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CHLOROPLAST DEGENERATION AS A CONSEQUENCE OF "HYBRID NECROSIS" IN WHEAT

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With 8 figures

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ABSTRACT

The results of a cytological study of hybrid necrosis in wheat are presented. Two types of necrosizing of the leaves have been found viz. necrosis via dull green discoloration, and necrosis via a yellow intermediate colour. In the first case the conglomeration of the cell contents starts when the size of the chloroplasts is only little reduced. In the second case the chloroplasts become very small before forming small lumps or bunches. It is suggested that the conglomeration of the cell contents is more disastrous for the plant than the decrease in size of the chloroplasts. The problem of the genetical and physiological causes of hybrid necrosis is discussed.

INTRODUCTION

Hybrids from crosses between normal parents are mostly normal. Some crosses however give rise to weak and abnormal hybrids. This phenomenon is called *hybrid weakness* (DOBZHANSKY, 1951, STEBBINS, 1950). It has been found in many plant genera: *Aegilops* (ROY, 1955), *Antirrhinum* (HARRISON, 1960), *Clarkia* (HIORTH, 1948), *Crepis* (HOLLINGSHEAD, 1930), *Gossypium* (STEPHENS, 1950, GERSTEL, 1954, HUTCHINSON, 1946), *Hordeum* (WIEBE, 1934), *Lycopersicum* (SAWANT, 1956), *Nicotiana* (BRIEGER, 1929), *Oryza* (OKA, 1956), *Streptocarpus* (LAWRENCE, 1948), *Triticum* (see HERMSEN, 1962) and *Vigna* (SAUNDERS, 1960). In *Triticum* at least four different forms of hybrid weakness occur of which hybrid necrosis has been studied most extensively (HERMSEN, 1963 a-c).

Hybrid necrosis is the premature gradual death of leaves and leaf sheaths in wheat hybrids. Emergence and early growth of the hybrids are normal. The first symptoms may appear in any growth stage of the plants from the 1–2 leaf stage onwards, dependent on the nature of the cross. These first clearly recognizable symptoms consist of dull green or light green spots (mottling) which become larger and larger and more numerous until they join and form larger spots or zones. The colour changes which occur in the meantime are in some hybrids green → dull green → brown (necrotic). In other hybrids the colour changes from green → light green → yellow → brownish yellow → necrotic. In this article it will be demonstrated that these two types of colour changes reflect two different ways in which the chlorophyll apparatus of the necrosizing leaves may degenerate.

Material and method

Hand-made cross sections of healthy and of necrosizing leaves were examined under the microscope. The healthy leaves were taken from the wheat varieties Koga, Sambo, Kharkof and *Triticum macha*. The necrosizing leaves were from the crosses Koga × Canad. 3842 with dull green and Koga × Zanda with yellow as intermediate colour between normal glossy green and necrotic (see introduction).

After preliminary experiments using tap water, different sugar solutions, alcohol and chromic acid the following method evolved as the most suitable one. The leaves collected in the field were thoroughly saturated with water under reduced air pressure and then stored in water at 2°C over the night. Sections of about two cell layers cut in water were most favourable for study. Most cells were cut and contained only a structureless mass of chloroplasts and protoplasm destroyed by the action of the water. Observations were made on the remaining intact cells which showed the chloroplasts and their arrangement beautifully.

Results

The figures 1–4 inclusive present cross sections of one necrosizing leaf from the gross Koga × Canad. 3842. This leaf may be described as follows: leaf base normal, clossy green; leaf tip necrotic; centre dull green; the transition zone from centre to base was dull greenish spotted and from centre to the tip light brownish spotted. The glossy green leaf base had normal mesophyll cells with numerous large green chloroplasts lying near the cell walls. Due to tight packing the chloroplasts were often somewhat angular (fig. 1).

The first deviation from normal is that the cell contents are loosened from the cell walls while the chloroplasts have become somewhat smaller (fig. 2).

Soon the cell contents conglomerate into a greenish mass showing the chloroplasts only in vague outline, the cell walls being still intact (fig. 3). This is the picture of the cells in the dull green tissue. The cell walls are still intact then. Presumably the fading of the glossiness of the leaf is caused by turgor loss of the mesophyll cells.

In the final stage (brown necrotic tissue) the cell contents exist of angular lumps in which the chloroplasts can hardly be recognized. The cell walls now easily tear to pieces even when cut carefully (fig. 4).

The figures 5–8 inclusive present cross sections of one necrosizing leaf from the cross Koga × Zanda. The symptoms on this leaf were as follows: leaf base light green with scattered dark green patches; leaf tip necrotic; centre yellow with a transition zone to the base showing yellow to light green mottling, and to the tip yellow to light brown mottling. Most mesophyll cells of the leaf base contained light green chloroplasts which were smaller than normal. Figure 5 shows at right two cells with normal chloroplasts (arrows). Figure 6 (same picture but different focussing) shows at left a group of cells with small chloroplasts.

In the yellow to yellowish green zone of the leaf the cells with very small light green chloroplasts predominate (fig. 7). From cell to cell some variation occurs in the size of the chloroplasts. Within one and the same cell however they are fairly uniform.

Also in the yellowish brown tissue some cells occur with small scattered chloroplasts but here cells with greenish orange-brown lumps are most frequent. The outlines of the small chloroplasts are still vaguely visible (fig. 8 lower right). Compare the green and larger lumps in figure 3.

The cells with orange-brown lumps also dominate in the brown necrotic tissue. Peculiar however is the fairly frequent occurrence of intact cells with globular continuously moving particles with clear outline, sometimes conglomerated like a little bunch of grapes, or in a looser group of globules (fig. 8 the cell at extreme right in the centre). The size of these little spheres is often fairly uniform in a cell but cells with large and small ones together also occur, as is shown in figure 8 (second cell from right in centre, rather vague).

Discussion

The characteristic difference between necrosis via dull green discoloration, and necrosis via yellowing is that in the first case the conglomeration of the cell contents starts already when the size of the chloroplasts is only little reduced while in the second case the chloroplasts first become very small, later forming small lumps or bunches. In hybrids with dull green as intermediate colour necrosis mostly sets in rather quickly. Many of these hybrids are lethal (do not produce seed). Among the hybrids with yellow as intermediate colour lethality has not been found until now. The conglomeration of the cell contents therefore seems to be more disastrous for the plant than the decrease in size of the chloroplasts.

In discussing the cause of hybrid necrosis a genetic and a physiological cause may be distinguished, keeping in mind that both must be closely related. The problem of the genetic basis of hybrid necrosis in wheat has been studied extensively (Hermsen, 1963 b). Two complementary genes Ne_1 and Ne_2 are the basis of necrosis-as-such. The occurrence of dull green or yellow as an intermediate colour depends on the alleles of Ne_1 and Ne_2 involved, on the dosage of these alleles and on the genetic background. No such systematic study is known to the authors about the physiological basis of hybrid necrosis. However different hypotheses have been proposed for a physiological explanation of hybrid weakness. Sears (1944) considered a shortage of some essential substance the most probable cause in his material. According to Gerstel (1954) a surplus of some harmful product were the most obvious explanation of his red lethals in cotton. McCalla (1961) suggests that in his recessive necrotic mutant in corn an accumulation of poisonous products takes place. Hadorn (1955) formulated a theory on the physiological action of complementary lethal genes in general. According to Hadorn the action of complementary genes may be a. *cumulative:* both genes produce the same substance which becomes harmful not before a certain threshold concentration is reached; b. purely *complementary:* each of the genes governs the production of a different substance. Separated these substances are harmless, only in combination the harmful effect occurs.

The action of complementary genes may be either a *direct* one (when the harmful product deranges the formation, replacement or survival of the chloroplasts) or an *indirect* one (when the harmful product inactivates a substance which is necessary for a normal functioning of the chlorophyll apparatus). In the case of hybrid necrosis it is still impossible even to decide which of these hypotheses is the most probable one.

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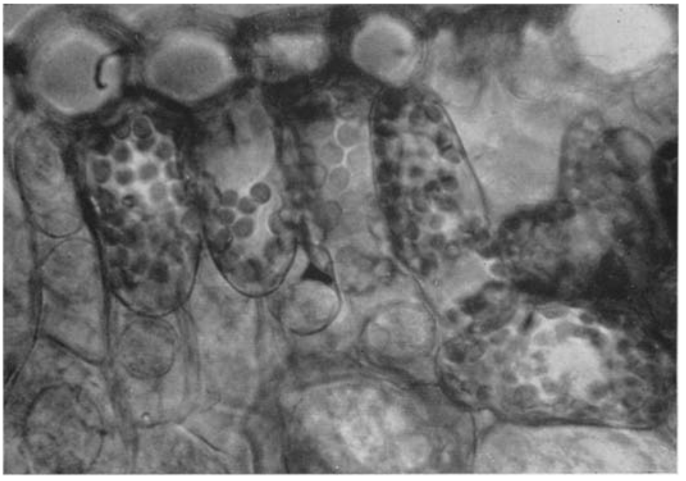

FIG. 1. Normal mesophyll cells from the base of a necrosizing leaf of Koga × Canad. 3842. See text. Magnification 3/4 that of the figures 2, 3 and 4.

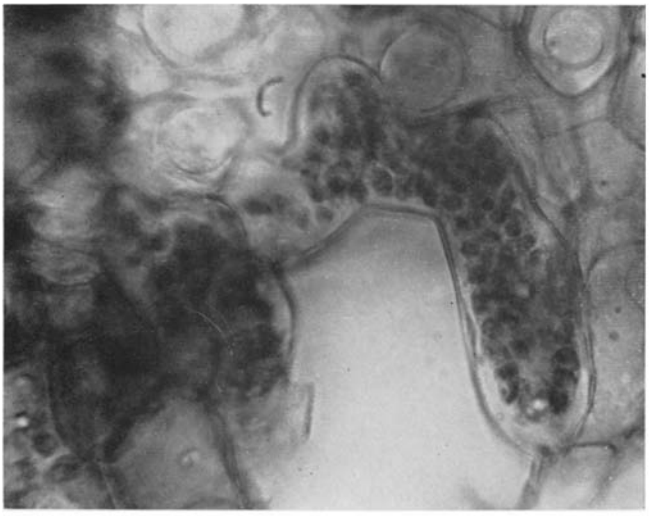

FIG. 2. Mesophyll cells from the mottled region between normal and dull green. Same leaf as fig. 1. See text.

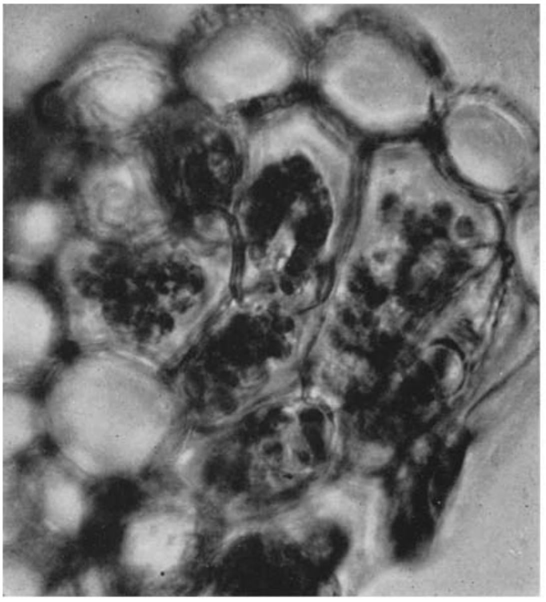

FIG. 3. Mesophyll cells from the dull green region. Same leaf as fig. 1. See text.

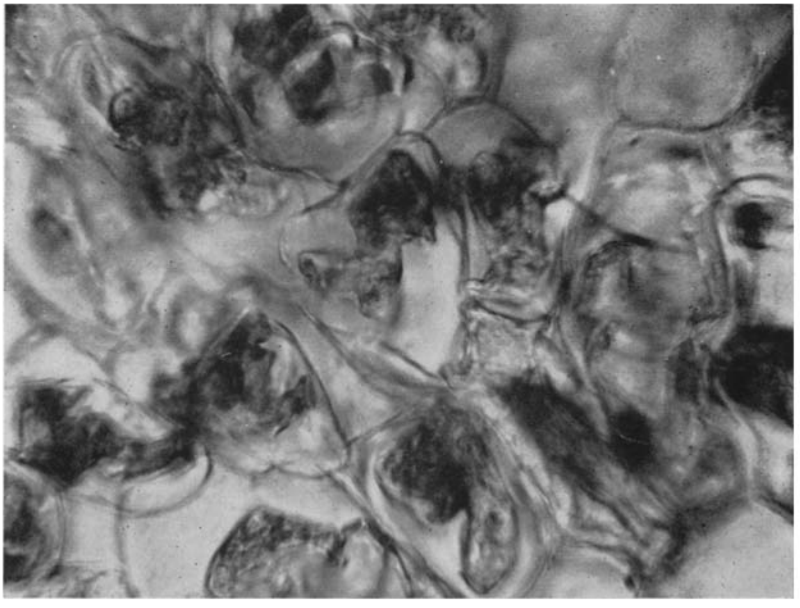

FIG. 4. Mesophyll cells from the necrotic leaf tissue. Same leaf as fig. 1. See text.

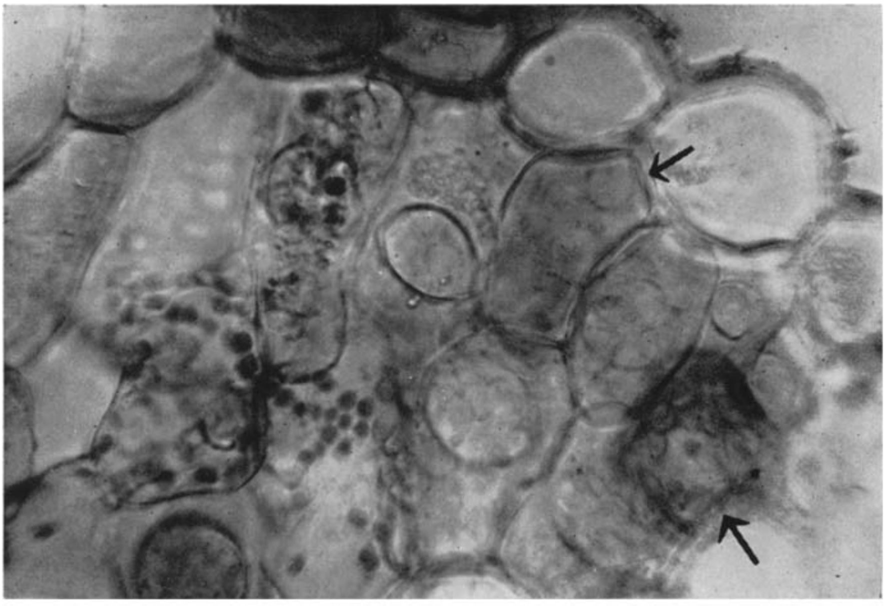

FIG. 5. Mesophyll cells from the spotted leaf base of a necrosizing leaf of Koga × Zanda. See text. Same magnification in the figures 5 to 8 inclusive.

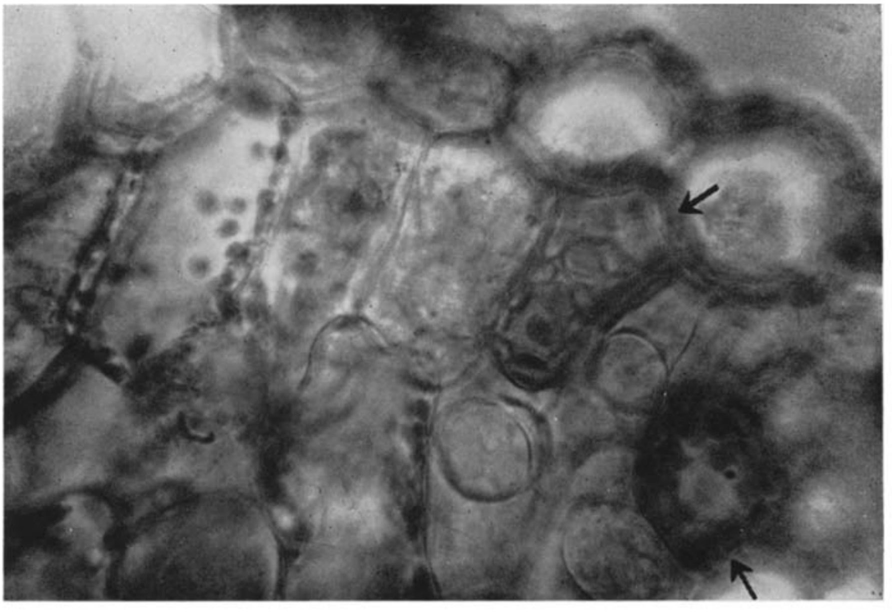

FIG. 6. Same cells as fig. 5, but other focussing. See text.

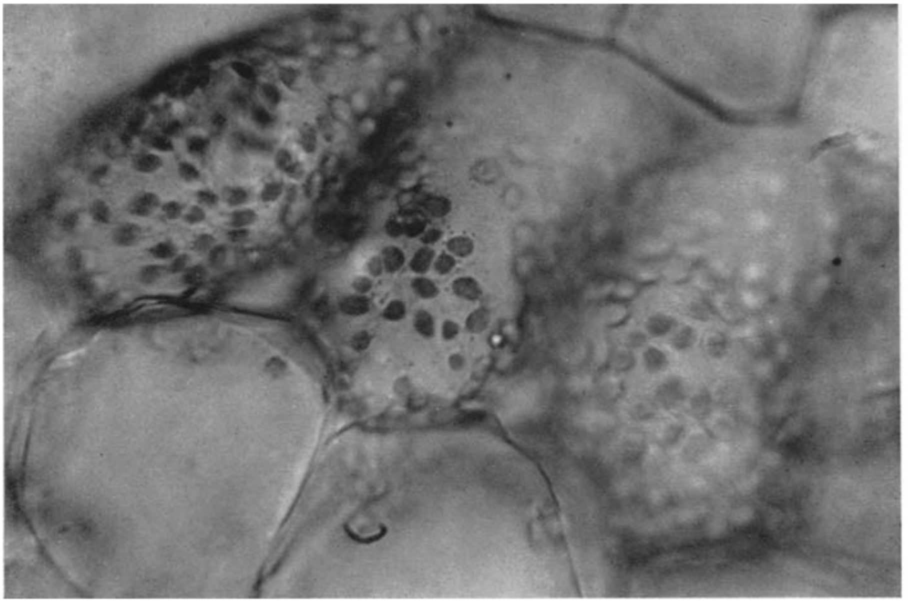

FIG. 7. Mesophyll cells from the yellow to yellowish green region. Same leaf as fig. 5. See text.

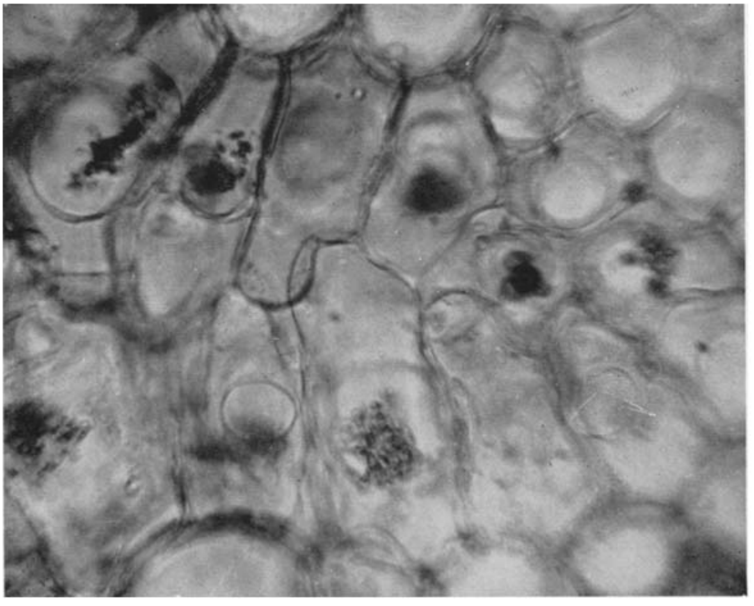

FIG. 8. Mesophyll cells from the yellowish brown part of the same leaf as in fig. 5. See text.