Carbohydrate and Nitrogen Content of a Virus-Diseased Tobacco Plant

Introduction. Previous data show that virus infection usually affects the carbohydrate and nitrogen content of the diseased plants; increased soluble sugar content of beet leaves infected with virus yellows was noticed by STANGE[1]. GEROLA and GILARDI[2] proved an increase in amylase activity during the sprouting of virus-infected potato tubers. On the other hand, virus-diseased plants tend to accumulate organic acids and in particular amino acids, oxalic, malic, and citric acids (COMMONER and NEHARI[3] working with tobacco mosaic virus; GOVINDJEE et al.[4] using yellow vein mosaic of Hibiscus).

The following is an endeavour to elucidate the effects of tobacco mosaic virus (TMV) on the carbohydrate and nitrogen content of tobacco leaves.

Materials and methods. Plants of *Nicotiana tabacum* were grown under natural conditions in a glass-house. They were sprayed weekly with 0.0015 p.p.m. pholidol for protection against insect pests. When the plants were about 20 inches high, half of them were inoculated with tobacco mosaic virus through rubbing the lower leaves with a dilute water solution of the partially purified virus containing a small amount of carborundum as abrasive. Three weeks later, the infected and healthy leaves were collected and each was randomized for uniformity in 10 samples, 5 leaves each. Samples were weighed, frozen at 0°C for 10 days, thawed rapidly (BAWDEN and PIRIE[5]) and triturated in a mortar. The sap was passed through cheese-cloth and made up to volume. The residue and extract were analysed for their carbohydrate fractions according to the method adopted by NAGUIB[6,7]. The soluble nitrogen fractions estimated in the tissue extract were ammonia-, amide-, amino-, nitrate, nitrite, and total soluble nitrogen; the difference between the latter and the sum total of the first 5 fractions was called the peptide-N.

Amides, proteins, and total soluble nitrogen were estimated through their conversion to ammonia either by hydrolysis in 2.5 *N* H_2SO_4 solution at 100°C for 4 h for the first fraction or by digestion for the last two fractions. The estimation of ammonia was carried out using Nessler's solution prepared by DELORY[8]. Nitrates and nitrites were estimated according to PAECH and TRACEY[9], while RUSSELL's method[10] was applied for the determination of amino nitrogen.

Results and discussion. Analysis of both healthy and diseased leaves for their different carbohydrate and nitrogen fractions proved the absence of galactose, soluble disaccharides, and nitrites from the tissues.

Effect of tobacco mosaic virus on the nitrogen content of tobacco leaves. Table I records the mean value of the different nitrogen fractions present in the healthy and diseased tobacco leaves. The Table shows clearly that the infected leaves contained a significantly high level of total nitrogen that resulted from the increased protein and peptide nitrogen contents while the other fractions were more or less similar to those of the control. These results agree with those of STANGE[1], who found that the virus yellows increased the nitrogen content of sugar beet blades. Furthermore, FIFE[11] showed that amino acids,

[1] L. STANGE, Z. Phytopath. *21*, 214 (1953).
[2] F. M. GEROLA and E. GILARDI, Nuovo G. Bot. Ital. *62*, 384 (1955).
[3] B. COMMONER and V. NEHARI, J. gen. Physiol. *36*, 791 (1953).
[4] M. GOVINDJEE, M. LALORAYA, and T. R. RAO, Exper. *12*, 180 (1956).
[5] F. C. BAWDEN and W. W. PIRIE, J. gen. Microbiol. *4*, 482 (1951).
[6] M. I. NAGUIB, Z. Zucker *15*, 351 (1962).
[7] M. I. NAGUIB, Z. Zucker *16*, 15 (1963).
[8] G. E. DELORY, *Photoelectric Method in Clinical Biochemistry* (1949), p. 76.
[9] K. PAECH and M. V. TRACEY, *Modern Method of Plant Analysis* (Springer-Verlag, Berlin 1955), vol. 1, p. 481.
[10] F. D. SNELL and C. T. SNELL, *Colorimetric Methods of Analysis* (D. Van Nostrand Co. Inc., London 1954), vol. IV, p. 109. – E. G. FRAME, J. A. RUSSELL, and A. E. WILHELMI, J. biol. Chem. *149*, 255 (1943). – J. A. RUSSELL, J. biol. Chem. *156*, 467 (1944).
[11] J. M. FIFE, J. Am. Soc. Sugar Beet Technol. *9*, 207 (1956).

Table I. Nitrogen fractions in healthy and diseased tobacco leaves (mgN/100 g fresh weight)

Treatment	Ammonia and amide	Amino	Nitrate	Peptide	Total soluble nitrogen	Insoluble proteins	Total nitrogen
Healthy	4.09 ± 0.41	1.19 ± 0.09	2.81 ± 0.22	14.23 ± 0.77	22.32 ± 1.28	13.74 ± 0.47	36.06 ± 1.76
Diseased	3.57 ± 0.45	1.21 ± 0.12	2.59 ± 0.27	21.94 ± 0.83	29.31 ± 1.51	18.86 ± 0.85	48.17 ± 0.26

Table II. Carbohydrate fractions in healthy and diseases tobacco leaves (mg sugar/100 g fresh weight)

Treatment	Soluble fractions						Insoluble fractions						Total carbohydrates
	Glucose	Fructose	Total hexose	Pentose	Total monosaccharides	Total soluble sugars	Glucosans	Fructosans	Galactosans	Total hexosans	Pentosans	Total polysaccharides	
Healthy	18.75 ± 1.43	39.5 ± 3.05	58.25 ± 3.3	1.92 ± 0.21	60.17 ± 4.08	60.17 ± 4.08	75.23 ± 4.74	43.68 ± 3.29	81.00 ± 4.79	199.91 ± 7.22	203.89 ± 8.11	403.80 ± 13.71	463.97 ± 17.80
Diseased	21.82 ± 1.07	39.3 ± 3.07	61.12 ± 3.14	2.30 ± 0.27	63.42 ± 3.25	63.42 ± 3.25	103.74 ± 2.71	43.21 ± 3.22	67.36 ± 4.4	214.31 ± 5.02	211.86 ± 11.32	426.17 ± 11.34	469.59 ± 18.39

particularly arginine, aspartic and glutamic acids, tend to accumulate in sugar beet leaves affected by the curly top virus, while DIENER[12] showed that virus-infected peach leaves contained higher proline content than the healthy ones. REINDEL and BIENENFELD[13] proved that the leaf roll induced a high soluble nitrogen content of potato leaves mostly phosphorus containing peptides.

Effect of tobacco mosaic virus on the carbohydrate content of tobacco leaves. Table II represents the mean values of the different carbohydrate fractions present in both healthy and diseased leaves. The Table shows clearly that virus infection seemed to have no significant effect on the different soluble carbohydrate fractions of tobacco leaves. Furthermore, in spite of the fact that the total polysaccharide content was more or less similar in both diseased and healthy leaves, yet the glucosan content was higher and the galactosan content was lower in the former than in the latter.

These results indicate that the only effect of the virus on the carbohydrate metabolism seemed to be the activation of starch phosphorylase accompanied by retardation of hexose isomerase. This contradicts the observations of STANGE[1], who found that the concentration of sucrose, glucose and fructose was always higher in the sugar beet leaves affected with virus yellows than in the healthy ones.

Zusammenfassung. Der Tabakmosaikvirus vermehrte den Gehalt an Protein- und Peptidstickstoff in den befallenen Tabakblättern, wirkte aber weder auf die totalen Kohlehydrate noch auf die Kohlehydratfraktionen. Hingegen wurde eine gewisse Beschleunigung der Glukosanbildung nachgewiesen, ein Phänomen, das von einer gleichzeitigen Abnahme der Galaktosanbildung begleitet war.

JOSEPHINE K. ESKAROUS and M. I. NAGUIB

Botany Department, Faculty of Science, Cairo University, Guiza (Egypt), April 1, 1964.

[12] T. O. DIENER, Phytopathology *50*, 141 (1960).
[13] F. REINDEL and W. BIENENFELD, Hoppe-Seyler's Z. *303*, 262 (1956).

Chlamydospore Germination and Artificial Culture of *Protomyces macrosporus* Unger

Coriander (*Coriandrum sativum* L.) suffers recurrently from a 'stem gall' disease, incited by *Protomyces macrosporus* Unger which is widely distributed in the country. The infection stimulates development of linear to fusoid, coalescent gall-like swellings over the stem surface and twirling 'goose-neck' shoots during preflowering period of the host. Abundant chlamydospores develop intercellularly in the hypertrophied tissues providing primary inoculum for the pathogen in the following crop season.

During the course of investigation, mature galls were collected in March–April 1963, stored under low temperature conditions and a portion was weathered out of doors. Periodical tests for chlamydospore germination gave negative results. Earlier, POPTA[1], VON BUEREN[2], and BUTLER[3] reported their germination, while TUBAKI[4] indicated termination of the dormancy period as prerequisite for it. Chlamydospores were fixed onto the slides by alternate wetting and drying and inverted over wet cotton for moisture condensation[5,6]. Less than 25% chlamydospores from both the lots kept for 4 months in storage germinated at room temperature (22 to 24°C) after prolonged incubation. Immersion of the spores in water for over a week was favourable to help break the thick exospore[3,4]. The hard thick exospore was possibly blocking the germination which was apparently construed for a prolonged dormancy. Steeping in acidulated or weakly alkalined distilled water (pH 5 to 5.5 and 8 to 8.5 respectively) for a short period helped soften the exospore and hastened their germination to over 90%. Mature chlamydospores developed in the current season also yielded a high germination percentage when similarly pretreated.

Pretreated chlamydospores, prior to bursting their exospores, were transferred to sterile moist filter paper strips stuck inside the cover of a petri plate and inverted over potato-dextrose-agar medium as suggested by TUBAKI[4]. The plates were incubated at room temperature. The spores germinated, ejecting their contents over the agar substrate, which developed into small colonies. Isolated colonies were transferred to potato-dextrose-agar slants. They appeared like glistening bacterial colonies externally, consisting of unicellular budding spores. Two types of cultures could be differentiated by their pigmentation. One of them was dull, creamy white, and the other bright, deep salmon-red in colour.

Several lots of month-old host seedlings were inoculated by spraying separately with the two culture types and retained in moist chambers for 48 h. Typical galls developed on the inoculated stems in about 2 weeks in both cases. Further work is in progress.

Zusammenfassung. Die Keimruhe der Chlamydosporen von *Protomyces macrosporus* Unger, welcher auf *Coriandrum sativum* L. Stamm-Gallen erzeugt, kann durch künstliches Aufweichen der Exospore gebrochen werden. Der Pilz ist auf Kartoffel-Dextrose-Agar kultivierbar.

A. N. MUKHOPADHYAY and M. S. PAVGI

College of Agriculture, Banaras Hindu University, Varanasi (India), March 6, 1964.

[1] C. POPTA, Flora *86*, 1 (1899).
[2] G. VON BUEREN, Mycol. Centralbl. *3*, 12 (1913).
[3] E. J. BUTLER, *Fungi and Disease in Plants* (Thacker Spink & Co., Calcutta 1918), p. 547.
[4] K. TUBAKI, Mycologia *49*, 44 (1957).
[5] M. J. THIRUMALACHAR and M. S. PAVGI, Indian Phytopath. *3*, 177 (1950).
[6] M. J. THIRUMALACHAR and M. J. NARASIMHAN, Mycologia *45*, 461 (1953).