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KINETIN TREATMENT OF STOLBUR
DISEASED TOMATO PLANTS (*LYCOPERSICON
ESCULENTUM* L.) AND THE POSSIBILITY OF
ITS APPLICATION IN CHEMOTHERAPY

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In this study we investigated the influence of kinetin (6-furfurylaminopurine) on stolbur infected tomato plants. By using electron microscopy we analysed the starch accumulation and the thylakoid membranes in the chloroplast and we quantified the photosynthetic pigments by spectrophotometry. Consideration was also given to the growth and general appearance of the plants. We proved that in stolbur infected plants kinetin provoked the degeneration of MLO (mycoplasma like organism), the synthesis of photosynthetic pigments, regenerated the thylakoid membranes, and increased the hydrolysis of starch accumulated in the chloroplast. After kinetin treatment, diseased sterile plants produced normal flowers, fruit, and viable seeds which gave healthy progeny.

Introduction

Stolbur of the tomato and potato is one of the most important diseases in southeast Europe. In 1968 and 1969 many papers appeared concerning MLO etiology of stolbur.

Ever since the discovery of MLO as a pathological agent of yellows diseases (Doi et al. 1967) the prevention and control of the disease has been a great problem. Although it is generally believed that tetracyclines, first time used by Ishii and coworkers in 1967, induced only temporary remission of symptoms (McCoy 1972, Raychaudhuri and Rishi 1981), it is still the only chemotherapeutic treatment of MLO infection. Since the criteria of disease remission are subjective, and sterility is a common feature of MLO disease, Maramorsch (1974) proposed, as a

criterion for complete cure of MLO disease, the obtainment of a viable seed from treated plants.

Taking into consideration already known symptomatology of the stolbur infection, i.e. chlorosis, starch accumulation in the leaves, changes in chloroplast structures (Plavšić et al. 1976), sterility, proliferation of axillary buds, and hyperplastic and necrotic changes in the phloem (Mihajlova 1934, Samuel et al. 1933, Cousin and Grison 1966, Plavšić-Banjac 1967), we supposed that kinetin, owing to its specific features could have some influence on MLO infection.

The properties of kinetin are inhibition of the destruction of chlorophyll in cut leaves (Osborne and McCalla 1961), of the thylakoid membranes of the chloroplast (Kursanov et al. 1964, Svešnikova and Hohlova 1969), and of the mitochondrial and endoplasmic reticulum membranes (Kursanov et al. 1964). Kinetin shows effects on cytokinesis and cell differentiation, induces organogenesis and participates in apical domination. At high temperature kinetin, like other cytokinins, has a protective roll in plants. Kinetin also shows stimulative effects on RNA, DNA, and protein synthesis (Kulaeva 1973, Latham et al. 1978). Because of the features mentioned above kinetin has been given the name »juvenile substance« (Reunov et al. 1977).

Material and Methods

The transmission of the disease was performed by grafting from a naturally infected potato plant to healthy tomato plants. The infected as well as the healthy control plants were grown in a greenhouse under controlled conditions.

Kinetin used in our investigation was synthetic, obtained from Koch-Light Laboratories Ltd. London.

The effectiveness of the kinetin treatment was evaluated on the basis of its influence on: 1) MLO agent, 2) chloroplast structure, 3) concentration of photosynthetic pigments, and 4) general appearance of treated plants with special attention to reproductive organs.

Investigations in 1 and 2 were done by using electron microscopy. Leaf samples were fixed in buffered glutaraldehyde (pH 7.3), and post-fixed in buffered osmium tetroxide (pH 7.3). Dehydration was done in series of ethanol and propylene oxide. For embedding we used Epon 812. Cutting was done with diamond knife using an LKB ultramicrotome. The sections were contrasted on grids with uranyl magnesium acetate and lead citrate. The samples were examined under a JEM 100B electron microscope. The analysis of chlorophyll (a + b) was carried out by spectrophotometric method (Arnon 1949), and of carotenoids also by spectrophotometric method (Wettstein 1957).

The kinetin treated diseased plants, as well as healthy treated and untreated control plants were kept together with diseased untreated plants in a greenhouse for comparative observations.

The kinetin treatment was carried on as follows. The top part of the tomato plants, a sprout containing 3 to 4 leaves, was cut and placed in Knop's solution until roots appeared. Afterwards kinetin was added to the concentration of 0.05 mg/l. The treatment lasted 11 days when the treated plants were transferred to soil and placed in the greenhouse. Electron microscopy and spectrophotometry were run parallelly using identical samples for both processes.

Results and Discussion

Four weeks after grafting the tomato plants showed a prominent symptom of stolbur infection — the »big bud« (Samuel et al. 1933) (Fig. 1). Electron microscopy of the phloem showed MLO in the sieve tubes (Figs 1 — insert, 2), which was taken as direct confirmation of the MLO infection. The photosynthetic apparatus, compared with that of healthy control plants, was significantly changed (Fig. 4A, B). The thylakoid membranes were extremely reduced. Chloroplasts were full of starch and their membranes desintegrated. In the same plants the level of photosynthetic pigments (chlorophylls and carotenoids) was decreased (Table 1-B). There was a correlation between the structural changes and the decreased level of pigments.

The enzyme chlorophyllase located in the chloroplasts induced the first step of chlorophyll destruction in the virus infected plants (Goodman et al. 1967). In the tissues with the biggest loss of chlorophyll the strongest activity of the chlorophyllase was demonstrated. Consequently, in the tissues with low losses of chlorophyll the activity of chlorophyllase was poor (Peterson and McKinney 1938). It is difficult to discuss these results with respect to our findings since the alteration of the chlorophyll was induced with two different agents, virus and mycoplasma.

Because of high starch accumulation in stolbur infected tomato the chloroplasts were unable to function. This is probably due to suppressed hydrolysis and also disturbances in the phloem (Samuel et al. 1933, Mihajlova 1934, Cousin and Grison 1966, Plavšić-Banjac 1967). Although we are not able to explain the mechanisms of the changes described in the photosynthetic apparatus of stolbur infected plants, we can say that they are in direct connection with the »yellowing« symptom which is specific of MLO infection. Proliferation of axillary buds and flowers might also be a consequence of a hormonal imbalance (Krivokapić et al. 1978). These results support the previous finding that stolbur symptoms are induced by changes in the metabolism of organic substances (Blattny 1956, Valenta et al. 1961).

Some of the tomato plants were investigated after the ninth day of the kinetin treatment. Electron microscopy of the phloem of the diseased treated tomato plants showed degenerative forms of MLO (Fig. 3). In comparison with the MLO in the untreated plants (Fig. 2) it was clear that kinetin caused destructive changes in this procariotic microbe, i.e. no DNA in the procarion and no ribosomes in the procytoplasm. Most of MLO cells were empty. Electron microscopy of chloroplasts after the ninth day of treatment showed the regeneration of normal thylakoid membranes (Fig. 4D). There was no trace of abnormal starch accumulation and the chloroplast membrane clearly separated the chloroplast from the surrounding cytoplasm. Spectrophotometric analysis of the diseased treated plant leaves showed a remarkable increase in the photosynthetic pigment concentration (Table 1—D). That was not only the case in the diseased treated plants but also in the healthy treated ones (Table 1—C).

After 11 days of treatment with kinetin, all of the plants were transferred into soil and placed in a greenhouse. A month and a half later diseased treated plants, previously showing the »big bud« symptom

Table 1. Changes in the content of the photosynthetic pigments (chlorophylls and carotenoids) on the ninth (9) day after treatment with kinetin (0.05 mg/l) in healthy and diseased tomato plants.

	Chlorophylls (a + b) (mg/g fresh weight)	Carotenoids (mg/g fresh weight)
A — Healthy untreated plant	1.122	0.440
B — Diseased untreated plant	0.074	0.050
C — Healthy plant after kinetin treatment	2.028	0.460
D — Diseased plant after kinetin treatment	0.952	0.243

started to produce normal flowers, normal fruits, and viable seeds (Fig. 5). The plants originating from those seeds were completely healthy (cf. also Fig. 6). Their progeny has been observed for 3 years without the slightest sign of MLO infection present.

Concerning the healthy treated plants our opinion is that they are more vital and green than the healthy untreated plants.

Obviously kinetin strongly induced both the synthesis of thylakoid membranes and synthesis of photosynthetic pigments. The stimulative kinetin effect was present in the diseased treated as well as in healthy treated plants (Fig. 4C). As a result there was very intense greening of the plants, which is in distinct contrast to yellowing — a typical symptom of »yellows« disease.

In our experiments kinetin showed a destructive effect on MLO agent. The mechanism of this is difficult to explain. Srivastava (1968) found that 12% of kinetin—8—C¹⁴ is incorporated in the nucleic acids. We can suppose that kinetin, as a purin, is able to incorporate itself into the MLO nucleic acid and in that way »disinforms« the metabolism of the pathogen.

From our investigation we conclude that kinetin besides having favourable features already known also has distinct therapeutic ability.

Taking what is known up to now about this biologically active substance we would like to make the following statements. The properties which were attributed to »juvenile substance« (Reunov et al. 1977) were also evident in the treated plants of our investigation. We also consider kinetin to be a useful substance in increasing photosynthetic production in plants.



Fig. 1. »Big-bud« symptom, and its agent MLO — insert.

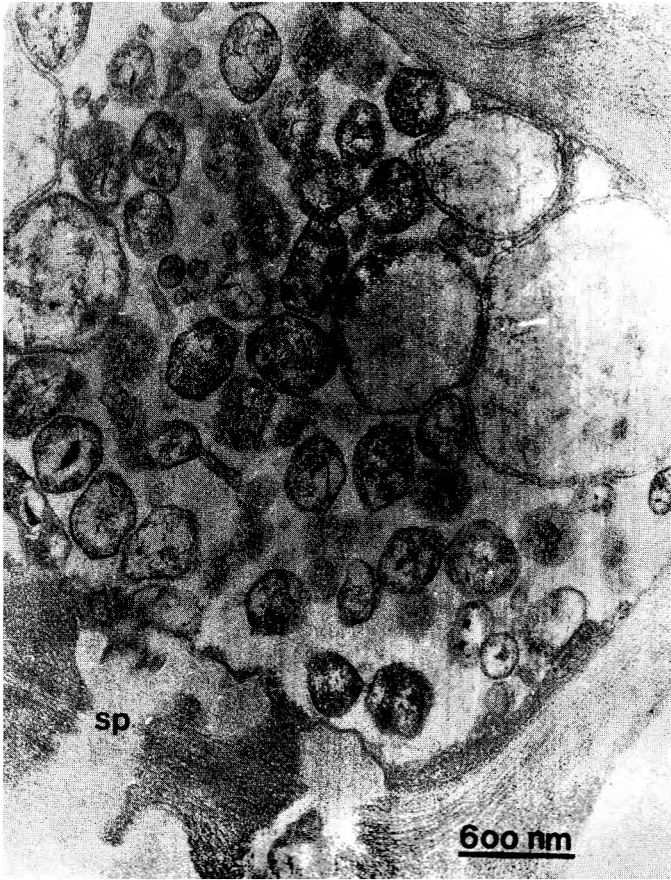


Fig. 2. MLO in sieve tube of stolbur diseased tomato.

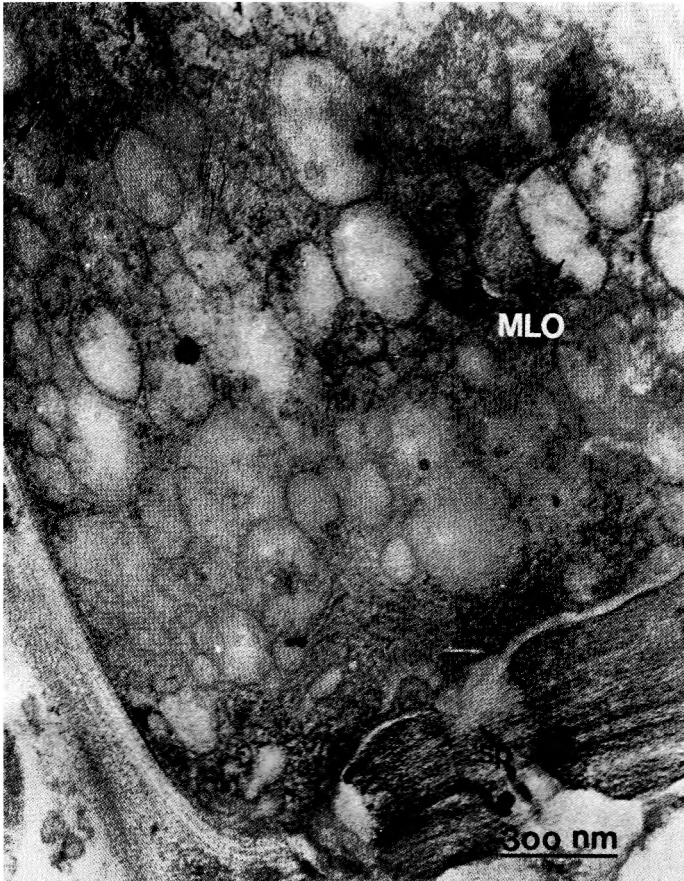
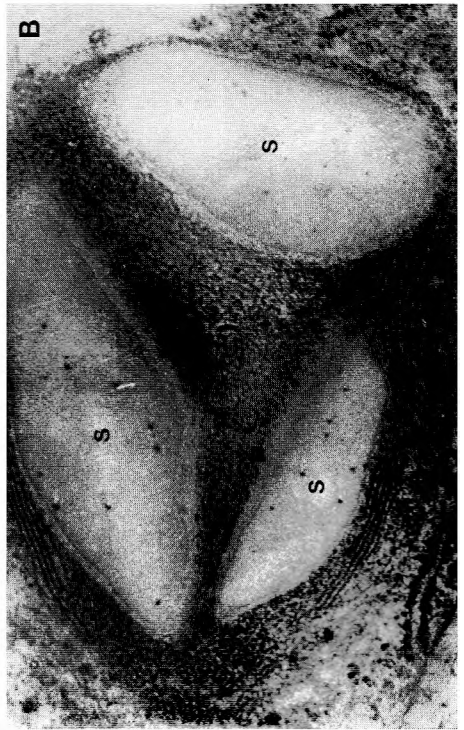
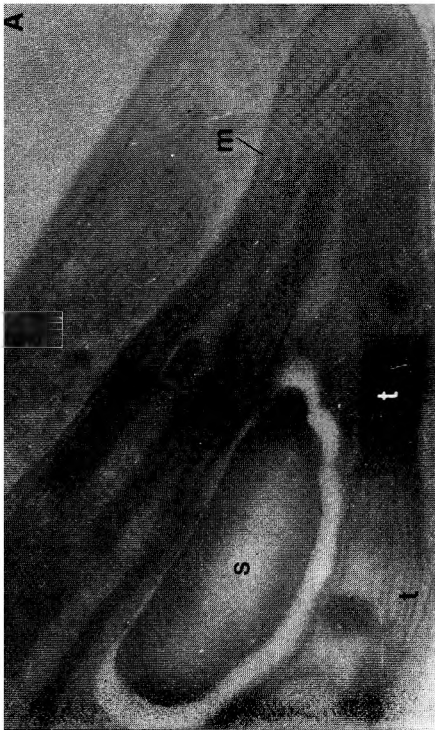
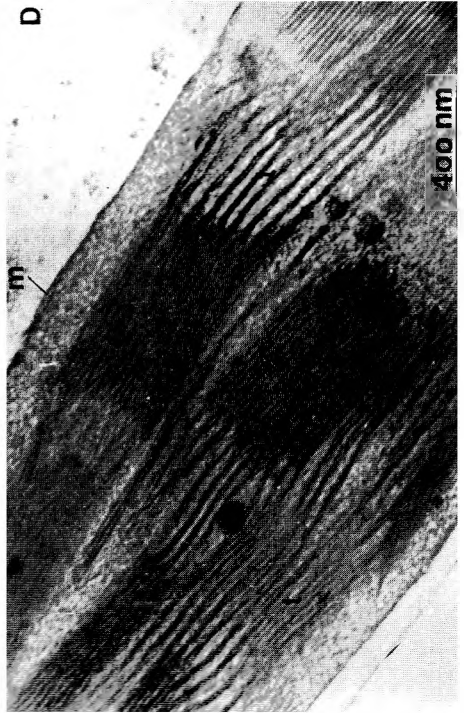
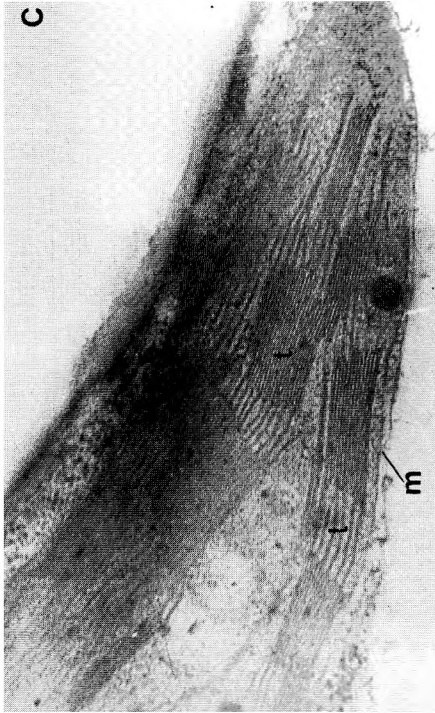


Fig. 3. MLO in sieve tube of stolbur diseased tomato treated with kinetin.
sp — sieve plate.



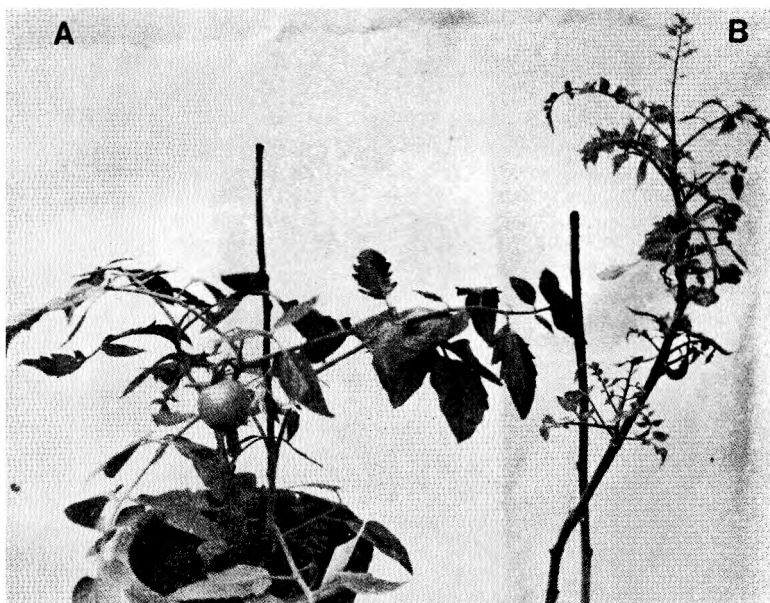


Fig. 5. A Diseased treated plant. B Diseased untreated plant.

Fig. 4. A Chloroplast of healthy untreated control plant. B Chloroplast of diseased untreated control plant. C Chloroplast of healthy treated plant. D Chloroplast of diseased treated plant.
m — chloroplast membrane, t — thylakoides, s — starch.



Fig. 6. *A* Plant from seed of diseased treated plant. *B* Healthy untreated control plant.

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SAŽETAK

TRETMAN STOLBURNE RAJČICE (*LYCOPERSICUM ESCULENTUM* L.) KINETINOM I MOGUĆNOST NJEGOVE PRIMJENE U KEMOTERAPIJI

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Ispitivali smo djelovanje kinetina (6-furfurilaminopurina) na stolburnu infekciju rajčice. Analizirali smo elektronsko-mikroskopski akumulaciju škroba i tilakoidne membrane u kloroplastima, a spektrofotometrijski određivali smo koncentraciju fotosintetskih pigmenata.

Izvršena su zapažanja habitusa ispitivanih biljaka. Utvrdili smo da kinetin izaziva degeneraciju OSM (organizama sličnih mikoplazmi), stimulira sintezu fotosintetskih pigmenata, regenerira tilakoidne membrane i pojačava hidrolizu akumuliranog škroba u kloroplastima.

Nakon tretmana kinetinom bolesne sterilne biljke normalno su procvjetale, dale su plodove i vitalno sjeme koje je dalo zdravo potomstvo.

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