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Desynapsis and the Blockage of Meiosis in *Pennisetum orientale* Rich.

PREM P. JAUHAR and UDAI SINGH

Division of Genetics, Indian Agricultural Research Institute, New Delhi

Summary. Meiotic behaviour of a desynaptic plant of *Pennisetum orientale* Rich., which seems to have arisen through spontaneous gene mutation/s, is recorded. The plant showed an extremely rare type of desynapsis in which the precocious falling-apart of the homologous chromosomes was accompanied by the blockage of the meiotic course from metaphase I onwards. Different factors which may have led to the failure of chromosome synapsis and to the blockage of the meiotic division are discussed. The possibility of the participation of different enzymes in the initiation and continued progress of chromosome synapsis as well as for the different meiotic steps is considered. It has been inferred that the absence or later inactivation of these genetically-controlled enzymes might have resulted in the meiotic abnormalities observed in the present material. Another plausible regulatory mechanism based on the existence of a switch — through gene-repressing effect of histones — has been hypothesized and its possible evolutionary significance indicated.

Introduction

It is now well established that the meiotic events leading upto, and including, formation and dismissal of chiasmata are the most susceptible to change by intra- and/or extra-cellular factors. During meiosis in microsporocytes of some organisms, the chromosomes pairing normally at the early prophase stages tend to fall apart precociously at diplotene. Thus, the homologous partners lie unpaired at diakinesis and metaphase I. This phenomenon which is commonly referred to as 'desynapsis' (LI, PAO and LI, 1945) is known to occur in a large number of plant species (RILEY and LAW, 1965). During the course of an extensive study of the genus *Pennisetum*, a plant of the diploid species, *P. orientale* ($2n = 18$), was found to be desynaptic and showed interesting meiotic behaviour. Desynapsis in this material was associated with the blockage of the second meiotic division, a situation extremely rare in the known cases of desynaptics. In the present report meiotic behaviour of the desynaptic is recorded and some such factors which may have led to this anomaly are discussed.

Material and Methods

Pennisetum orientale, an important forage grass, has several chromosome races with $2n = 18$, 22, 34 and 36 (PATIL, HARDAS, O'CONNOR and VOHRA, 1962). A cytological survey of the diploid cytotype ($2n = 18$) showed the presence of accessory chromosomes in some plants (JAUHAR and SINGH, 1968). Out of the 12 plants which were raised from seeds from a single ear of a normal plant, one was found to be desynaptic. Detailed meiotic study of this plant was done.

Buds were fixed and stained according to the schedules standardized earlier for grass chromosomes (JAUHAR and JOSHI, 1966). Analysis of meiotic stages was done from temporary slides. Pollen fertility was studied by ascertaining their stainability with 1.5 per cent acetocarmine.

The atmospheric temperature at the time of fixations was 29 °C, the minimum and maximum temperatures recorded during that week being 24.5 °C and 33.4 °C, respectively.

The data on chromosome pairing at the two stages, viz., diplotene and diakinesis were separately tested for goodness of fit for Poissonian distribution. The data at metaphase I could not be tested because no degrees of freedom are available when data in the lower classes are pooled to make a minimum frequency of five.

Observations

Meiosis in the desynaptic plant

The desynaptic plant showed partial to complete failure of meiotic pairing at stages from diplotene onwards. While the normal plants showed regular pairing of chromosomes (Fig. 1), mostly univalents were observed in the desynaptic plant (Figs. 2 and 3). Although pachytene stages in this plant could not be analysed accurately, yet some paired and unpaired regions in some bivalents were discernible. At diplotene the homologues tended to desynapse, their falling-apart being rather rapid so that at diakinesis they were almost completely unpaired. It is significant to note, however, that the desynapsed chromosomes tended to lie juxtaposed together (Figs. 2 and 3), showing thereby some sort of residual attraction between the potential partners. The data on chromosome pairing at diplotene, diakinesis and metaphase I are presented in the Table.

It appears that in the desynaptic plant there is considerable extent of pairing at pachytene, but as the diplotene stage progresses the homologous partners tend to dissociate progressively. Late diplotene, therefore, appears to be the 'critical' stage where a considerable failure of synapsis starts. The Table shows that the frequency of the occurrence of bivalents at diplotene and diakinesis follows a Poisson

Table. *Frequency of bivalents in the desynaptic plant*

Stage	Number of PMC's analysed	Number of cells with bivalents					Average number of bivalents per cell	χ^2	Fitness for Poisson distribution
		0	1	2	3	4			
Diplotene	38	15 12.28	9 13.88	9 7.84	4 2.95	1 0.84	1.13	2.713	Very good (χ^2 at 0.05 p = 3.840)
Diakinesis	85	60 54.20	15 24.39	7 5.49	3 0.82	0 0.09	0.45	6.246	Good (χ^2 at 0.01 p = 6.635)
Early metaphase I	64	53	7	4	0	0	0.22		

The upper figures indicate the number of cells actually observed; the lower figures show the numbers expected on Poisson distribution.

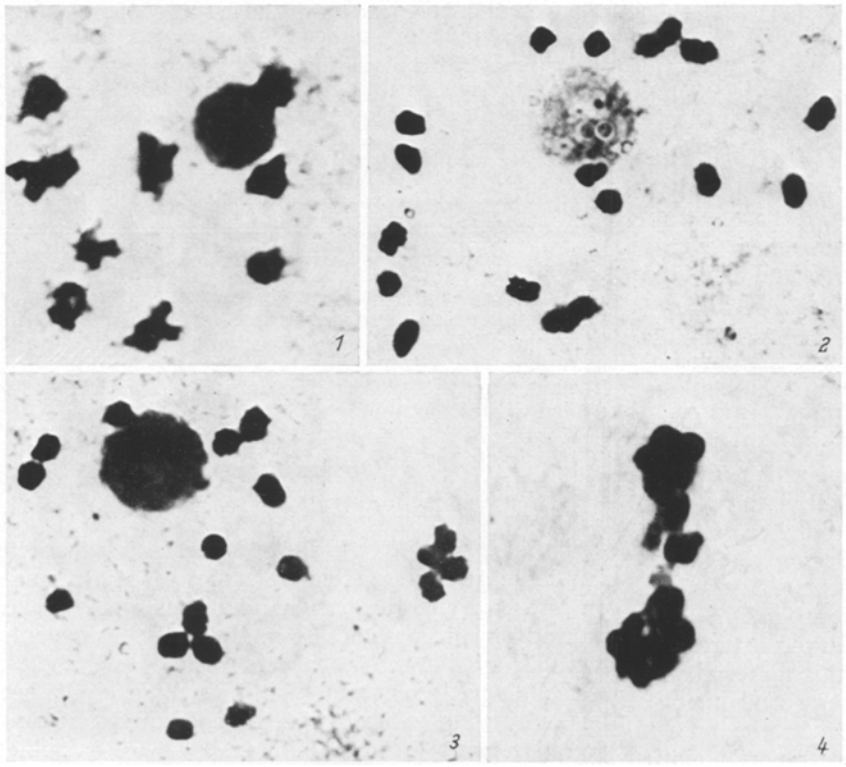

Fig. 1. A PMC of a normal plant of *Pennisetum orientale* showing normal diakinesis with 9 bivalents

Figs. 2 to 4. Cells showing meiotic stages in the desynaptic plant. — Fig. 2. Diakinesis with 2 rod-bivalents and 4 univalents. — Fig. 3. Diakinesis with 18 univalents some of which are showing close juxtaposition, and secondary associations. — Fig. 4. An extremely irregular anaphase I

distribution. The probability of getting cells with 4 or more bivalents is very low at diplotene and is almost negligible at diakinesis.

At metaphase I, the spindle formation seemed to have failed. The univalent chromosomes also appeared to have lost their centromeric activity and looked almost completely disorganized.

Secondary association of univalents

An interesting feature observed was the secondary association of univalents at diakinesis (Fig. 3). The chromosomes tended rather consistently to lie in groups. Two univalents were associated with the nucleolus. The secondary associations of bivalents in the normal, diploid plants of *P. orientale* have been reported and their evolutionary implication discussed by JAUHAR (1968).

Blockage of meiosis

Anaphase I and telophase I stages of normal meiosis appeared to have been blocked in the desynaptic plant. The movement of univalents to the poles was not only extremely rare but highly irregular also (Fig. 4). In 95 per cent of the PMC's (out of a total of 220 analysed) the univalents were scattered haphazardly at the centre. Thus, more often the chromosomes clumped together thereby forming first division restitution nuclei and ultimately monads (Figs. 5 and 6). No cytokinesis was observed. The stages of second meiotic division seemed to have been completely arrested and the first division restitution nuclei apparently gave rise to giant microspores directly. Such spores were quite evident and were deeply stained with acetocarmine when pollen was examined. About 85 per cent of the pollen was completely sterile.

Discussion

A finding of great cytogenetic and phylogenetic significance is the recent evidence that the specificity of chromosome pairing can be widened or narrowed by gene action (RILEY and LAW, 1965). More recently, it has been inferred that chromosome pairing and in fact all events of meiosis may be under some

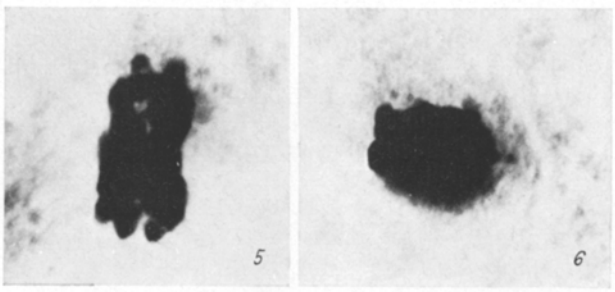

Fig. 5. Full chromosome complement clumping together and forming a compact block in the restituted nucleus resulted from failure of polar movement

Fig. 6. Full, compact block of chromosomes forming a monad

form of genetic control (RILEY, 1966). Among the recorded deviations from normal chromosome pairing, desynapsis constitutes one of the most interesting meiotic phenomena. The genetics of desynapsis in several plant species is now fairly well known. It has been shown, in most of the cases, to be due to the double recessive condition of one or two genes. It is likely that the desynaptic phenomenon observed in the present study has a genetic basis. The fact that only one of the 12 plants of *P. orientale* raised from seed from a single ear was found to be desynaptic indicates the possibility of the occurrence of spontaneous gene mutations which led to desynapsis, and blockage of the meiotic course from metaphase I onwards.

Since the discovery of the asynapsis gene located in chromosome 1 of maize (BEADLE, 1930), a considerable number of mutant genes are now known which reduce chiasma frequency. They have been found, among others, in *Drosophila* (GOWEN, 1933), in wheat (HUSKINS and HEARNE, 1933; LI, PAO and LI, 1945), in *Crepis* (RICHARDSON, 1935), in *Nicotiana* (GOODSPEED and AVERY, 1939), in *Oenothera* (CATCHESIDE, 1939), in rye (PRAKKEN, 1943), in *Hordeum vulgare* (ENNS and LARTER, 1960), in *Oryza sativa* (CHAO, LI and HU, 1960) and in *Schistocerca gregaria* (JOHN and NAYLOR, 1961). In most of these cases chromosome failure has been shown to be due to a simple Mendelian recessive. The type of desynapsis and blockage of meiosis seen in the present material of *P. orientale*, however, seems to have been reported very rarely, although somewhat similar cases of suppression of meiosis in asynaptic *Scilla* (REES, 1952) and failure of cytokinesis after one or both meiotic divisions resulting in the formation of giant, diploid or tetraploid sporads in maize (MILLER, 1963) are known.

External factors, like the temperature, are also known to produce desynapsis (LI, PAO and LI, 1945; EHRENBERG, 1949; SOOST, 1950). The effect of environmental factors in bringing about the meiotic abnormalities reported in the present study can, however, be ruled out because of the fact that the buds from the rest of the plants which showed regular synapsis, were also fixed either on the same day or the following day in the same week (the maximum and minimum temperatures being 33.4 °C and 24.5 °C). Moreover, a reinvestigation on the meiosis from the normal and the desynaptic plants showed the consistency of results reported above.

Several explanations have been given for the phenomenon of desynapsis. CELARIER (1955) and ROSS, SANDERS and FRANZKE (1960) suggested the failure of chiasma formation as the major factor involved in desynapsis. It had been earlier proposed that in crossing over proteolytic enzymes may be involved, the diminution or altogether absence of any one of which may well interfere with this process (EHRENBERG, 1949). Such a hypothesis is quite compatible with the simplicity of the genetic control of desynapsis and also takes care of the influence of external factors on chiasma formation. In the present material although the stages prior to diplotene could not be studied critically, yet the presence of some bivalents at diplotene and rarely at diakinesis and even at metaphase (Table) suggests the possibility of considerable extent of pachytene pairing. However, most of the bivalents observed at the post-diplotene stages were rather loose and of the rod-type (Figs. 2 and 3). It appears, therefore, that the initial failure of chiasma formation at early prophase stages may not be responsible for the failure of pairing at diakinesis and metaphase I in the present material.

In view of the known direct genetic control of chiasma formation and crossing over (REES, 1955, 1961; SMITH, 1966) and the inference of RILEY (1966) that all events of meiosis are under some form of genetic control, it can be supposed that several specific enzymes are essential for the onset and continued progress of meiosis and for chiasma formation. Since gene action is an enzyme-mediated process, it is quite logical to implicate enzymes in chromosome pairing and in chiasma formation and even for the different meiotic steps. That gene products are responsible for chiasma formation has been elegantly shown by the induction of crossing-over in male *Drosophila* through treatment with ovarian extracts from the females which normally form chiasmata in this Dipteran (REDDI, REDDI and RAO, 1965). Moreover, genetically-controlled enzyme deficiencies are also known (HOROWITZ, BONNER, MITCHELL, TATUM and BEADLE, 1945). It would, then, be reasonable to assume that in the present material the desynaptic gene mutation/s arose subsequent to the onset of synapsis so that at pachytene stage there is almost regular pairing and as meiosis progresses to metaphase I, the enzyme/s starvation of the PMC's is the maximum so that the homologues tend to fall apart rather rapidly (Table) and the further meiotic course gets almost blocked (Figs. 5 and 6).

Alternatively, the gene-controlled enzyme/s, essential for the initiation and maintenance of synapsis, may be normal at pachytene and earlier stages but as the diplotene and the subsequent stages approach, get/s progressively inactivated due to some abnormal intra- or extra-cellular conditions. That such a progressive inactivation of the enzyme/s may be occurring is suggested by the progressive falling apart of the homologous partners at these stages (Table). Also, this set-back to the microsporocyte, resulting from an abnormal cellular situation, is reflected in the blockage of the further meiotic course leading to the formation of first division restitution nuclei and then monads (Figs. 5 and 6). That such restitution nuclei give rise to giant, tetraploid microspores becomes clear on examination of pollen stained with acetocarmine. Another possibility that the enzyme/s required for these subsequent stages is/are not synthesized due to errors either at the translation or transcription level, also deserves consideration.

Still another point of great significance is an observation (ANSLEY, 1957, 1958) that certain biochemical changes are associated with the failure or success of chromosome pairing in *Loxa flavicolis*. ANSLEY's work, wherein it was shown that DNA: histone ratio was 1:1 in synaptic cells at meiosis, but in asynaptic cells the ratio was 2:3, provides a sort of experimental evidence as to the causal basis of meiotic synapsis. SINHA (1959) also feels that a critical balance between DNA and RNA, and between DNA and histones is essential for the onset and normal progress of meiosis in maize. Now that it has been known that an altered histone: DNA ratio can disturb chromosome pairing and that gene action can be repressed by the association of histones with chromosomal DNA (*see* BONNER, 1965; CLEVER, 1968), it is tempting to believe that chromosome pairing in the present desynaptic material may be similarly regulated. Although such a plausible regulatory mechanism based on the existence of a switch may be operating in a number of desynaptics, it is difficult to put it to experimental test. Interesting inferences regarding the importance of histones in regulating chromosome pairing have been drawn by RILEY (1966) who feels that synapsis may be dependent upon the DNA constituent of the chromosomes, but whether or not it is realized in a particular cellular situation is determined by how the DNA is complexed with histones.

If the aforesaid mechanism is occurring it provides a good tool for the production of syndiploid (*see* NYGREN, 1946) spores which may be a chief cause of polyploidy in plants (DARLINGTON, 1937) particularly in grasses; such a polyploidization can help them resist the hazards of environment. Thus, such a gene-repressing effect of histones may have evolutionary significance.

A further study of desynaptics like the one reported here may provide a valuable means of investigating the functional regulation of the meiotic course and may give a clue to a better understanding of the genetic and physiological basis of chiasma formation and terminalization.

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Zusammenfassung

Bei einer desynaptischen Pflanze von *Pennisetum orientale* Rich., die wahrscheinlich durch spontane Genmutation entstanden ist, wird die Meiose beschrieben. Die Pflanze weist eine sehr seltene Form der Desynapsis auf, bei der das vorzeitige Auseinanderfallen der homologen Chromosomen mit einer Blockierung des Meioseablaufs von der Metaphase I ab verbunden ist. Verschiedene Faktoren, die möglicherweise den Ausfall der Synapsis und die Blockierung der meiotischen Teilung verursacht haben könnten, werden diskutiert. Die mögliche Beteiligung verschiedener Enzyme sowohl bei der Auslösung und dem weiteren Verlauf der Synapsis als auch bei verschiedenen meiotischen Teilprozessen wird in Betracht gezogen. Es wird gefolgert, daß das Fehlen oder die spätere Inaktivierung solcher genetisch gesteuerten Enzyme die im vorliegenden Material beobachteten meiotischen Anomalien verursacht haben könnten. Ein weiterer denkbarer Regulationsmechanismus, beruhend auf der Existenz einer Umschaltung des Meioseablaufs infolge eines Gen-Repressions-Effektes der Histone, wird als Hypothese aufgestellt und hinsichtlich seiner möglichen evolutionären Bedeutung umrissen.

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Dr. PREM P. JAUHAR and UDAI SINGH
Division of Genetics
Indian Agricultural Research Institute
New Delhi-12 (India)