INFLUENCE OF WHITE LEAF DISEASE OF SUGARCANE ON THE CHLOROPLAST DEVELOPMENT AND CHLOROPHYLL BIOSYNTHESIS.

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White leaf disease of sugarcane was first discovered in Taiwan in 1958. It has gradually spread out and become a very serious disease in the plantations of Taiwan Sugar Company, especially in the southern part of this island.

Although the symptom of white leaf disease is typically albino, it sometimes appear as variegation and later may recover to become green. Usually, therefore, on a single leaf blade one may find three kinds of symptoms, i. e. (1), "albino", an area completely lack of chlorophyll, (2), "mottled", characterized by scattered green islands and (3), the recovered green area.

The purpose of this research was to investigate the pathological effects of sugar cane white leaf disease on the chloroplast development as well as the chlorophyll biosynthesis.

Materials and methods

Leaf materials used in this investigation were collected from sugarcane variety N:Co 310. Three types of diseased tissues, namely albino, mottled and recovered areas, were separately treated. Healthy leaf blades were pretreated in the following two ways before harvested: (1) itiolated seedlings germinated from stalk were grown in darkness for ten days, and (2) after 10 days in darkness, some of the seedlings were followed by 24 hrs illumination.

For the electronmicrscopical examination the diseased and healthy leaf tissues were fixed in glutaraldehyde (6% in phosphate buffer at pH 7.0) followed by 1% aqueous solution of OsO_4 in phosphate buffer at pH 7.0 (ENGELBRECHT, A. H. P. and T. E. WELER, 1967). After fixation the specimens were dehydrated in a graded series of ethyl alcohol. Porpylene oxide was employed in Araldite embedding. Thin sections were post stained with 2% osmic acid on grids. They were observed under a Hitachi HU-11A electronmicroscope at 75 KV.

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The chlorophylls were extracted with acetone and ethyl ether for the quantitative assessment. Before extraction the fresh leaf tissues were immersed in 80°C water bath for three minutes to inactivate the enzymes and therefore prevent chlorophyll from further degradation. One hundred milligrams of leaf tissues were ground with quarts sand and extracted with 5 ml of acetone (80%). The supernatant was mixed with the same volume of ethyl ether and was washed several times with distilled water. Two grams of anhydrous sodium carbonate was added to the ethyl ether extract to remove the residual water. After these treatments the chlorophyll were maintained in ether phase. The three chlorophylls, i. e. chlorophyll a, b, and protochlorophyll were quantitized using ODS of 663 mμ, 664 mμ and 624 mμ respectively. Quantitative analysis of chlorophyll a, b and protochlorophyll were conducted according to Koski's formula (1950).

Results and discussion

Electronmicroscopical examination demonstrated that the plastids in healthy leaf blade grown in darkness and those of albino leaves were alike. Circular profiles (SAC) and prolamella body (PB) were observed in proplastid envelope Fig. 1, 2, and 3).

In mottled leaf tissues, the plastids were observed in four types representing different developmental stages. (1) The proplastid stage is characterized by having only prolamellae in the plastid envelope (Fig. 4). (2) A more advanced stage where the lamellae are loosely arranged (Fig. 6). (3) The third type is the chloroplasts with a more elaborated lamellae system arranged toward the plastid envelope (Fig. 5). (4) The last type is the normal chloroplast (Fig. 7, 8).

The chlorophyll contents of three types of diseased tissues are listed in Table 1. The chlorophyll contents of albino leaves are about ten percent those of the recovered green blades.

Table 1. *The optical-density and chlorophyll contents in healthy leaf and in different symptomatic areas of affected sugarcane leaf blades.*

Tissue / Optical density / Wavelength	Healthy	Diseased		
	Green	Albino	Mottled	Recovered
663 mμ	0.870	0.052	0.256	0.501
644 mμ	0.261	0.022	0.099	0.165
624 mμ	0.191	0.015	0.063	0.166

Tissue / Content (mg/l) / Chlorophyll	Healthy	Diseased		
	Greeen	Albino	Mottled	Recovered
chlo. a.	9.0425	0.5343	2.6396	4.9079
chlo. b.	2.0910	0.2360	1.0050	2.0070
pro. chlo.	1.1250	0.3120	0.4120	2.6160

The low chlorophyll contents in albino blades could be due either to weak biosynthetic activities or to the poor capacity of the tissues to retain the synthesized substances (Koski and Smith 1951).

Our results indicated that the chlorophyll contents are closely related to the developmental stages of plastids. The symptom expression of the white leaf diseased sugarcane therefore is considered to be due to the pathological effects on the chloroplast development, namely, the albino tissues are due to the inability of the blades to permit normal development of the plastids to mature ohloroplasts.

Shumway and Weller (1967) reported that the aberrant chloroplasts in white stripes of Iojap maize is the result of early plastid developmental blocks. The condition in white leaf disease of sugarcane could be suggestive to that of the maize.

Summary

The fine structure of chloroplasts in the affected leaf blades of white leaf disease of sugarcane were studied. The proplastids in albino tissues were very similar to those in the itiolated sugarcane leaves.

Four developmental stages of chloroplasts were recognized in mottled and recovered leaf tissues. Fully developed chloroplasts are present in the completely recovered green leaf blades.

A small quantity of protochlorophyll, chlorophyll a and chlorophyll b were present in albino leaf tissues. The chlorophyll contents in the other types of diseased leaf tissues were directly proportional to the degree of chloroplast development.

The symptom expression of white leaf disease is considered to be directly related to the development of chloroplasts and chlorophyll biosynthesis.

甘蔗白葉病對葉綠體發育以及對葉綠素合成影響之研究

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以電子顯微鏡觀察甘蔗白葉病，病株葉片不同病徵部份葉綠體的構造。發現在白色病徵內的葉綠體，均呈現葉綠體前趨體（Proplastid）的形狀，如圖 1. 所示。在斑紋病徵部份的葉綠體，其構造則呈現四個不同的發育階段（圖 4, 5, 6, 8）完全康復呈綠色的部份其葉綠體的構造與正常發育者無異(圖 7)，由觀察的結果顯示，甘蔗白葉病病徵的表現乃是由於葉綠體在發育的初期受到抑制所致。

用乙醚（Ethyl ether）抽出不同病徵葉片組織內的葉綠素，發現葉綠素的含量隨着不同病徵葉片組織內的葉綠體發育的程度而增加（見第一表）。在白色病徵葉片內葉綠素的含量，約爲完全康復組織中含量的十分之一。這些葉綠素乃是在光照下於葉綠體之前趨體中所合成。在病徵組織中葉綠素含量之顯著減少，是由於此葉綠體前趨體無貯存葉綠素的能力以致合成後再被光照所分解，或其合成的能力亦因而減低。

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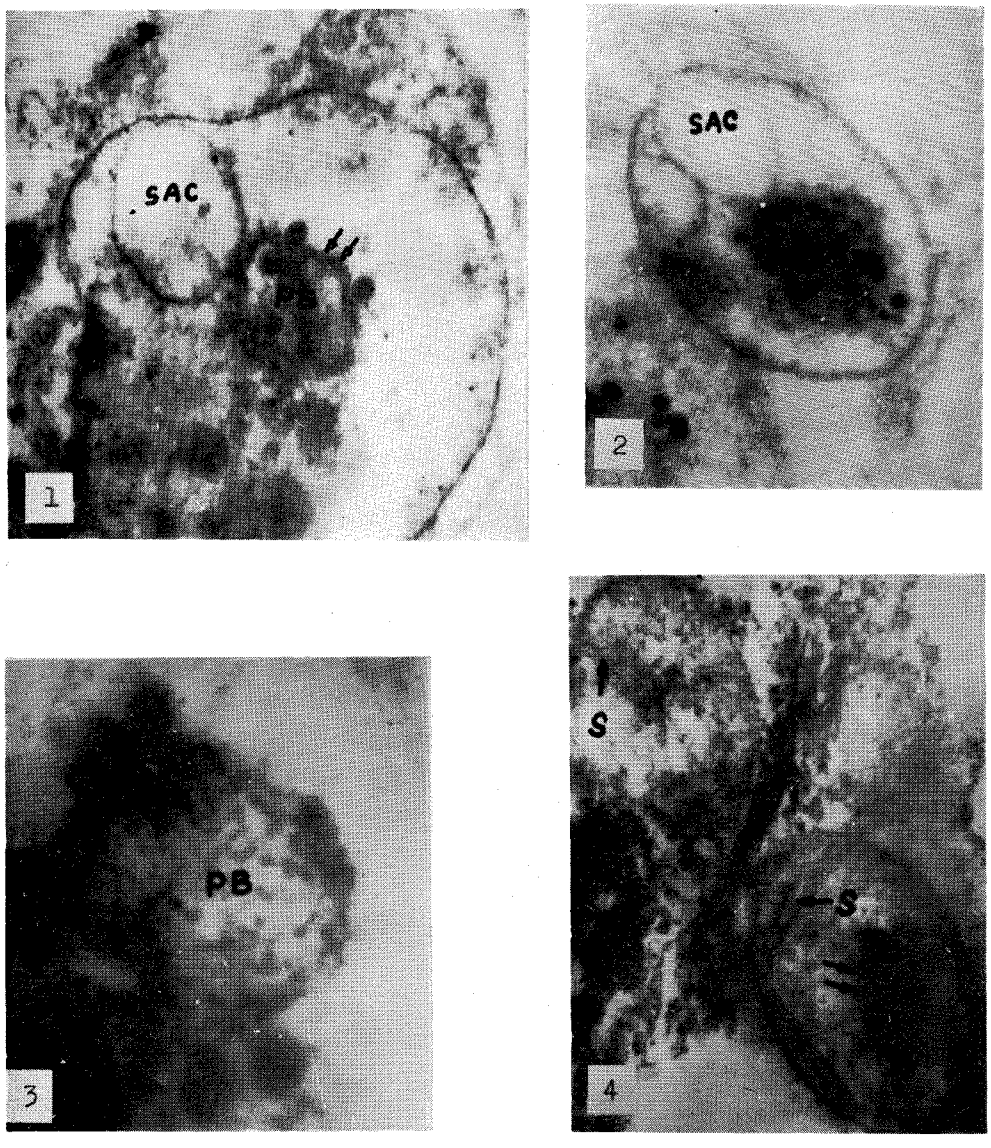


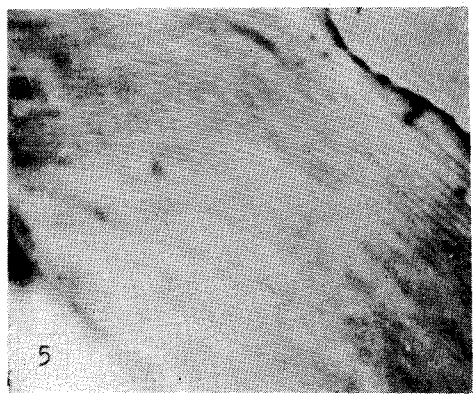
Plate I

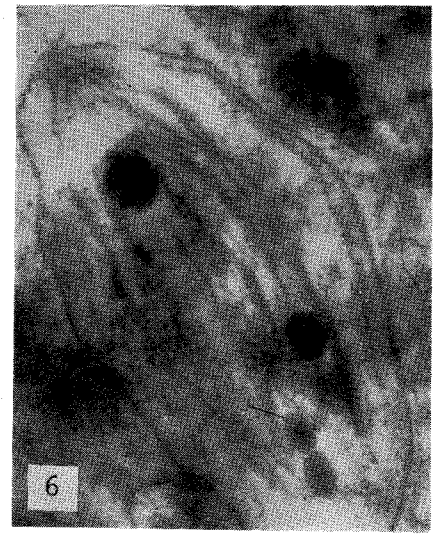
Fig. 1. A proplastid with a circular profile (SAC) and an electron dense area of prolamellar body (PB) in the albino symptomatic leaf blade. (X15,000)

Fig. 2. A proplastid in healthy leaf blade which was planted in complete darkness for ten days after germination from stalk. The circular profiles and the prolamellar body can be seen in the proplastid envelope. (X 12,000)

Fig. 3. The crystalline prolamellar body can be observed in the proplastid of the healthy leaf blade which was planted in complete darkness for ten days after germination from stalk. (X 20,000)

Fig. 4. The proplastids with profile tubes (S) and an electrondense area (double arrow) which presented in mottled symptomatic leaf blade of sugarcane. (X 12,000)





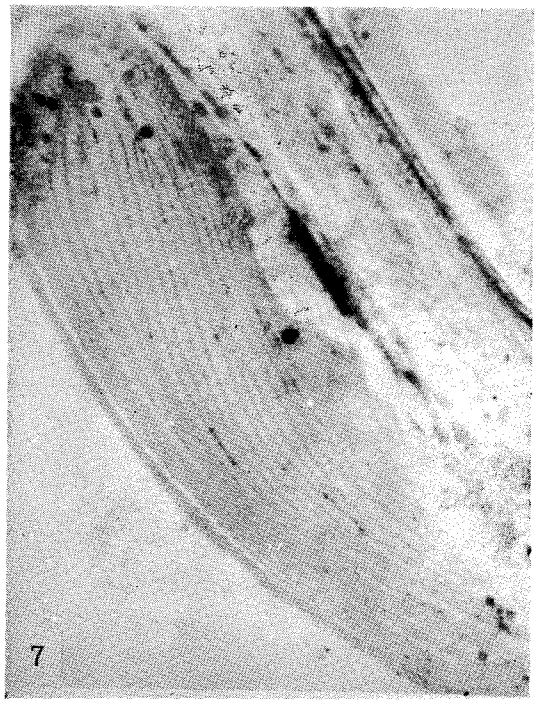


Plate II

Fig. 5. The more differentiated chloroplast containing many densely parallel lamella near the plastid envelope in recovered mottled tissues of affected leaf blade of sugarcane. (X 15,000)

Fig. 6. The more differentiated chloroplast with the loosely parallel lamella in the recovered mottled area of affected leaf blade of sugarcane. (12,000)

Fig. 7. The full developed chloroplast in the healthy leaf tissue of sugarcane after ten days of growth in complete darkness and followed by 24 hours of illumination. (X 15,000)

Fig. 8. A full developed chloroplast in green recovered area of white leaf disease affected leaf blade of sugarcane. (X 15,000).