Short Communication

Fine Structural Changes of the Nuclei of Degenerating Microspores in Triploid Tradescantia

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The triploid mutant, obtained from a population of Tradescantia paludosa, is highly male-sterile, and most of its microspores degenerate at a stage either just before or after microspore mitosis (Nakazawa, 1962). It has been supposed that the degeneration of the microspore in the triploid mutant may be due to an unbalance of the genome (Darlington, 1965), but nothing definitive is known about the direct causes of male sterility. To examine the mechanism of microspore degeneration, it is necessary to clarify the fine structural changes of the degenerating microspores. However, few papers have been published so far on this phenomenon. De Vries and Ie (1970) reported that the degenerating microspores of male sterile wheat revealed little cytoplasmic structure, but they described nothing about the degenerative processes. Horner and Rogers (1974), who reported an electron microscopic study of microsporogenesis in cytoplasmic male sterile pepper, did not also observe the fine structure of the degenerating microspore, because the development of the microspore was arrested by the abnormality of the tapetum.

The present study deals mainly with a comparison of the fine structural changes in the nuclei of degenerating microspores of triploid Tradescantia, and the normal development in male fertile tetraploid.

Triploid Tradescantia from the garden of our institute provided the raw materials for these experiments. Comparisons were made with T. reflexa as the control. To determine the developmental stage of the microspore, one anther of a bud was tested by the aceto-carmine smear method. The process of microspore development was divided into 10 stages, P₁ to P₁₀, in the same manner as done in earlier papers (Nakazawa, 1957, 1962). These stages are outlined as follows: P₁ just after the tetrad stage, P₂ an early one-nucleate microspore stage, not yet vacuolated — the nucleus being located at the center, P₃ an early vacuolation stage — the ellipsoidal nucleus displaced to one side of the cell, P₄ a later vacuolation stage — the nucleus found rather round, P₅ just before mitosis — the large nucleus returned to the center, P₆ microspore mitosis, P₇ just after mitosis, P₈ a spheroidal generative nucleus, P₉ a spindle-shaped generative nucleus, P₁₀ a mature pollen grain.

The remaining anthers were fixed for 2 hr using 3% glutaraldehyde in 1/15 M

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phosphate buffer (pH 7.0), before they were post-fixed in 2% OsO₄ for 2 hr. The materials were treated with 3% uranyl acetate and Reynold’s lead citrate, and examined under a Hitachi HU-11 electron microscope.

In the normal or fertile microspore, the cell organelles such as ribosomes, endoplasmic reticulum and Golgi apparatus do not develop noticeably during the period from the tetrad to the P₂-stage, but they begin to develop rapidly after the P₂-stage when a main vacuole is formed in the cytoplasm (Niki and Nakazawa, 1974). There is no distinctive difference between fertile and sterile microspores until the P₂-stage, except that the cytoplasm of the sterile appears to contain fewer ribosomes.

The most remarkable event generally found at the beginning of degeneration in the sterile cells is the vacuolation of the nucleus occurring at stage P₂ or later. Several small vacuoles, which develop directly under the nuclear envelope (Fig. 1), expand into the nucleus (Fig. 2), causing the nuclear envelope to disintegrate (Fig. 3), the nuclear substances to scatter in the cytoplasm and finally to autolysé. Some of the degenerating nuclei form no vacuoles but the peripheral part under the nuclear envelope loses its structure (Fig. 4) and the chromatin gradually disintegrates (Figs. 5, 6).

Electron microscopy, like light microscopy, distinguishes 2 types of chromatin structures in the nuclei destined for degeneration. One is a coagulated type, in which chromatin threads are thick and coarse (Fig. 4), and the other a dispersed one, in which they are fine and scattered (Fig. 7). An extreme case of the coagulated type presents reticulate or massed shapes (Fig. 8), in which, though the nucleolus has usually disappeared, at times it is still observed to exist buried in the chromatin (Fig. 9). While nuclear degeneration generally coincides with cytoplasmic degeneration, sometimes the nucleus or the cytoplasm degenerates more quickly than the other (Fig. 6).

The scheme observed for the degeneration processes in the microspore of the male sterile triploid *Tradescantia* is inclusively shown in Fig. 10, although a few exceptional or intermediate types have been observed.

It is clear from our study that: (1) the first sign of nuclear degeneration arises at the stage when the vacuole and other organelles begin to develop in the cytoplasm; (2) the degeneration of the nucleus, either through vacuolation or loss of structure, begins to occur in an area directly under the nuclear envelope; and (3) 2 types of chromatin structures are generally distinguished in the nuclei destined for degeneration, the