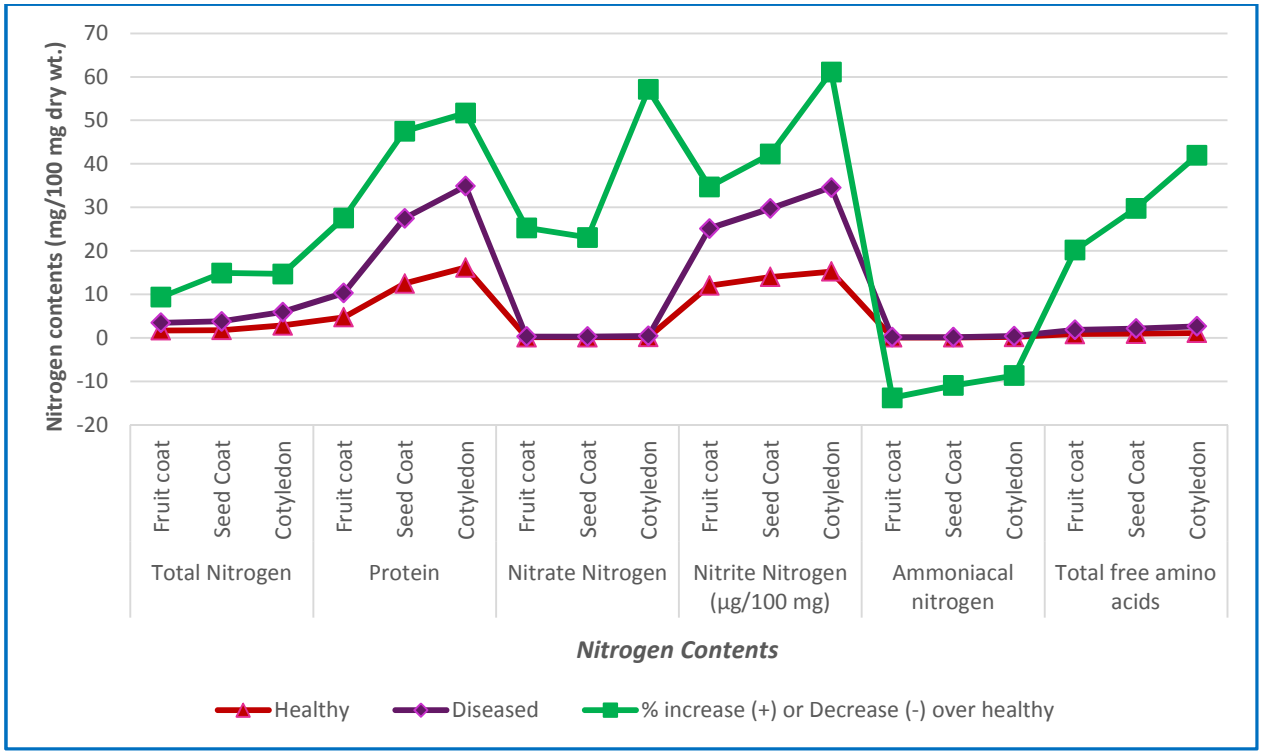

Figure 3
Nitrogen contents of *cowpea* cv. *Pusa Dofasali* fruit parts infected with CABMV

Phosphorus Contents

The data presented in the Table 3 shows that total phosphorus and organic phosphorus contents were higher in healthy fruits but inorganic phosphorus was more in diseased fruits than their healthy counterparts. The accumulation of total phosphorus and organic phosphorus were more in cotyledon followed by fruit coat and seed coat but in the case of inorganic phosphorus, accumulation was found more in fruit coat followed by seed coat and cotyledon.

Table 3
Phosphorus contents (mg/100 mg dry wt.) of cowpea cv. Pusa Dofasali fruit parts infected with CABMV

Phosphorus Contents	Fruits Parts	Healthy	Diseased	% increase (+) or Decrease (-) over healthy
Total Phosphorus	Fruit coat	0.172	0.134	-22.09
	Seed Coat	0.082	0.080	-2.44
	Cotyledon	0.385	0.231	-14.02
Inorganic Phosphorus	Fruit coat	0.037	0.040	+8.10
	Seed Coat	0.030	0.035	+16.66
	Cotyledon	0.025	0.027	+8.00
Organic Phosphorus	Fruit coat	0.135	0.094	-22.96
	Seed Coat	0.052	0.045	-13.46
	Cotyledon	0.360	0.204	-43.33

DISCUSSION

An increased concentration of nitrogenous fraction in the virus infected plant parts of cowpea was observed. Unless previously fixed nitrogen was effectively used (or) transported elsewhere, the presence of excess nitrogenous compounds could alter the C/N ratio, which might inhibit the normal rate of nitrogen fixation.

Carbohydrate Contents

A reduction in total sugars, reducing sugars, non-reducing sugars, and starch Figure 2 in the nodules of diseased plants has been noticed in the present

experiment. These findings are supported by the work of Rajagopalan and Raju (1972) on DEMV infected D. lablab plants, who also noticed, decrease in carbohydrate in the nodules of diseased plants.[7] Decrease in the carbohydrate content of the nodules of diseased plants may be a result of reduced carbohydrate contents of leaves due to CABMV infection (Figure 2).

Nitrogen Contents

The data presented in Figure 3 revealed a striking disparity in the contents of nitrogenous fractions in nodules of healthy and CABMV infected cowpea plants. The nodules of virus-infected plants contained higher total nitrogen than their healthy counterparts. Singh has also reported accumulation of nitrogen in nodules due to viral infection (Singh *et al.* 1976) in arhar (Pigeon Pea) mosaic virus infected sunn hemp, pigeon pea, urd bean, mung bean, cowpea, gram, pea, masur and berseem.[8] A few viruses such as TMV and PVX multiply to such an extent that virus protein contributes significantly to the total protein of the host and as a consequence, there is an increase in the total nitrogen. It is believed that the increase in total nitrogen and decreases in leghaemoglobin content are signs of accumulation due to inefficient nitrogen utilization in CABMV infected *cowpea.* [9] Infection with a virus brings about the diversification in the type of protein synthesized by the host. In addition to the viral proteins, other proteins like X- proteins and virus-related proteins have also been isolated from the infected tissues (Mathilde Fagard *et al.* 2014).[10] The increase in nitrate and nitrite nitrogen of diseased nodules of *cowpea* indicates that virus infection increases the nitrogen demand of the host plant resulting into enhanced absorption of nitrate nitrogen and its speedy conversion into utilizable forms like nitrate nitrogen. The increase in the nitrate reductase activity in the diseased nodules and low soil nitrogen percentage support the view. The decrease in ammoniacal nitrogen content in infected nodules further strengthens this idea. Suggested that virus is synthesized from ammonia and non-protein carbon sources.[11]

Phosphorus contents

Phosphorylated compounds have fundamental functions in many metabolic processes and therefore, virus induced disturbances of any of these compounds could have far reaching effects on the metabolic machinery of the cell. In the present case, CABMV being a systemic virus, its virus specific nucleic acid would have accumulated in the infected cowpea nodule, which as a result showed increased total phosphorus and organic phosphorus contents (Table 3). Due to the same reason, accumulation of both the phosphorus fractions was also noticed in the leaf, stem, and roots of infected plants. The low level of inorganic phosphorus in diseased nodule probably reveals the quick conversion of this fraction (absorbed from the soil) into organic form for its incorporation into viral nucleic acid as shown by the increase in nitrogenous fractions and total, organic phosphorus in the diseased nodules.

CONCLUSION

The total nitrogen protein, nitrate nitrogen, nitrite nitrogen, and total free amino acids are higher in CABMV infected cowpea cv. Pusa Dofasali plant parts i.e. leaves, stems and roots than their healthy counterparts. However, the ammoniacal nitrogen was lower in infected plant parts than the healthy ones. The maximum amounts of total nitrogen, protein and total free amino acids was recorded in diseased leaves, nitrate nitrogen, and nitrite nitrogen in diseased roots and ammoniacal nitrogen in healthy leaves. The minimum amounts of total nitrogen, protein, nitrate nitrogen, nitrite nitrogen, ammoniacal nitrogen, and total free amino acids were present in the stem. Generally, nitrogenous fractions showed an increase up to 80th day of inoculation and afterwards, their concentration decreased. The concentration of total nitrogen, protein, nitrate nitrogen, nitrite nitrogen and total free amino acids was higher in diseased seed but ammoniacal nitrogen was higher in healthy seeds than diseased ones. An increased concentration of nitrogenous fraction in the virus infected plant parts of cowpea was observed.

AUTHORS CONTRIBUTION STATEMENT

Dr. Raj Kumar Yadav, conceptualized and gathered the data with regard to this work, the methodology and results. Mr. Avshesh Kumar and Dr. Nouratan Singh analyzed these data and necessary inputs were given towards the designing of this manuscript and contributed to the final manuscript.

CONFLICT OF INTEREST

Conflict of interest declared none.

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