

INFLUENCE OF CATION BINDING BY TOBACCO MOSAIC VIRUS ON NITROGEN AND CARBOHYDRATE CONTENTS IN TOMATO PLANTS

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ABSTRACT

*The effect of tobacco mosaic virus (TMV) and TMV mixed with 10⁻³ M Aluminium chloride or Calcium chloride infection on the nitrogen and carbohydrate contents and also on the total fresh and dry weights of *Lycopersicon esculentum* L. cv. Hilda has been studied. TMV alone or TMV mixed with cations lowered the total fresh and dry weight as compared to healthy ones. The total nitrogen content was increased in all treatments compared with healthy plants. On the other hand, virus alone showed marked increase in carbohydrate contents in infected plants, while virus mixed with cations produced great reduction in carbohydrate values.*

INTRODUCTION

It has previously been found that most, perhaps all plant viruses contain calcium - binding sites with some of the properties to be expected for a switch controlling disassembly (Durham *et al.*, 1977 a, Durham and Haidar, 1977b). Ferrario *et al.* (1985) showed that calcium is a regulator of many metabolic processes and has biphasic influence on TMV multiplication.

The studies on the interaction of cations such as Ca⁺² or Al⁺³ with virus on the virus - infected plants especially on their nitrogen and carbohydrate contents are limited. Tu *et al.*, 1970 a; b, found higher nitrogen content in the soybean mosaic

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virus infected soybean nodules. Rajgopalan and Raju, 1972, also noticed higher nitrogen content in Dolichos enation mosaic infected Dolichos lablab L. plants. Singh and Mall, 1974, reported that arhar mosaic virus strains (ASM and AMM) lowered the growth and fresh weight of cowpea, mung and urd plants in comparison to healthy plants. Tu, 1986, proved that Ca^{+2} plays a vital role in counteracting the effect of IAA or kinetin and promoting local lesion formation and development in bean infected with alfalfa mosaic virus.

The present investigation was undertaken to study the effect of infection of tobacco mosaic virus alone or mixed with Ca^{+2} or Al^{+3} on nitrogen and carbohydrate content and also on the total fresh and dry weight of *Lycopersicon esculentum* L. cv. Hilda plants.

MATERIALS AND METHODS

a) preparation of Virus Inoculum

A virus was isolated from naturally infected *Nicotiana glauca* plants grown in Egypt. It induces mosaic mottling and some abnormalities including deformation of infected leaves and was identified as a new strain of tobacco mosaic virus (Eskarous *et al.*, 1975). Tobacco mosaic virus (TMV) was partially purified from frozen infected *Nicotiana tabacum* L. var. samsun leaves. The leaves were ground in 0.06 M phosphate buffer pH 7.0 with a little washed sand. Then, the leaves were squeezed through muslin and centrifuged at 2000 xg for 15 min. the supernatant was kept at room temperature overnight to precipitate proteinaceous inhibitors, and then partially clarified by further centrifugation. The supernatant virus solution was stored at -25 °C.

b) Preparation of Assay Host Plant

Seeds of *Lycopersicon esculentum* L. cv. Hilda were planted in 15 cm pots filled with good soil. Five plants per pot were grown. Pots were divided into 4 sets. After two months the plants became suitable for inoculation. Seedling of the first, second and third set were inoculated with TMV mixed with water, TMV mixed with CaCl_2 and TMV mixed with AlCl_3 respectively. The fourth set of seedlings was inoculated with water only and used as control. For inoculation, the leaves were dusted with 600 mesh carborundum as abrasive. The inoculated leaves were harvested after 45 days of inoculation.

c) Determination of Total Soluble Nitrogen

A known aliquot of borate buffer extract was well digested using 50% sulphuric acid followed by little dilute perchloric acid for complete oxidation, till the whole mixture turned pale green or colourless. This was made up to volume before a known amount was neutralized and its ammonia content was estimated by the Bertholt reaction. The values, obtained by this procedure, denoted all soluble nitrogen components. Nitrate and or nitrite nitrogen which were of insignificant amounts or even absent were estimated from the extracts.

d) Determination of Total Insoluble Nitrogen

A known volume of the sodium hydroxide residue extract was well digested and its nitrogen content determined following the previously mentioned procedure.

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e) Estimation of Total Monosaccharides (Direct Reducing Values -DRV)

This was estimated in the cleared phenol -water extract. 2 ml solution were mixed with 3 ml modified Nelson's solution and the mixture was kept in boiling water bath for 15 min (Naguib, 1964). After cooling 3 ml arsenomolybdate solution were added and the mixture was shaken till effervescence stopped. The coloured solution was made up to volume before photometrically measured at 700 nm.

f) Estimation of Sucrose

1.5 ml clear extract were mixed with 0.5 ml of 1% invertase solution. The mixture was incubated for one hour, at 37 °C, before its reducing value was determined according to the procedure for direct reducing value. The difference between the value, obtained by this procedure, and that obtained for the total monosaccharides is the estimate of sucrose, in the terms of glucose.

RESULTS

Results in table (1) indicated that TMV mixed with water and TMV mixed with Ca^{+2} or Al^{+3} ions markedly reduced the total fresh and dry weight of *Lycopersicon esculentum* L. cv. Hilda plants as compared with healthy plants. The virus mixed with ions showed less effect on the fresh and dry weights in comparison to the virus mixed with water.

Table (1) : Effect of TMV mixed with water and TMV mixed with Ca⁺² or Al⁺³ ions for one hour on the total fresh and dry weights of *Lycopersicon esculentum* L.

Treatment	Total fresh weight	% decrease	Total dry weight	% decrease
Healthy plant	60.45	-	7.00	-
TMV + Water	37.40	38.13	3.93	43.85
TMV + Ca ⁺²	53.50	11.50	5.88	16.00
TMV + Al ⁺³	55.25	08.60	5.80	17.14

Results in table (2) and (3) showed that virus mixed with water or with cations (Ca⁺² or Al⁺³) increased total nitrogen percentage in comparison to healthy plants. On the other hand, virus in water showed marked increase in total carbohydrate percentage. In contrast, the carbohydrate values in infected *Lycopersicon esculentum* L. cv. Hilda decreased significantly when TMV was mixed with calcium or aluminium chlorides for one hour before inoculation.

Table (2) : Effect of TMV mixed with water for one hour on nitrogen and carbohydrate contents of *Lycopersicon esculentum* L.

Treatment	Healthy plant	TMV mixed with water	%
Total Nitrogen	00.276	00.36	+31.50
Total DRV	21.70	30.80	+41.93
Total sucrose	06.30	11.50	+82.54
Total Starch	56.00	73.30	+30.89

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Table (3) : Effect of TMV mixed with Ca⁺² or Al⁺³ ions for one hour on nitrogen and carbohydrate contents of *Lycopersicon esculentum* L.

Treatment	Healthy plant	TMV mixed with Ca ⁺²	%	TMV mixed with Al ⁺³	%
Total Nitrogen	00.276	00.283	+02.54	00.336	+21.74
Total DRV	21.70	0.90	-57.60	17.20	-20.74
Total Sucrose	06.30	02.80	-55.55	03.30	-47.62
Total Starch	55.00	43.50	-22.14	41.80	-25.36

% (-) = Percentage decrease.

% (+) = Percentage increase.

DISCUSSION

The effect of viruses on fresh and dry weight ratio of some plants have been reported. Chant (1960) found that tobacco mosaic and cowpea yellow mosaic viruses increased the fresh and dry weights of infected cowpea plants. In contrast, Harrison (1935) and Reddy and Chenulu (1970) reported that bean mosaic and cowpea mosaic viruses respectively decreased the fresh and dry weight of their respective susceptible plants. These results suggest that the variation in fresh and dry weights of infected plants may be due to the differences in susceptibility of such plants. Our results agreed with these last point of view .

The total nitrogen value increased significantly in infected *Lycopersicon esculentum* L. cv. Hilda plants either inoculated with TMV mixed with water or mixed with cations (Ca⁺² or Al⁺³). Reports on nitrogen content of plants infected with virus showed that, most virus infected plants showed an increase in nitrogen content (Kreitlow *et al.*, 1957, Nambiar and Ramakrishnan, 1969). The higher nitrogen value in infected *Lycopersicon esculentum* L. cv. Hilda plants can be

explained on the observation of Takahashi and Ishii (1953) who indicated that infected plants contain in addition to virus, varying amounts of abnormal proteins serologically related to TMV. Such suggestion was also supported by Singh and Mall (1974). Tu et al (1970 b) and Rajgopalan and Raju (1972) also found higher nitrogen content in soybean mosaic virus infected soybean nodules and *Dolichos lablab* L. respectively. They believe that higher nitrogen in the nodules is due to insufficient utilization of nitrogen by the infected plants.

Total carbohydrate level increased in infected plants inoculated with virus alone but decreased markedly in plants inoculated with TMV mixed with Ca^{+2} or Al^{+3} for one hour before inoculation. These results suggest that the rate of carbohydrate consumption when virus mixed with cations exceeds the rate of accumulation by the plant as compared to healthy one. However, virus in water showed carbohydrate accumulation in the infected plant. Brants (1964) suggests that the increased carbohydrate content of host infected leaves resulted in increased respiration and a subsequent increase in susceptibility.

The carbohydrate content in infected tomato plants depended on cations associated with virus. Further accurate investigations are now necessary to understand the influence of Ca^{+2} or Al^{+3} on TMV multiplication and their direct effect on the storage of carbohydrate levels in infected plants.

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تأثير اتحاد فيروس تبرقش الدخان مع الكاتيونات على المحتوى النيتروجيني والكربوهيدراتى لنباتات الطماطم

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فى هذا البحث تم دراسة تأثير العدوى بفيروس تبرقش الدخان بمفرده وأيضا وهو مختلط بمحلول كلوريد الألومنيوم أو كلوريد الكالسيوم على المحتوى النيتروجينى والمحتوى الكربوهيدراتى وأيضا على الوزن الرطب والوزن الجاف لنباتات الطماطم "هيلدا".

ولقد وجد أن العدوى بفيروس تبرقش الدخان بمفرده أو مختلط بالكاتيونات تقلل فى الوزن الرطب أو الوزن الجاف وذلك بالمقارنة بالنباتات السليمة.

كما تبين أن المحتوى النيتروجينى قد زاد فى جميع المعاملات بالمقارنة بالنباتات السليمة.

ولقد أثبت البحث أيضا أن العدوى بالفيروس بمفرده تزيد من المحتوى الكربوهيدراتى للنباتات المصابة بينما العدوى بالفيروس مختلطا بالكاتيونات تسبب نقصا شديدا فى المحتوى الكربوهيدراتى للنباتات المصابة .