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Playing with the Interaction of Viruses with Plant Metabolism in the Mid-1950s: Reminiscences

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In this historical paper, I present my involvement and stories, with photographs, during the mid-1950s, on research on the effects of virus infection on the amino-acid metabolism of various plants (Croton, tobacco, and many others), using a novel multi-sector circular paper chromatography method. This research, including the one published in Nature (London), done in the laboratory of S. Ranjan, with M.M. Laloraya, T. Rajarao, and Rajni Varma, is then related to those done by others.

Keywords: *Abelmoschus esculentus*, *Acalypha indica*, F.C. Bawden, *Carica papaya*, Circular paper chromatograph, K.V.Giri, *Nicotiana tabacum*, Shri Ranjan, M. Toyota, *Trichosanthes anguina*, C.Wong.

INTRODUCTION

After completing my M.Sc. (Botany) in mid 1954, from the University of Allahabad, I was appointed as a Lecturer to teach plant physiology — having been initially trained by Shri Ranjan, who had been a graduate student of Felix Frost Blackman (1866-1947)— known for basic discoveries in photosynthesis. For Blackman, see https://en.wikipedia.org/wiki/Biographical_Memoirs_of_Fellows_of_the_Royal_Society; for Ranjan, see Laloraya (1970). Instead of working on photosynthesis (that was in the pipeline for my later studies with Robert Emerson, and with Eugene Rabinowitch, at the University of Illinois at Urbana-Champaign; see Govindjee, 2019), I began to delve in the area of the interaction of viruses with plant metabolism, specifically amino acids. For this, I collaborated with my class fellow Manmohan Manohar Laloraya (for his life and contributions, see Govindjee et al., 2023), and Tadimeti Rajarao (who had just joined Ranjan's research group; see: Govindjee et al., 2022, for his life and contributions); in 1955, we were joined by Rajni Varma; for a 1955 photograph of this group of four, see Fig. 1 (top); for a photograph of the same group, in 1998, see Fig. 1 (bottom).

By the end of 1954, just within a six-month period, Laloraya and I completed a detailed paper on the effects of virus infection on the amino-acid metabolism of *Croton sparsiflorus* (Ranjan et al., 1955), as well as a brief communication on the effects of virus infection on the amino acid metabolism of *Acalypha indica* (Laloraya et al., 1955), the latter including Rajarao. These two papers were immediately followed by a paper in Nature (London), which was on the effect of virus infection on *Nicotiana tabacum* (Laloraya and Govindjee, 1955). After this, in 1956, we published three more reports on differences, in the amino acids, between the healthy and virus-infected plants of (i) *Trichosanthes anguina* (Rajarao et al., 1956); (ii) *Carica papaya* (Laloraya et al., 1956; it was here that Rajni Varma was with us); and (iii) *Abelmoschus esculentus* (Govindjee et al., 1956).

First, we summarize below stories and details of our observations in the first three papers, just mentioned above, which was followed by the three, published in 1956 (see below). For a paper by another research group, on this topic, see e.g., John (1963). We, however, note that our work had been recognized by many including Kiraly and Farkas (1959) and Magyarosy et al. (1973). For the current status of the field of viruses and plants, we refer the readers to excellent overviews by van derWant and Dijkstra (2006), Lefeuvre et al. (2019), and Wu et al. (2019).

On Our First Three Papers, Published in 1955, on the 'Amino Acid Metabolism' of Virus-Infected and Healthy Plants

It is important to begin by giving the story about our success in separating all the amino-acids from the healthy and the diseased leaves of plants by circular paper chromatography (also see: Govindjee et al., 2022, 2023). It was in late 1954 September that

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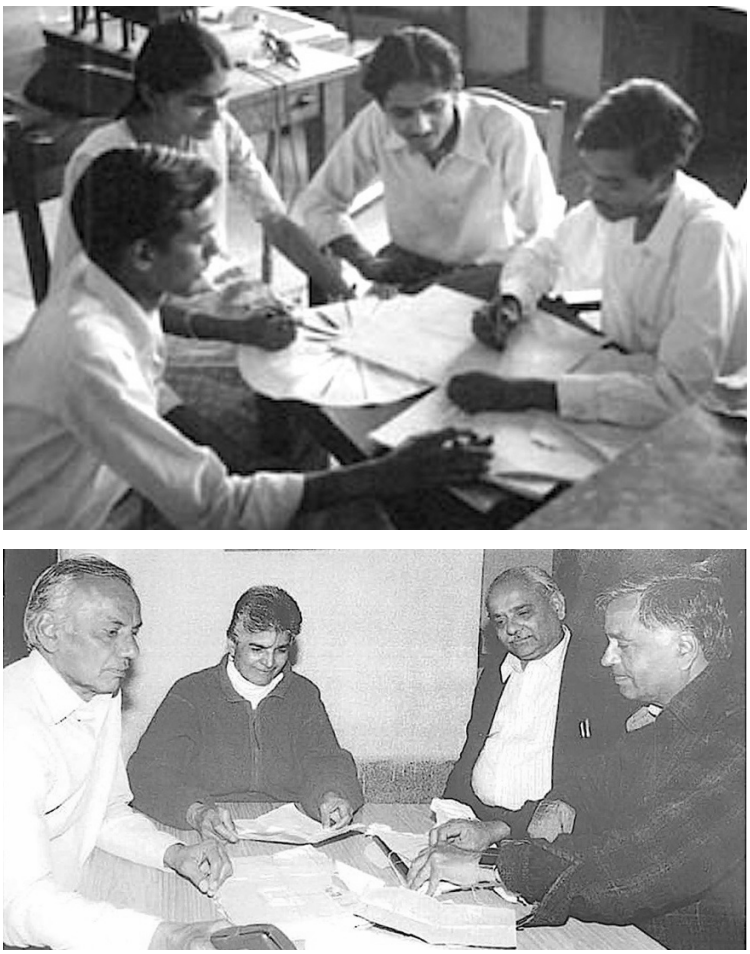
Laloraya and I—after working almost all night—had success! We were so excited that the very next morning, at 8 AM, we went to the home of Professor Shri Ranjan, carrying the circular paper chromatogram, wrapped up in a newspaper, that showed results from the healthy and the virus-infected leaves (also see Laloraya, 1970; and Govindjee, 2019). Prof. Ranjan's first words were : "It is beautiful" and he was so excited that he drove us in his Ford car to the laboratory in the Botany Department, Allahabad University, to see, first- hand, our entire set-up. And, right away, he granted us funds to purchase anything we needed for our research. This led to the publication, in 1955, of the three scientific journal articles mentioned above (for details, see below).

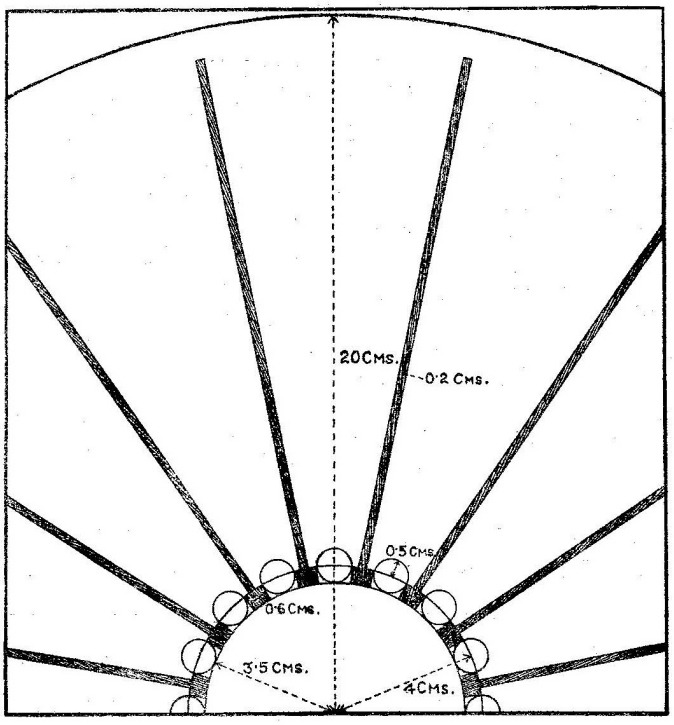


Source: <https://www.life.illinois.edu/govindjee/> (bottom): the four of us are sitting in the same order as above, in Indore, India, more than 40 years later, in 1998– in which we had a reunion. Source: Malini Laloraya; reproduced from Fig.6 in Govindjee et al. (2023)

Fig. 1: (top). Left to right: Tadimeti Rajarao, Rajni Varma, Manmohan Laloraya, and Govindjee, in Ranjan's Plant Physiology Laboratory, Department of Botany, University of Allahabad, 1955

What did the Virus-Infection do?

The Ranjan et al. (1955) paper, on *Croton sparsifloris*, was an elegant detailed, first of its kind, using a paper chromatographic method, where 12-16 samples were run, simultaneously, with radial cuts to separate them, and the levels of all the amino acids could be separately measured. (For a diagram, see Figure 2.)



Diagrammatic representation of a portion of Whatman No. 1 filter paper—40 cm. (diameter). The shaded areas are cut-off. The arrows pointing towards the centre mark the position of the wick.

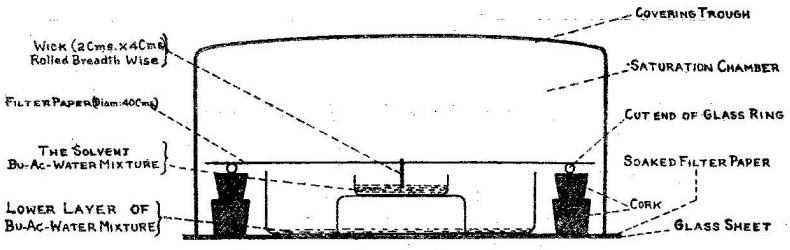


Fig. 2: A diagrammatic representation of part of the circular paper chromatogram, reproduced from Ranjan et al. (1955)

We would like the readers to know that the order of authors was decided by Professor Shri Ranjan who communicated it to the Proceedings of the National Institute of Science of India. As far as I remember, the experiments were done by Laloraya and Govindjee and the paper was written by Govindjee and Laloraya—however, the idea of the radial cuts on the chromatograms was that of T. Rajarao, who was thanked in the acknowledgment section. As this paper was being "finalized" by Ranjan, as Ranjan et al. paper, he told us that we can, from now on, publish our observations on other virus-infected plants — without his name!

The new observations here in our first paper, on the leaves of *Croton* sp. leaves, clearly showed that the virus infection caused an accumulation of the free amino acid (s) histidine/lysine. This was a very new finding and provided the first clear information on what viruses do, as Bawden (1954) had just been asking and speculating on this topic. Almost in parallel to the work on *Croton sparsiflorus*, Laloraya et al. (1955) looked not only on the changes in the amino acids, but also in the sugars, in the leaves of a common weed *Acalypha indica.* Here, in the virus-infected leaves, the content of many amino acids increased, but that of the sugars decreased in the 'mosaic' over that in the

normal leaves. Although we did not quite understand the importance of it all, we were thrilled by these observations.

What Changes Did the Virus-Infection Make to the Amino Acids of *Nicotiana tabacum?*

Soon thereafter, Laloraya and Govindjee (1955) had new observations on differences in the amino-acids between the normal and virus-infected leaves of tobacco (*Nicotiana tabacum*). *But, first, a story!* Since Ranjan had told us (see above) that we can independently (i.e., without his name) submit papers, we did that for the tobacco paper and mailed it to Nature (London), the topmost journal. As soon as we received the acceptance letter, we were so excited that we rushed to Ranjan's office to show him the letter from the editor of "Nature". Hearing this, Prof. Ranjan spoke to us in a stern voice: *"Sit down, you boys!"*– Laloraya was a "research scholar" on Ranjan's research grant, and I was a "lecturer" (equivalent to an "assistant professor"). Ranjan added : "I did not know that you will submit your paper to "Nature" ; from now-on, you will submit all your papers through me !" Laloraya, immediately, agreed, but I kept quiet since I was working on another independent project, which was on the effects of X-rays on the metabolism of *Cicer arietinum* (see Govindjee, 1956, 1957).

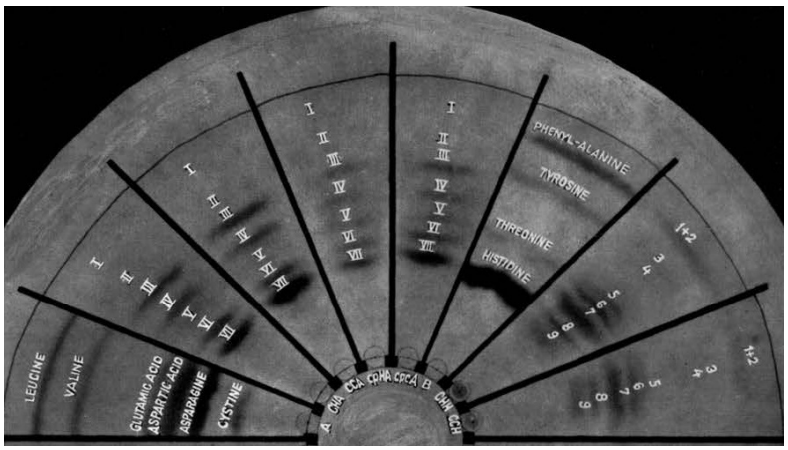
We summarize below our observations on the amino-acids of virus—infected tobacco leaves. We were aware of yellow and sick-looking tobacco plants. We brought, to the Laboratory, the leaves of the plants that were infected by "mosaic" and "leaf curl" viruses-based on their appearances, along with those from the healthy ones. Laloraya and Govindjee (1955) showed that the virus had caused an increase in asparagine (an amide), and several amino acids, especially aspartic acid, and histidine/lysine. In addition to providing new information, our work supported the notion that virus infection may cause the formation of new proteins in the infected plants. For results on virus effects on the metabolism of *Phaseolus vulgaris,* see Laloraya (1955). Our research provided specific information on the idea that virus infection is associated with increase in soluble proteins in tobacco (see e.g., Martin et al., 1938, 1939; Wildman et al., 1949; Meneghini and Delwiche, 1951, as well as Commoner et al., 1952, 1953; and Commoner and Nehari, 1953) ; also see Takahashi and Mamuro (1952). We note that all of this was possible because we had remarkably "improved upon" the earlier paper chromatographic methods of Giri and Rao (1952), Giri et al. (1952a, 1952b) and Ganguly (1954). Our method had incorporated the advantages of both circular paper chromatography and strip-paper chromatography wherein amino acids appeared in arcs instead of spots. Since some others had reported the presence of lysine along with other amino-acids in the nitrogen content of tobacco mosaic virus, we considered it possible that a band (having a Rf value of 0·20) might be the lysine of tobacco mosaic virus protein. However, the formation of asparagine and the increase in the content of other free amino acids, especially aspartic acid, observed by Laloraya and Govindjee (1955) in the diseased tobacco leaves clearly supported the view of Bawden (1954a, 1954b) that virus infection leads to the formation of a new range of proteins (also see: Pirie, 1950).

On our next three reports on the effects of virus infection on the amino acids in *Carica papaya*, *Trichosanthes anguina,* and *Abelmoschus esculentus*

Following the methods mentioned above, Laloraya et al. (1956) observed increased formation of asparagine in 'Carica-curl' virus infected papaya leaves (a result similar to that in tobacco), as well as of alanine- and γ amino-butyric acid, but there was a decrease in the content of insoluble leaf proteins. On the other hand, Govindjee et al. (1956) showed, in *Trichosanthes anguina*, the absence of valine + methionine and aspartic acid, as well as a decrease in the amount of some other amino acids in virus- infected leaves; we were unable to decide if this indicated their utilization in the synthesis of virus protein or is just a decrease in their content. About the same time, Rajarao et al. (1956) showed that in *Abelmoschus esculentus* ("hibiscus" in English; "bhindi" in Hindi) using the virus-infected leaves had an increase in the content of the amide asparagine as well as of several free amino acids. Although increase in the formation

of several free amino acids had been observed in many cases, asparagine formation had been known only in just a few cases. Further, the concentration of some amino acids (e.g., tyrosine) was lower, but of many others (leucine, isoleucine, phenylalanine, tyrosine and aspartic acid) higher in virus-infected leaves.

As an example of data, obtained in the above research, we show a photograph of the paper chromatogram on the amino acids from Carica curl – virus infected and healthy leaves (Laloraya et al., 1955) as Figure 3 reproduced here mainly for history since all the four shown in Figure 1 were co-authors of this paper.



Source: Laloraya et al. (1956)

Fig. 3: A Chromatogram showing Amino Acids of Healthy and 'Carica-eurl' infected Leaves of Carica papaya (Solvent: butanol- acetic acid-water: 4 : 1 : 5). See the bottom semi- circle for the symbols: A and D- Reference Solutions; CHA—Alcoholic Extract of Healthy Leaves; CCA - Alcoholic Extract of Carica-curl infected Leaves; CpHA—Alcoholic Extract of healthy petiole; CpCA—Alcoholic Extract of 'Carica curl' infected petiole; CHH—Acid Hydrolysate of Healthy Leaves; CCH—Acid Hydrolysate of 'Carica-curl' infected Leaves

EPILOGUE

What do the above observations — during the above brief period in our early lives — mean to the history of this field must be left to the current authorities in the field to tell us. Furthermore, for the development all in all, the two years (1955-1956) of my research life — when I was a Lecturer in Botany at the University of Allahabad, was great fun and a learning experience on how to do research, how to formulate objectives, and how to go about doing it all in a team. Although I made no real lasting "dent" in the field it led all of us: Manmohan Laloraya (Govindjee et al., 2023); Tadimeti Rajarao (Govindjee et al., 2022); Rajni Govindjee (Balashov et al., 2023); and myself (https://www.life.illinois.edu/govindjee/90th-birthday.html) into our own directions. For some of the very early papers, related to viruses and plants, see Mayer (1886), Beijerinck (1898), Stanley (1935) and Bawden et al. (1936); for its components, see Frenkel-Conrat and Williams (1955); and for virology, through the ages, we refer the readers to read an excellent overview by Zuo et al. (2024).

I end my educational perspective by citing a personal communication from Xiaofei Cheng, a current authority in Plant Virology. He wrote, in November, 2024 : "I have carefully read this historical perspective, more specially, the story about the pioneering work on the amino acid changes during plant virus infection. I really enjoyed reading it. I would like to add, from my own perspective that some amino acids actually act as key hormones in plant immunity, e.g., glutamate (cf. Toyota et al., 2018) and lysine (cf. Wang et al., 2018); this clearly links the early discoveries, described in this paper, with the progress being made, around the World, now, on plant immunity."

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I thank Dr. Xiaofei Cheng (of the College of Plant Protection, Northeastern Agriculture University, China), a leading international authority in Plant Virology, for reading this manuscript and for his comment, cited above. I dedicate this historical paper to one of the fathers of Plant Virology, Frederick Charles Bawden (1908-1972); https://doi.org/10.1098/rsbm.1973.0002.

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