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**Effect of Kinetin on Some Aspects of Senescence of Tobacco Leaf Disks**

Wpływ kinetyny na niektóre aspekty starzenia się dysków wyciętych z liści tytoniu

Влияние кинетина на некоторые аспекты строения дисков, вырезанных из листьев табака

INTRODUCTION

Excised leaves from some plants, when kept in darkness, gradually turn yellow. It is known that this process can be delayed by exogenous cytokinins (14, 21, 25). The senescence-retarding effect of kinetin is demonstrated by inhibiting the drop in the level of chlorophyll, proteins and RNA (2, 3, 23, 27, 30) and even by the stimulation of protein synthesis (5, 18, 29, 31) and nucleic acids (22). Kinetin also inhibits the degeneration of plastids in ageing leaves (6, 18, 20, 31).

The aim of the present work was to study the influence of kinetin on senescence of tobacco leaf disks in terms of the content of plastid pigments, galactolipids and photosynthetic oxygen evolution as well as chloroplast ultrastructure.

MATERIAL AND METHODS

Tobacco plants (*Nicotiana tabacum* var. Virginia Puławska 70802) were grown in a greenhouse for ca. 4 months. Ageing but still green leaves were taken for analysis. They were detached, washed with distilled water and blotted with filter paper. Disks of 1.8 cm in diameter were punched from intervein areas of the leaves. The disks were placed with the back side down on filter paper in petri dishes soaked with distilled water or in water solution of kinetin at concentration 10 mg/l with addition of 2% sucrose and stored in darkness at 27°C for 6 days. After 0, 2, 4, 6 days of incubation the disks were washed with distilled water and

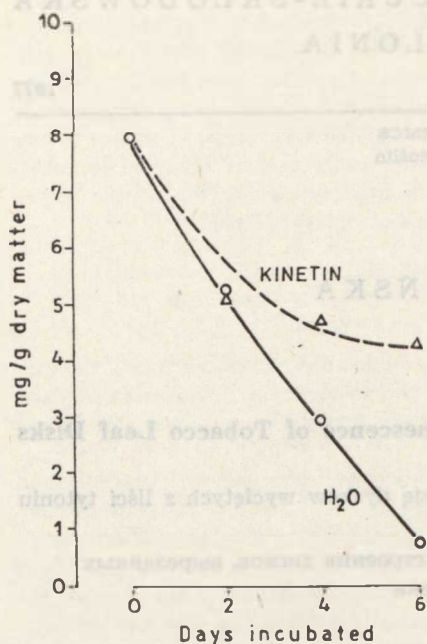


Fig. 1. The loss of chlorophyll by tobacco leaf disks in the presence and absence of kinetin over a period of 6 days in the dark at 27°C

their content of plastid pigments, galactolipids as well as the rate of photosynthetic oxygen evolution and chloroplast ultrastructure were studied.

Photosynthetic oxygen evolution was measured microrespirometrically according to Zurzycki (35) using a light intensity of  $3.4 \times 10^4$  ergs  $\times$  cm<sup>-2</sup>  $\times$  s<sup>-1</sup>. Chlorophyll was determined as described by Arnon (4). Carotenoids were chromatographed and estimated by the method of Hager and Bartenrath (9). Galactolipid analyses were carried out according to Pohl et al. (24). They were assayed by determination of galactose according to Roughan and Batt (26) and factors of 4.3 and 2.6 were used to convert galactose to MGDG (monogalactosyl diacylglycerol) and DGDG (digalactosyl diacylglycerol) respectively (13).

For investigation of the structure of chloroplasts small leaf segments were fixed in 4% glutaraldehyde in 0.07 M phosphate buffer pH 7.2, for 12 h at 4°C and stained in 1% OsO<sub>4</sub>. The fixed material was dehydrated through series of ethanol and propylene oxide washes and then embedded in Vestopal W. The polymerized material was cut on a Tesla ultramicrotome. The sections were counterstained with uranyl acetate and lead citrate. They were then photographed on Scientia Agfa-Gevaert plates at microscopic magnification 9800 $\times$ .

## RESULTS

The influence of kinetin on some aspects of senescence of tobacco leaf disks is shown in Figs. 1—5. Rapid chlorophyll loss is a very distinct feature of disk ageing (Fig. 1) The kinetin solution used at a concentration of 10 mg/l considerably inhibited this process. After a 6-day incu-

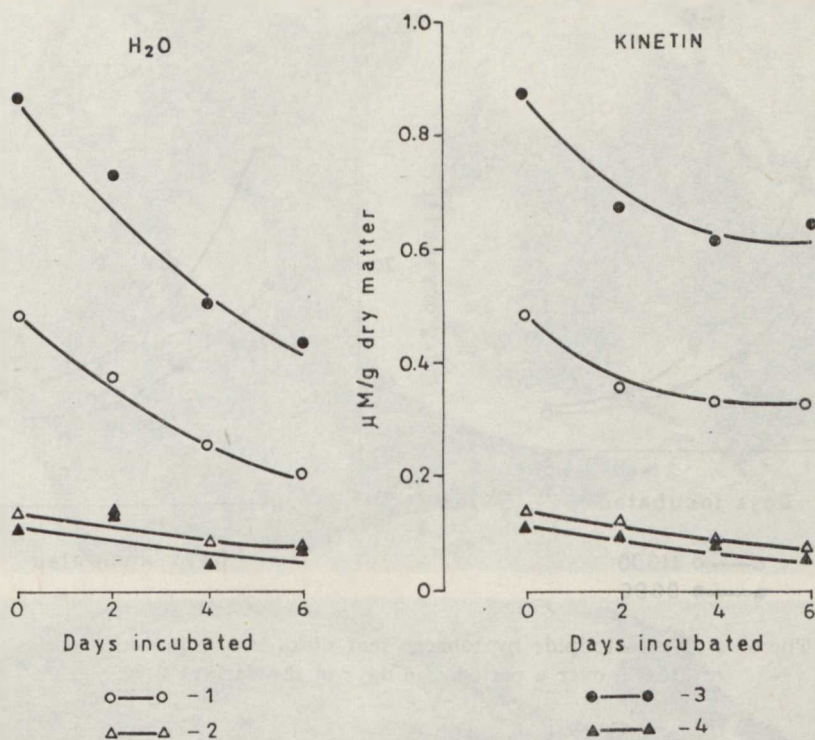


Fig. 2. The loss of carotenoids by tobacco leaf disks in the presence and absence of kinetin over a period of 6 days in the dark at 27°C; 1 —  $\beta$ -carotene, 2 — violaxanthin, 3 — lutein, 4 — neoxanthin

bation of the disks in kinetin solution, chlorophyll content decreased to about 50%, whereas in water to about 15% of the initial one. With senescence of the disks their content of  $\beta$ -carotene and lutein distinctly decreased (Fig. 2). The decrease in the content of both plastid pigments was inhibited by kinetin action. As regards lutein this effect was more distinct. The level of other carotenoids, viola- and neoxanthin underwent only slight changes. No significant effect of kinetin on their level in the disks was observed either.

It was found that galactolipids and particularly MGDG were rapidly decomposed (Fig. 3). Kinetin inhibited this process only to a small extent. The amount of photosynthetically evolved oxygen simultaneously decreased with the decrease in the content of the above-mentioned chloroplast components (Fig. 4). On the 6th incubation day of the disks in water, photosynthesis was not found. However, at the same time photosynthesis intensity of the disks incubated in kinetin solution was still 45% of that found on the first day of the experiment.



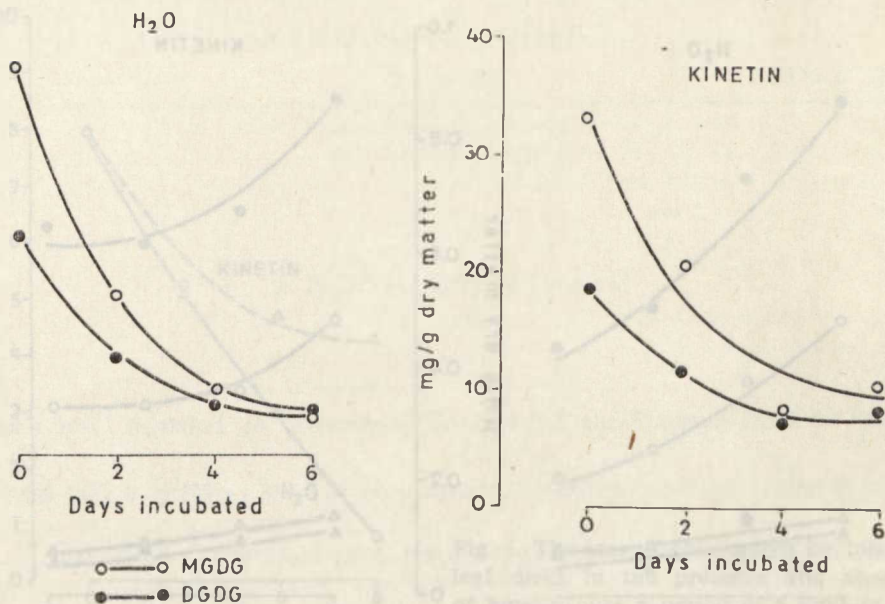


Fig. 3. The loss of galactolipids by tobacco leaf disks in the presence and absence of kinetin over a period of 6 days in the dark at 27°C

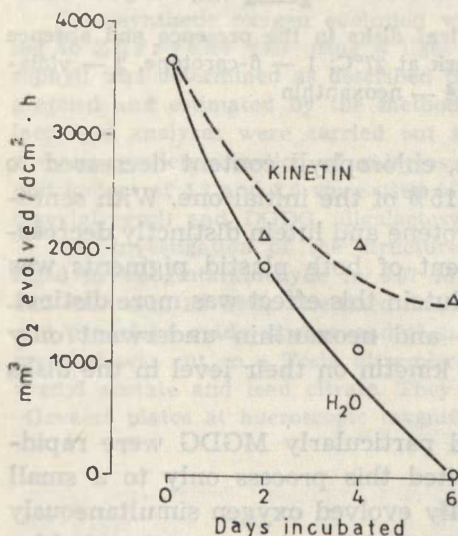


Fig. 4. Photosynthesis rate of tobacco leaf disks in the presence and absence of kinetin over a period of 6 days in the dark at 27°C

Chloroplasts of the leaves used for experiment were characterized by partially degraded stroma lamellae and the presence of large grains of starch (Fig. 5a). After a 6-day incubation of the disks in water the

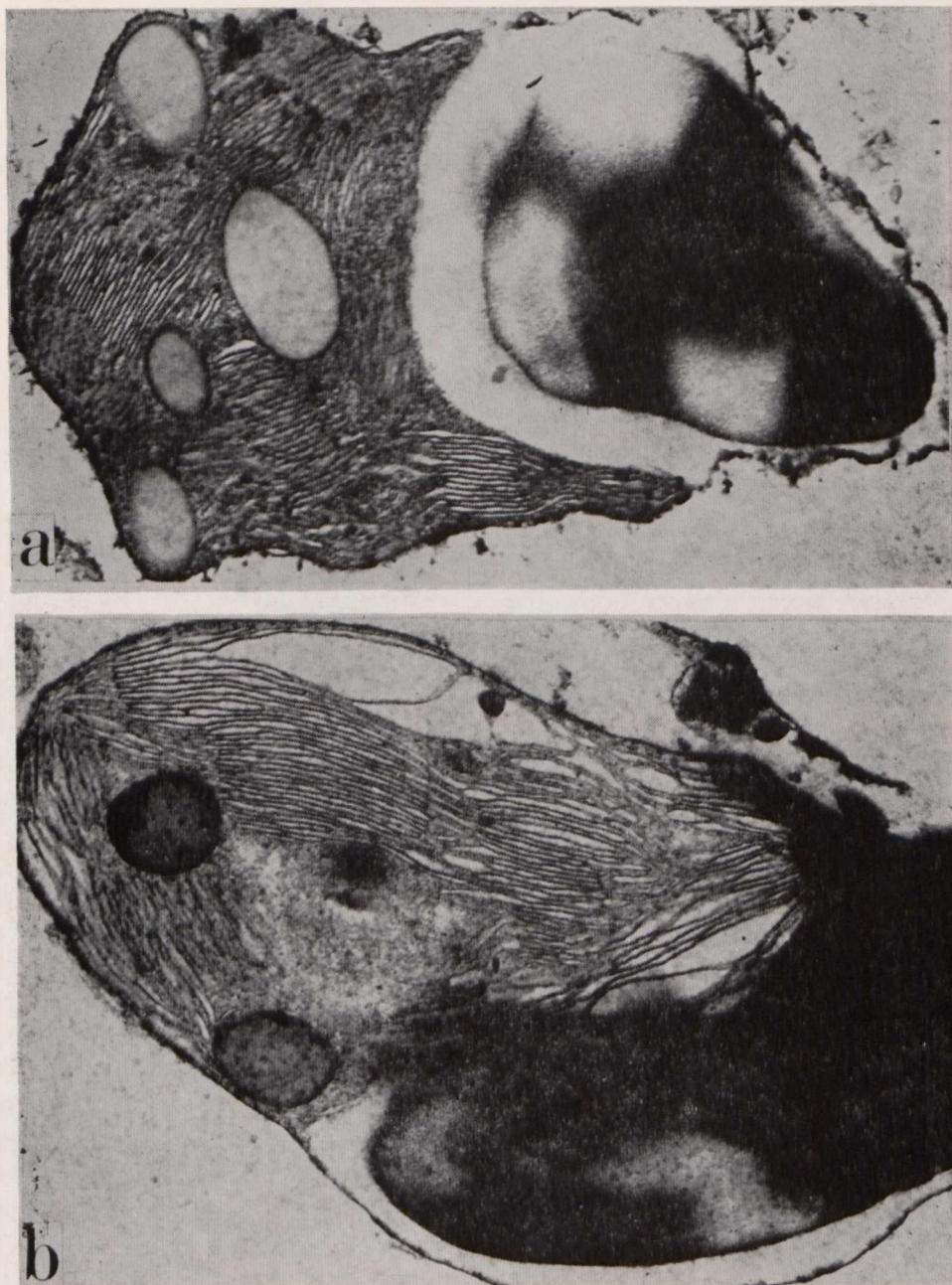


Fig. 5. Chloroplasts from tobacco leaf disks incubated in water or kinetin solution ( $\times 30\ 000$ ); a — chloroplast from initial material, partially degraded stroma lamellae, large starch grains; b — chloroplast of a leaf disk treated 4 days with kinetin; well-preserved grana (continued on the next page)



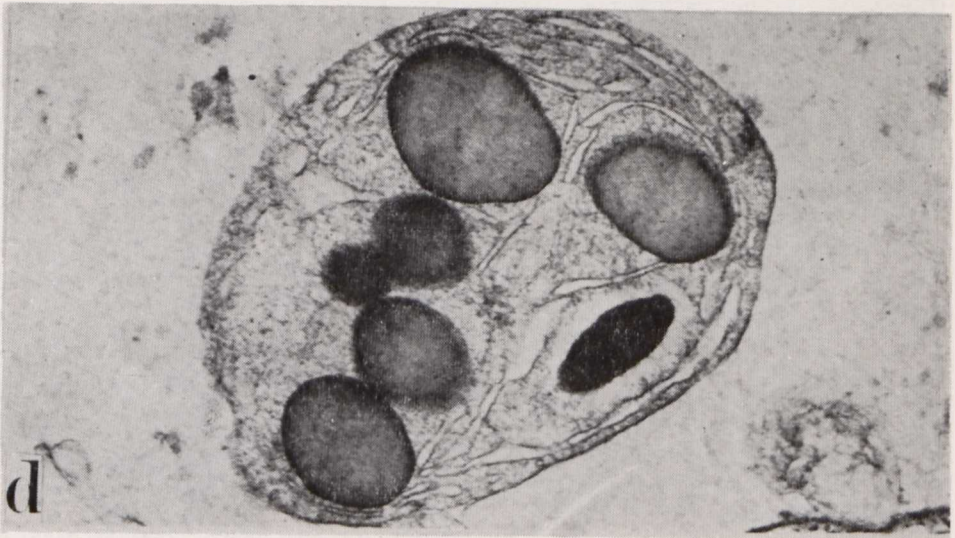
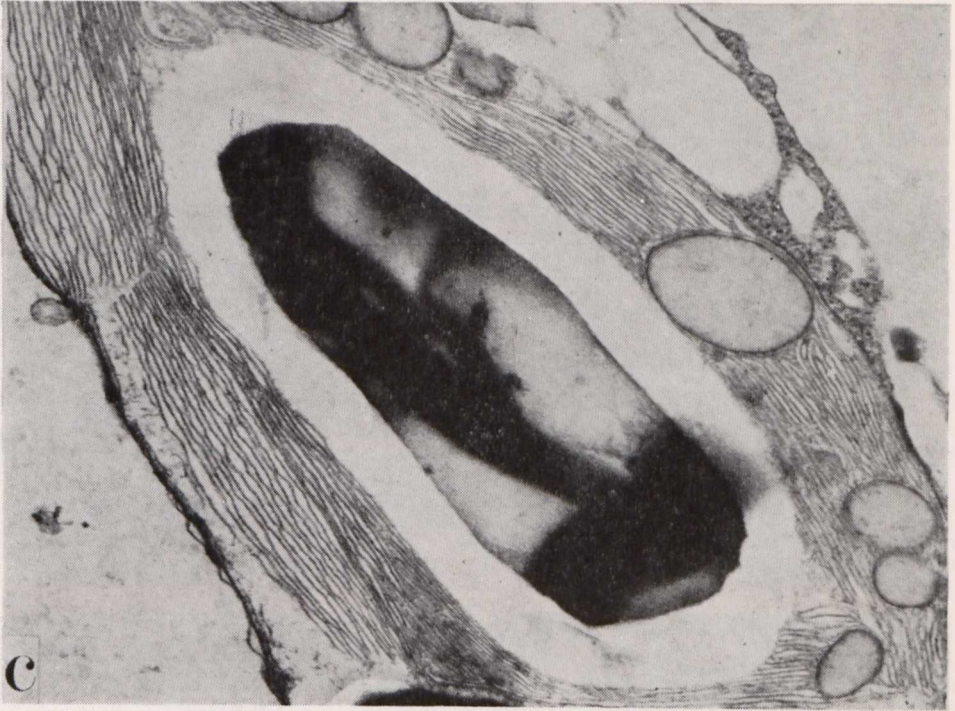


Fig. 5. c-chloroplast of a leaf disk treated 6 days with kinetin; partial degradation of the membranous system, large starch grains are still present; d — chloroplast of a leaf disk incubated 6 days in water; few swollen lamellae, large plastoglobules

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volume of chloroplasts considerably decreased. The chloroplasts which became spherical contained only a few swollen lamellae, small starch grains and large plastoglobules (Fig. 5d). Chloroplasts of the kinetin-treated material were larger than those of control one (Fig. 5b, c). Their grana were present till the fourth day of the experiment. However, on the sixth day, the chloroplasts were characterized by partial degradation which was manifested by certain reorganization of the membranous system. The long parallel lamellae occurred. Large starch grains, however, still remained, similarly as in the chloroplasts of initial leaves.

#### DISCUSSION

Rapid decomposition of chlorophyll, carotenoids and galactolipids as well as decreasing photosynthesis intensity and degradation of the lamellar system of chloroplasts in the disks excised from tobacco leaves and incubated in water in darkness — found in this investigation, was also observed by a number of authors on different plant material (7, 10, 11, 15, 20, 33, 34). An acknowledged factor delaying this process is kinetin (8, 18, 32). In numerous papers dealing with kinetin effect on senescence, attention was largely drawn to the ultrastructure of cellular organelles and analysis of the content of chlorophyll, protein and nucleic acids. It was shown in them that kinetin inhibits level decrease of these compounds in ageing leaves (8, 12, 25, 30). Another effect of kinetin is inhibition of chloroplast degradation (6, 17, 19, 20, 28). The disks excised from leaves of the studied tobacco variety reacted to kinetin on adding a small amount of sucrose to the medium. This was probably connected with lack of a required pool of endogenic sugar, with the presence of which, according to Sugiura et al. (30), the reaction to kinetin is connected. The lack of the required amount of sugar in some plants is supplemented by an addition of sucrose or by experiments conducted in weak light.

The studies presented in this paper have confirmed the inhibitory effect of kinetin on chlorophyll level decrease and degeneration of chloroplasts in senescence of tobacco leaf disks. Furthermore, it was shown that kinetin also inhibits the decrease of the level of carotenoids and galactolipids.

Dennis et al. (6), when studying the effect of another kinin, benzyladenine, also found its inhibitory action on decomposition of carotenoids in ageing leaf disks excised from leaves of *Brassica oleracea*. Adedipe et al. (1) showed even a gradual increase in the content of carotenoids and chlorophyll in attached primary bean leaves treated with benzyladenine. Kinetin action on ageing discs excised from tobacco leaves also



resulted in a much slower decrease in photosynthesis intensity, as compared with that of the control material. A slight increase in the rates of CO<sub>2</sub> assimilation in mature barley leaves after treating them with kinetin was observed by Meidner (16). Senescence inhibition of attached primary bean leaves by benzyladenine, according to the supposition of Adedipe et al. (1), is connected with the maintenance of photosynthetic activity. The results obtained in this paper indicate that this maintenance of photosynthetic activity results from inhibition of chloroplast structure degeneration as well as from inhibition of the decomposition of the plastid components studied.

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#### STRESZCZENIE

Wycięte z liści tytoniu dyski, trzymane w ciemności, stopniowo żółkną. Traktowanie kinetyną w stężeniu 10 mg/l w znacznym stopniu osłabia ten proces. Uwidacznia się to w wolniejszym obniżaniu poziomu karotenoidów, galaktolipidów i intensywności fotosyntezy w porównaniu z kontrolą na wodzie. Potwierdzono hamujący wpływ kinetyny na proces degeneracji chloroplastów i zmniejszanie się poziomu chlorofilu.

#### РЕЗЮМЕ

Хранимые в темноте диски, вырезанные из листьев табака, постепенно желтеют. Обработка кинетином концентрации 10 мг/л в значительной степени тормозит этот процесс, о чем свидетельствует медленное понижение уровня каротиноидов, галактолипидов и интенсивности фотосинтеза по сравнению с контролем на воде. Подтверждено тормозящее влияние кинетина на процесс дегенерации хлоропластов и уменьшение уровня хлорофилла.