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MONOFACTORIAL "MULTIPLOID SPOROCYTES" CONDITION INDUCED BY EMS IN PEARL MILLET

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"Multiploid sporocytes" condition was observed in the M_2 generation of *Pennisetum typhoides* raised from seeds treated with 0.3 percent EMS at pH 7. The pollen mother cells in the anther locules were devoid of individual boundaries and aggregated into plasmodium-like masses of various sizes in which the chromosomes were lying in groups. The number of chromosome pairs in these groups varied from 7 to 42 and in a few cases much larger groups were observed. At diakinesis and metaphase I, trivalents and quadrivalents were observed, which suggested that the chromosomes from different cells were in proximity at an early stage. Apparently cytokinesis was suppressed at the premeiotic divisions. Sometimes groups of chromosomes from several nuclei were together at metaphase I, the bivalents and multivalents co-orienting on a single spindle. Then the spindle was many times wider than normal, but the length was not greatly changed. Anaphase I segregation and second division were irregular. The microspores formed were of variable size. Sterility was high. This condition behaved as one controlled by a single recessive gene.

Introduction

SMITH (1942) has reported a case of spontaneously occurring "multiploid sporocytes" (symbol *mu*) in a six rowed barley variety. The mutant was formerly referred to as "contabscent anther". SMITH (1942) reported the presence, particularly in the lateral florets, of pollen mother cells with 14, 21, 28, 56, 112 and higher numbers of pairs of chromosomes. Apparently cell walls were absent in these cells, and syncytial masses of cytoplasm were formed which included all or part of the contents of a single anther locule. Whether cell walls were ever formed, or whether they disappeared in early prophase was not clear, but the presence of quadrivalents in some groups indicated that the chromosomes from different cells or nuclei were in proximity at an early stage and were able to synapse pre-

sumably because there was no barrier between them. It was possible that cytokinesis was suppressed in some premeiotic divisions. Mitotic divisions in the root-tip cells and the tapetal cells of *mu* plants appeared normal and those counted had only 14 chromosomes.

A similar mutation (*mu*) was detected by us in the M_2 generation of *Pennisetum typhoides* plants raised from seeds treated with ethyl methane sulphonate (EMS). One out of 53 plants raised showed this condition. Phenotypically these plants could be identified by the presence of prominent hairs on the leaves and tip sterile condition i.e. the top one third of the ear heads were sterile. There was high pollen sterility and on selfing the seed set was very low. Of the various premeiotic errors presently known, the *mu* condition is the only case which is single gene controlled, whereas other premeiotic errors are polygenically controlled (REES, 1961). As far as the authors are aware, this seems to be the only case of induced *mu* mutation and hence a detailed cytogenetic study was undertaken.

Materials and Methods

Dry seed of *Pennisetum typhoides* was treated with different concentrations of ethyl methane sulphonate at three different pH levels. The mutant plant was detected in the M_2 of the treatment with 0.3% EMS at pH7. For cytological study material from the mutant plant and its progeny obtained by selfing and open pollination was used. Inflorescences were fixed in alcohol-acetic acid 3:1, and acetocarmine squash preparations were made for the study of meiosis.

Results and Discussion

At diakinesis the PMC's revealed the boundaries of the individual cells to be indistinct i.e. the PMC's in an anther sac tended to form plasmodium-like masses of various sizes in which the chromosomes were lying in groups. These groups contained 7, 14, 28, 35, 42 pairs of chromosomes. As many as 56 pairs have been counted in a single group in some cases. At pachytene and prepachytene stages a large number of nuclei were observed to lie in a single plasmodial mass. When many nuclei were lying in the common plasmodial mass, in

some cases groups of chromosomes belonging to individual nuclei were lying separate from one another. In other cases there was a mixture of the nuclear material and this resulted in the formation of groups of 14, 21, 28, 35, 42 etc. pairs of chromosomes. At diakinesis and metaphase I plasmodium-like masses with a minimum of 7 pairs of chromosomes and a maximum of about 259 or more pairs of chromosomes, where it was not possible to count the number accurately, were observed. Generally these chromosome groups were composed of multiples of 7 pairs.

Occasionally were observed smaller plasmodial masses containing variable numbers of chromosomes; these had definite boundaries and appeared like polyploid cells of large size. Out of 125 such cells examined 6 had 28 pairs of chromosomes, 5 had 21 pairs, 106 had 14 pairs and 8 had 7 pairs of chromosomes. Apparently, groups of 14 pairs of chromosomes were most frequent. Where the number of chromosomes in a group was higher than the diploid number, a reduction in the size of the chromosomes was observed.

In the same plant the spikelets from the ear-heads on the main axis showed larger plasmodial masses, with larger aggregations of nuclei in each mass. In the spikelets from the tiller these aggregations were smaller, more often containing 7 chromosome pairs only.

At diakinesis, when 28 or more chromosomes were present, bivalents, trivalents and quadrivalents were observed. The bivalents were of the rod type only. The presence of multivalents suggests that the fusion of the cells should have occured before the process of synapsis had taken place.

At first metaphase, two different conditions of spindle organisation were observed. In one case the bivalents and multivalents from different nuclei aggregated and were regularly oriented on a single large metaphase plate within the plasmodial mass. In the second type groups of 14 or 28 chromosomes forming bivalents in the former condition and bivalents, multivalents and univalents in latter case were oriented on different metaphase plates in a single plasmodial mass. Independent of the formation of a single first metaphase plate or separate plates, univalents failed to orient themselves on the plate and appeared scattered on the spindle.

Anaphase I segregations were largely irregular except in cases where there were only 7 pairs of chromosomes. In those cases where

the first metaphase plate was very broad with a large number of chromosomes no definite recognizable poleward movement of chromosomes could be observed clearly. Telophase I groups of variable sizes and with chromosome numbers varying from a few to many, where it was difficult to count the exact number, were observed. From the size of the telophase I nuclei and the number of chromosomes found in them it could be seen that anaphase I segregation was highly irregular. A number of laggards were observed at telophase I and these led to the formation of micronuclei.

The second division also was irregular. Some of the dyad cells failed to undergo division and contained one to three micronuclei in addition to the normal nucleus. Sometimes out of the two dyad cells formed one was with and the other without any chromosomes. Failure of cytokinesis and of wall formation were also observed at the end of the second division. These phenomena along with the micronuclei formed at the end of the first division seemed to be responsible for the formation of microspores in different numbers and different sizes and also with variable number of chromosomes. Formation of four microspores each with a single nucleus was observed only in those cases where there was the diploid number of chromosomes in the PMC's.

Some ear-heads of the *mu* mutant plant were used for selfing and the other ear-heads were left for open pollination. When selfed there was very sparse seed set and the plants raised from these seeds showed *mu* condition. The plants raised from the seed obtained on open pollination were normal. When these plants were selfed, in the progeny, i.e. the M_4 generation, out of 78 plants raised, 21 plants showed the *mu* condition. This was a good fit for a 3:1 ratio ($\chi_1^2 = 0.1535$; $P = 0.05$), which shows that the *mu* condition is controlled by a recessive gene.

The *mu* condition in the M_4 progeny varied. In some cases the plasmodial masses were smaller, having 14 chromosome pairs, and others were larger having 35 to 42 pairs of chromosomes. Thus there seems to be a difference in the degree of expression of the mutant gene under different conditions.

A number of genetically determined meiotic abnormalities have been described previously (DARLINGTON, 1937; REES, 1961). These affect pairing, crossing over, chromosome contraction and chromo-

some size, spindle formation etc. These mutations were known to affect meiosis. But in addition to these a number of mutations are reported which induce premeiotic errors like chromosome breakage, spindle abnormalities, suppression of wall formation etc. These mutations are observed only in premeiotic mitoses and not elsewhere in the organism. Of these mutations which induce premeiotic abnormalities, the suppression of wall formation leading to coenocyte formation before meiosis is the only one which is single gene controlled, all others are polygenic.

The action of some of these genes, like those affecting chromosome pairing and chiasma frequency, is naturally restricted to meiosis. But some other phenomena like neocentric activity of chromosome 10 in maize, although a priori not typical for meiotic processes, are found only in meiosis and not in mitosis. It is generally accepted that interaction between the cytoplasm and the nucleus may control the expression of these genes. This leads to the assumption that expression of these genes and the onset of meiosis may depend on the cytoplasmic threshold at a particular stage of development (REES, 1961). The mutant genes, the expressions of which are manifest at premeiotic mitoses but not elsewhere in the meristematic tissues, are of interest as they give us an indication that changes take place in the cytoplasmic environment, as a result of which the genes are able to express themselves. There seems to be a difference between the premeiotic divisions and other mitoses, the former being more susceptible to abnormalities, at the same time being more similar to meiotic divisions. Thus it appears that the change from mitosis to meiosis is achieved by a cytoplasmic change that builds up gradually over a number of cell generations (REES, 1961). The same changes in the cytoplasmic environment obviously seem to be responsible for the expression of these mutant genes.

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REFERENCES

DARLINGTON, C. D. (1937). Recent Advances in Cytology 2nd ed. J. & A. Churchill Ltd., London.

REES, H. (1961). Genotypic control of chromosome form and behaviour. *Bot. Rev.* **27**: 288–318.

SMITH, L. (1942). Cytogenetics of a factor for multiploid sporocytes in barley. *Amer. J. Bot.* **29**: 451–456.