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The Causes of Genetic Male Sterility in 3 Soybaen Lines

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Summary. The cause of male sterility in 3 soybean lines, TGM 103-1, N-69-2774 and TGM 242-4 was studied. In TGM 103-1, which was both male and female sterile, two different abnormalities were associated with sterility. Precocious movement of a few chromosomes at the metaphase I stage resulted into the production of non-functional pollen while cells which underwent apparent normal meiotic division had disintergration of the tapetal cell wall immediately after the free microspore stage leading to the starvation and subsequent death of the developing microspores. In lines N-69-2774 and TGM 242-4, both of which were partially sterile, male sterility resulted from a failure of cytokinesis after the telophase II stage. Meiosis proceeded normally but the 4 microspores after telophase II failed to separate into pollen grains and degenerated thereafter.

Key words: Glycine max L. – Soybean – Male sterility – Meiosis

Introduction

Although genetic male sterility has long been known to exist in soybeans, *Glycine max* (L.) Merr. (Owen 1928), work on the different causes of this condition is quite recent. Oinuma (1952) and Sen and Vindyabhusan (1960) reported precocious movement of chromosomes at metaphase I, but found no supernumerary quartets at the tetrad stages. Prichard (1962), Burson and Bennett (1970) and Marechal (1973) observed a similar situation in *Paspalum yuguaronense* (Hanr.), *Paspalum nicorae* (Parodi) and in crosses between *Phaseolus vulgaris* and *Phaseolus coccineus*, respectively. Abnormal development of the tapetal cell wall, leading to male sterility, was reported by Singh and Hadley (1961) in sorghum, Kaul and Singh (1966) in barley, Chauham and Singh (1966) in wheat, Alam and Sandal (1967) in Sudan grass and Mian et al (1974) in barley. Failure of cytokinesis as a cause of male sterility was reported by Palmer and Albertsen (1975) in soybeans.

The cytological study reported in this paper was conducted to find out the causes of male sterility in three soybean lines at International Institute of Tropical Agriculture, Ibadan, Nigeria.

Materials and Methods

Three sterile lines viz: TGM 103-1, N-69-2774 and TGM 242-4 were used in this study. The plants of line TGM 103-1, which was discovered at the International Institute of Tropical Agriculture, Ibadan, Nigeria by Dr. Camacho in 1973, were maintained in pots in the greenhouse from stem cuttings. Seeds of the line N-69-2774 were obtained from North Carolina, U.S.A. in March 1975 as segregating material and were grown in pots in the greenhouse. Sterility in the line TGM 242-4 was discovered at IITA in January 1975.

Flower buds of about 1-2 mm in size from the 3 sterile lines and those from their fertile siblings were separately fixed into freshly prepared Cannoy's fluid (6 parts ethanol: 1 part glacial acetic acid: 3 parts chloroform) for 4 hours. They were then washed in water and transferred into 70% alcohol. Later, anthers were extracted from the buds of each line and squashed in a drop of acetocarmine on a microscope slide, examined under the high power microscope and photomicrographs taken.

Microtome sections were also taken for lines TGM 103-1 and N-69-2774. Whole anthers of fixed flower buds from these lines were extracted and dehydrated through a series of different alcohol concentrations from 10% to 100%. They were then cleared through a series of alcohol/xylene mixture, starting with 90% alcohol/10% xylene to 0% alcohol/100% xylene. After infiltration with molten paraffin, the anthers were embedded into a paraffin block and sectioned to 8 microns on a rotary microtome. They were then stained in Ironhaematoxylin and safranin (Johansen 1940). After mounting in Canada balsam and drying, the sections were examined under the high power microscope and photomicrographs taken.

Results and Discussion

Microscope examination results of line TGM 103-1 showed a number of abnormalities at different meiotic stages.

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At metaphase I (Fig. 1a and b) it was observed that a few chromosomes had started moving away from the equatorial plate, as shown by arrows, creating univalent chromosomes during early metaphase I. This meant that homology between some chromosomes would be lost. During anaphase I (Fig. 2), lagging chromosomes were observed and a number of univalents appeared to have moved randomly in different directions rather than moving systematically towards the poles. The univalents that precociously moved during metaphase I, as well as the laggards observed during anaphase I, never reached the

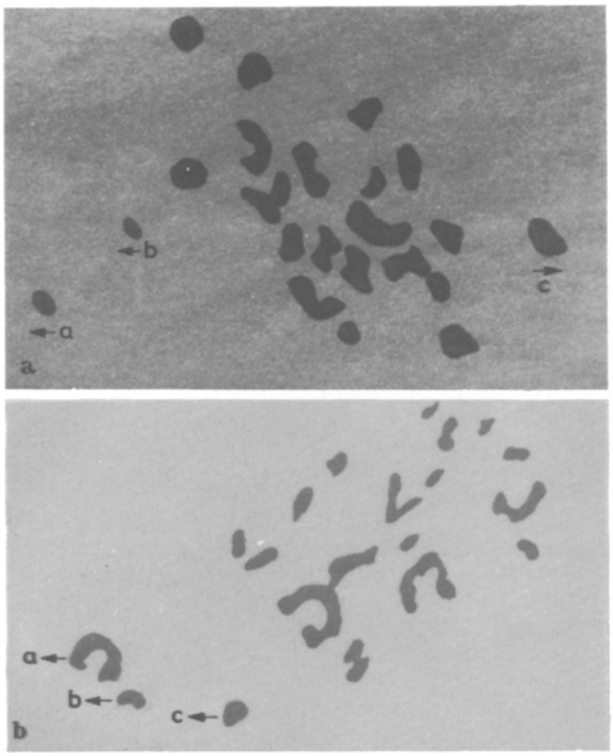

Fig. 1.a 'Precocious chromosome movement' in sterile TGM 103-1 at metaphase I, **b** Advanced metaphase I with 'Precocious chromosome movement' in sterile TGM 103-1

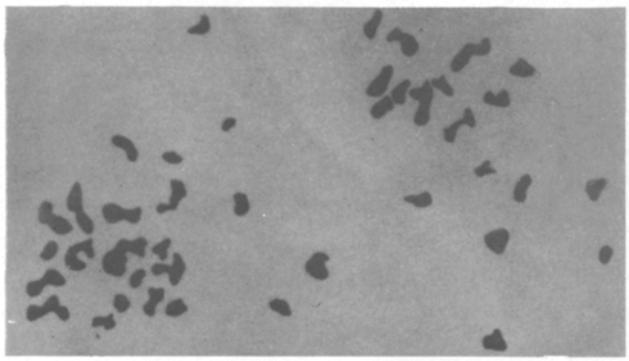

Fig. 2. 'Precocious chromosome movement' expressed at anaphase I

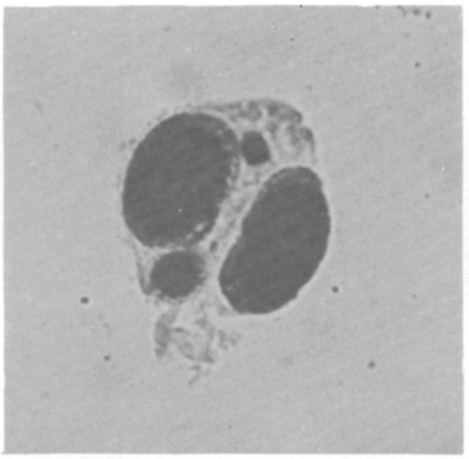

Fig. 3. Microcytes at the tetrad stage in sterile TGM 103-1

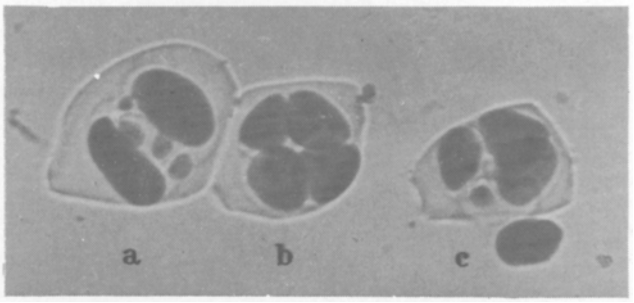

Fig. 4. Microcytes and normal tetrads (from the same anther) in sterile TGM 103-1

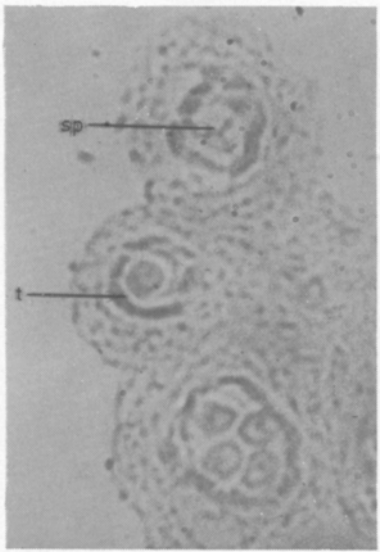

Fig. 5. Sporogenous cell wall stage and intact tapetum in anther section, in sterile TGM 103-1. sp = sporogenous wall, t = tapetum

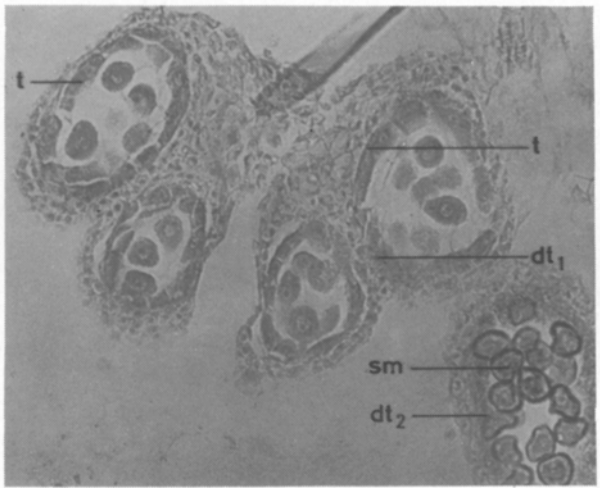

Fig. 6. Free microspore stage with tapetum beginning to disintegrate in TGM 103-1

poles but either collected together or remained separate, gathering bits of cytoplasm around themselves and forming one or more under-sized microspores (Fig. 3). Similar results were reported by Burson and Bennett (1970). These micronuclei were named microcytes by Marechal in 1975. The inclusion of a number of chromosomes into microcytes meant that the 'normal' appearing microspores (Fig. 3) lacked the correct genome of the species thus accounting for the inviability in the resulting pollen. It was observed, however, that not all cells undergoing meiosis, even within the same anther, had the precocious chromosome movement (Fig. 4). Although the three cells a, b, and c in Figure 4 came from the same anther, cells a and c had microsporocytes and microcytes, while cell b was normal. These cytological data clearly suggested that part of the sterility in line TGM 103-1 was caused by lack of the correct chromosome complement in the pollen grain. However, the fact that a number of dividing cells did not show the precocious chromosome movement and yet produced sterile pollen suggested that there was another mechanism responsible for sterility of pollen from such cells as this soybean line was found to be completely male sterile. Microtome anther sections at the sporogenous cell wall stage of development (Fig. 5) revealed that the tapetal cell wall was intact. Maheshwari (1950) suggested that the tapetal cell wall acted as the reservoir for food nutrients to the developing male gametophytes and its degeneration at any stage during the development process of the gametophytes led to their subsequent starvation and premature death. The tapetum was still persistent at the free microspore stage of development (Fig. 6) but had started to show signs of disintegration (dt_1), indicating that a second cause of male sterility in this line set in at this stage and was completed when tapetum completely degenerated (dt_2) and the developing gametophytes (Sm) started to shrivel. Similar results were re-

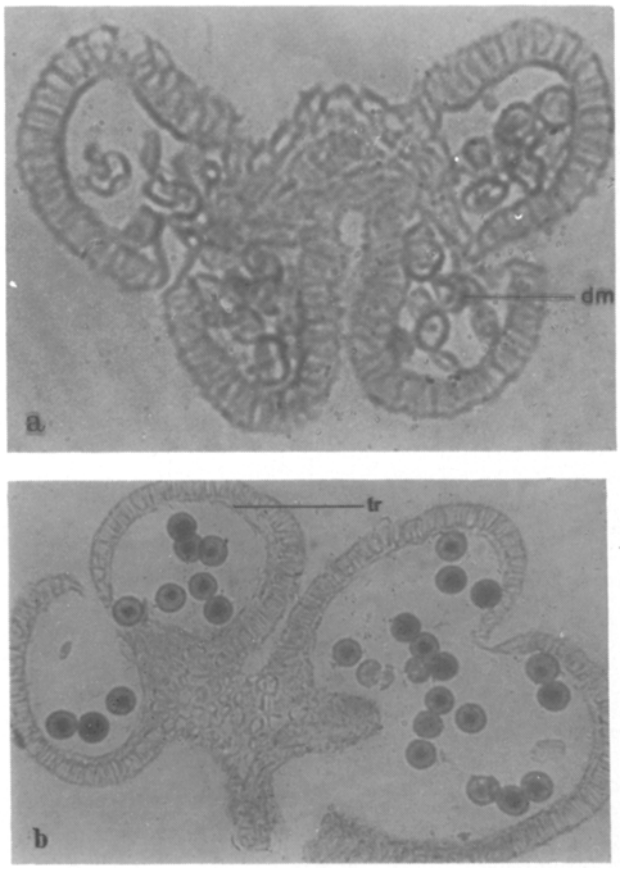

Fig. 7.a Dead pollen in mature anther section in sterile TGM 103-1 dm = dead microspores. **b** Fertile pollen in a mature anther section from a fertile sib of TGM 103-1. tr = tapetal remains

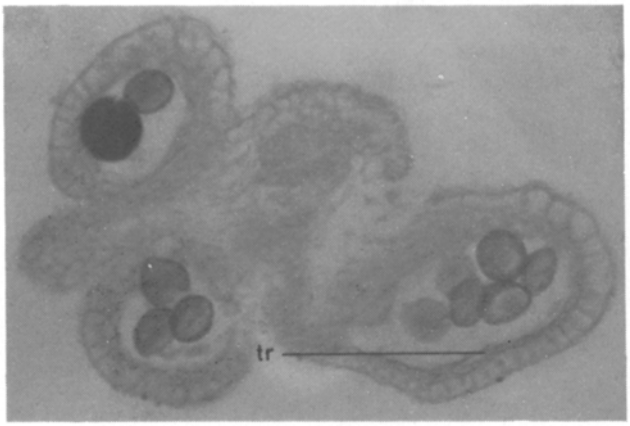

Fig. 8. Extra large sized 'pollen grains' in a sterile anther section of N-69-2774. tr = tapetal remains

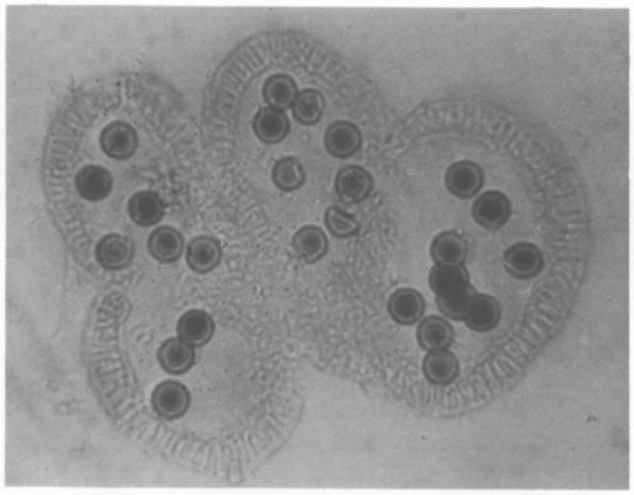

Fig. 9. Normal sized pollen grains in a fertile sib of N-69-2774

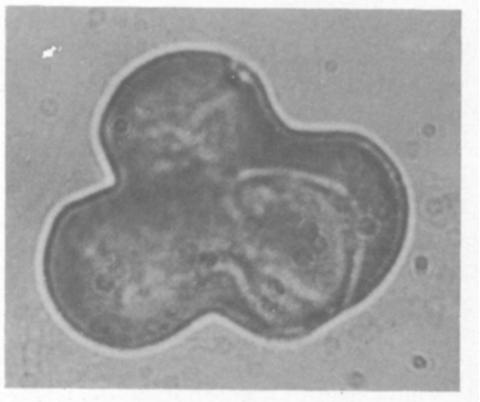

Fig. 10. Complete failure of cytokinesis at the tetrad stage in TGM 242-4

ported by Mian et al (1974) in barley. Tapetal degeneration went to completion as anthers proceeded to maturity and all the pollen grains were extremely misshapen (Fig. 7a) compared to those from a fertile segregant anther (Fig. 7b). The large nuclear material of the pollen grains indicated that they were approaching maturity and yet remains of the degenerated tapetum were still observable, suggesting that in order to produce viable pollen, tapetal degeneration should occur gradually from the time the microspore mother cells begins to divide to pollen maturity.

The results from meiotic studies of line N-69-2774 are shown in Figure 8. Tapetal degeneration in this material proceeded normally and gradually until pollen maturity, suggesting that normal pollen grains were produced. The size of the pollen grains, however, was much larger than that of the fertile grains shown in Figure 9 at the same magnification. The extra large sized 'pollen grains' were non-functional and were due to a complete failure of cytokinesis after telophase II. Singh and Patil (1975) reported similar results in this line. The four daughter microspores that resulted from meiosis remained encapsulated together within the same pollen wall. This meant that there was no free pollen for release, hence, the line was male sterile. Palmer and Albertsen (1975) reported similar findings in this crop and showed that the 4 nucleated non-functional pollen grains eventually degenerated. The cause of male sterility in line TGM 242-4 was similar to that in N-69-2774 (Fig. 10).

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