Cytogenetics of *Crotalaria* VI. Chiasma frequency and position, and univalent behaviour in a (partially) asynaptic mutant of *C. juncea*

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Abstract

In *Crotalaria juncea* (n = 8) a plant exhibiting partial asynapsis was isolated in the M_1 of a combined treatment of 50 kR gamma rays + 0.2% EMS. The majority (48.14%) of PMCs at diplotene, diakinesis and metaphase I had 16 univalents. The bivalents in the asynaptic mutant were always rod-shaped with one terminal chiasma. In comparison, controls had on the average 7.08 ring bivalents. The asynapsis is genetically controlled, monofactorially recessive, and it is concluded that chromosome pairing is interrupted at a very early stage. There is a possible correlation between the number of bivalents and the arrangement of the univalents at metaphase I. When there were less than four bivalents, the univalents tended to be polar, and when there were more than four, the univalents were more equatorial in arrangement. The arrangement of univalents was random and apparently not influenced by the bivalents, when their number (4) was exactly half the zygotic number.

Introduction

The normal behaviour of male and female meiosis is dependent on coordinated systems governed by genes and their interaction with external factors like temperature, water, nutrition. Mutation in the former or drastic change in the latter may lead the course of meiosis to deviate in various ways. The homologous chromosomes may fail to pair at zygotene (asynapsis), or else subsequent chiasma formation may fail (desynapsis). Desynapsis/asynapsis, besides being found spontaneously, has been induced by gamma and X rays (Martini & Bozzini, 1966; Bozzini & Martini, 1971; Gottschalk & Baquar, 1971; Gottschalk, 1978; Katiyar, 1977; Singh *et al.*, 1977; Srivastava, 1974), chemical mutagens (Bozzini & Martini, 1971; Sree Ramulu, 1973; Seetharam *et al.*, 1975; Singh *et al.*, 1977; Sharma & Reinbergs, 1974; Tyagi & Das, 1975) and by combined treatments of physical and chemical mutagens (Singh *et al.*, 1977). The cytogenetics of desynapsis/asynapsis has helped towards a better understanding of the intricate mechanisms involved in meiosis, as in *Pisum sativum* where Gottschalk (1978) has isolated 58 genes influencing meiotic behaviour.

Experiments were conducted to investigate the nature and extent of mutation induction by treating seeds of *Crotalaria juncea*, commercially known as Sunnhemp, with EMS, MMS, NMU and gamma rays separately and in combination, and their implications for meiotic behaviour. The present communication deals with the meiosis of a (partially) asynaptic mutant, which has some bearing on chromosomal pairing, exchange and orientation of univalents.

Material and methods

In a mutation experiment on *C. juncea* (2n = 16) several radiation doses and chemical mutagens in several concentrations, separately and in combina-

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tion were applied (unpublished). Flower buds of all M_1, F_1, F_2 and control plants were fixed separately in acetic alcohol (1:3) and, following the usual technique, the anthers stained in leuco-basic fuchsin were squashed in 1% iron acetocarmine. Microphotographs were taken from temporary preparations.

Observations

In the combined treatments of 50 kR gamma rays + 0.2% EMS, one of the five M_1 plants (survival 100%) raised was found to be partially asynaptic. No such behaviour was encountered in plants raised after separate and other combined treatments. As the mutant appeared as a whole-plant mutant in M_1 and in later generations segregated as a monofactorial recessive, there is reason to believe that it has not been induced by the treatment but occurred in the material as a spontaneous mutation arisen in an earlier generation.

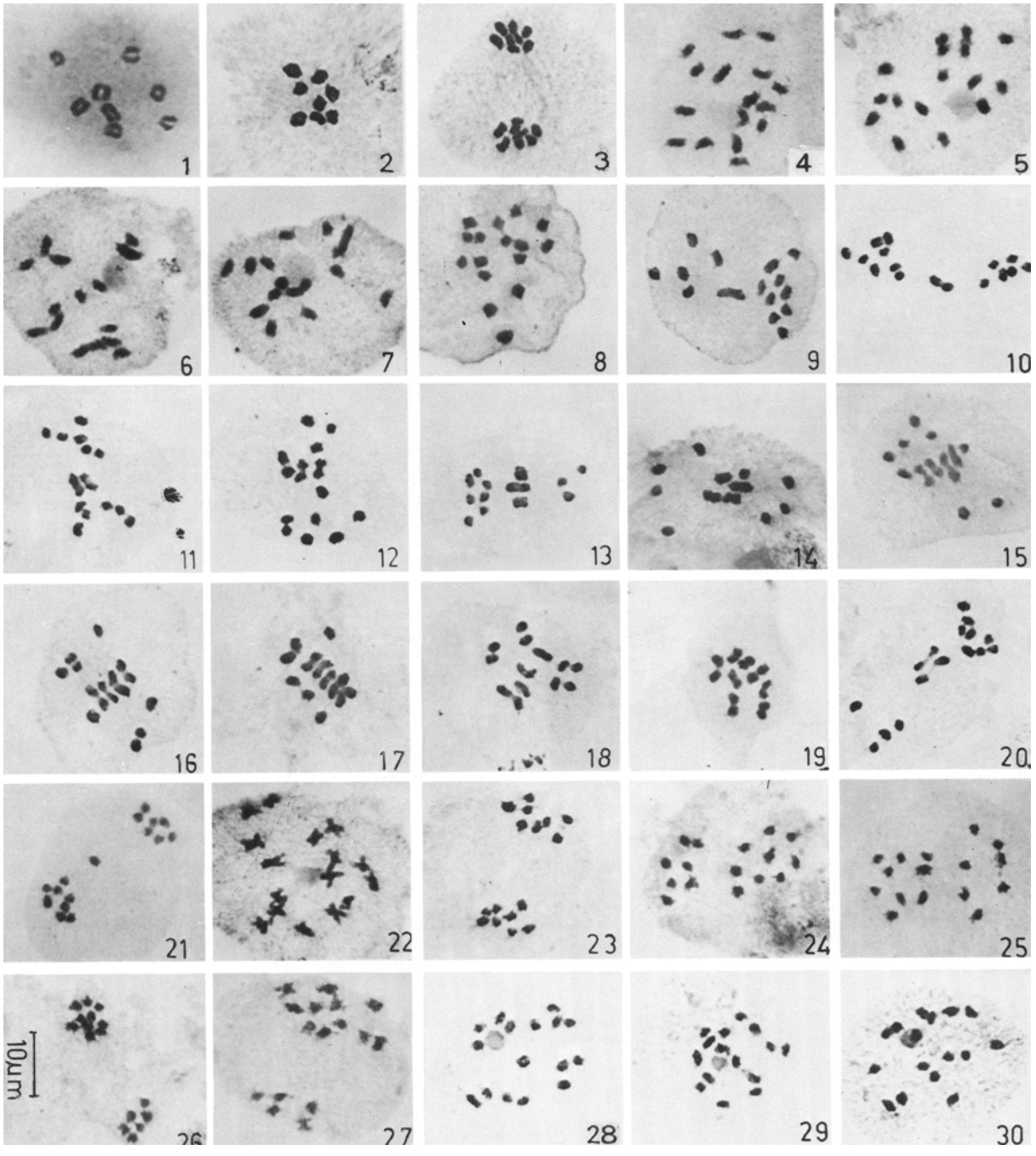
The observations have been summarized in Tables 1, 2, 3 and 4. The asynaptic plant was characterized by the presence of univalents at diplotene, diakinesis and metaphase I (Figs. 4–20, Tables 1–2). Twenty-nine (65.9%) out of 44 PMCs analysed at diplotene/diakinesis had 16I (Fig. 4). The other configurations observed were 3II + 10I (Figs. 6, 7), 2II + 12I (Fig. 5) and 1II + 14I in 9, 4 and 2 cells respectively. The bivalents were rod-shaped and the chiasmata were terminal. Sixty four cells were analysed at metaphase I and the most common (35.93%) association was 16I, followed by 4II + 8I, 3II + 10I, 2II + 12I, 6II + 4I, 7II + 2I, 1II + 14I, 5II + 6I, 8II in 14.06, 9.37, 9.37, 9.37, 7.81, 6.25, 6.25 and 1.56 per cent PMCs respectively (Figs. 8–20, Table 2). In all, 52 (48.14%) out of 108 PMCs had 16I (Figs. 4, 8) at diplotene, diakinesis and metaphase I. The predominant presence of the univalents at diplotene/diakinesis (14.3 per cell) and metaphase I (10.71 per cell) indicates, among other things, that the univalents may be produced by partial asynapsis. When the chiasmata are strictly terminal, there is a great chance that some of them may slip off precociously and convert the bivalents to pairs of univalents which move to the poles (Sybenga, pers. comm.). As is evident from the comparison between the numbers of chiasmata at diplotene-diakinesis and at metaphase I, such behaviour is not apparent in the present material which indicates that the association between chromosomes might be maintained by some sort of stickiness till they separate at anaphase I. A notable feature of the asynaptic mutant was that while the bivalents showed perfect congression at the

Table 1. Mean number of associations and chiasmata per cell at metaphase I in partially asynaptic *Crotalaria juncea.*

Source (Number of cells)	Chiasmata					Bivalents						Univalents	
	Total number	Freq-uency	Termi-nalized	Unter-mina-lized	Term. coeff.	Ring		Rod		Total			
						No.	Mean	No.	Mean	No.	Mean	No.	Mean
Control (25)	418	16.72 ±2.31	373	45	0.89	177	7.08 ±1.11	21	0.84 ±0.96	198	7.92 ±0.27	4	0.16 ±0.53
Asynaptic (64)	169	2.64 ±2.53	169		1			169	2.64 ±2.53	169	2.64 ±2.53	686	10.71 ±5.06

Table 2. Associations at metaphase I in 64 cells.

Association	Total	16I	1II + 14I	2II + 12I	3II + 10I	4II + 8I	5II + 6I	6II + 4I	7II + 2I	8II
Number of cells	64	23	4	6	6	9	4	6	5	1
Percentage	99.97	35.93	6.25	9.37	9.37	14.06	6.25	9.37	7.81	1.56



Figs. 1–30, Crotalaria juncea: (1–3) control, (1) diakinesis, 8II, (2) MI, 8II, (3) anaphase I, 8:8. Note eight ring bivalents in (1), seven ring and one rod bivalent in (2); (4–27) Asynapsis in M_1, (4–7) diakinesis, (4) 16I, (5) 2II + 12I, (6–7) 3II + 10I, (8–20) MI, (8) 16I, (9–10) 1II + 14I, (11), (20) 2II + 12I, (12–13) 3II + 10I, (14–16), (18) 4II + 8I, (17) 6II + 4I, (19) 8II. Note rod bivalents in (5–7), (9–20). Note polar orientation of univalents in (9–13), (20), non-polar arrangement in (17) and some non-polar, some polar arrangement in (14–16), (18); – (21–27), anaphase I, (22–23) 8 : 8, (24–25) 9 : 7, (26) 10 : 6, (27) 11 : 5. Note lagging univalent in 21; – (28–30) Asynapsis in F_2, diakinesis, (28) 16I, (29–30) 1II + 14I.

Table 3. Anaphase-I distribution in 50 cells.

Chromosome distribution	8:8	9:7	10:6	11:5	12:4	8:1*:7	7:4*:5	5:3*:8	6:6*:4	6:4*:6	9:2*:5
Number of cells	13	18	6	4	1	1	1	1	2	2	1
Percentage	26	36	12	8	2	2	2	2	4	4	2

* The middle values are the numbers of lagging univalents.

Table 4. Orientation of univalents in relations to number of bivalents.

Number of bivalents	Number of cells	Univalents			
		Pole I	Pole II	Not polar	Random
0	23				16
1	1	8	6		
1	1	10	4		
1	1	9	5		
1	1	7	7		
2	1	8	4		
2	2	7	5		
2	3	6	6		
3	4	5	5		
3	2	7	3		
4	3	6	2		
4	1	4	2	2	
4	1	4	3	1	
4	2	3	2	3	
4	1	5	3		
4	1	4	4		
5	4			6	
6	6			4	
7	5			2	
Total	63			19	23

equatorial region, at metaphase I, the orientation of univalents was influenced by the number of bivalents in the cell. When the number of bivalents was lower than 4, the univalents tended to be polar (Figs. 9–13, 20; Table 4). When there were more than four bivalents, the univalents remained much closer to the equator (Fig. 17; Table 4). With four bivalents the arrangement of the univalents was polar or incompletely so, or equatorial (Figs. 14–16, 18; Table 4). The univalents showing polar movement were not always distributed in equal numbers over the two poles (Figs. 9, 10, 12, 13, 20).

Drastic reduction in the number of chiasmata per bivalent was noticed. No ring bivalent was observed in the mutant, whereas in the control, on the average, 7.08 out of 7.92 bivalents were rings with at least two chiasmata per bivalent (Figs. 1, 2; Table 1). All the bivalents observed in the asynaptic mutant were rod bivalents with only one chiasma, which was terminal even at diplotene. As is evident, on the average there are 2.64 rod II and 10.71 I per cell at metaphase I, whereas the corresponding values for the control are 7.92 (7.08 ring + 0.84 rod) and 0.16 respectively (Table 1). It could not be ascertained whether the chiasma, in submetacentric chromosomes, was formed in long or short arms. Chiasma frequency per cell was 16.72 and 2.64 in control and asynaptic mutant respectively (Table 1).

Anaphase-I distribution (Figs. 22–27; Table 3) was quite abnormal and in 16% a few univalents were left at the equatorial region (Fig. 21; Table 3). In a few cells lagging bivalents were observed. Other abnormalities like bridge formation, chromosome fragmentation at anaphase I, observed in *Scilla* (Rees, 1952), *Allium* (Darlington & Haque, 1955), *Sorghum* (Magoon *et al.*, 1961) and precocious division of univalents at anaphase I were completely absent.

The PMCs which had no bivalents at prometaphase/metaphase I and the univalents randomly distributed, were distinguished from anaphase-I cells by the presence of separating chromatids in each chromosome, in the latter (Figs. 21–27). The separation, except at the centromeric region, was either complete (Figs. 22, 27) or was in the process of completion (Figs. 21, 23–26). The pollen fertility by carmine staining was found to be 35%. The pods generally had no seeds and the seed number never exceeded one per pod. Six seeds collected from the asynaptic plant in the M_1 generation were sown for an M_2 generation. They did not survive beyond the seedling stage, due to albinism. However, two seeds

were collected from a successful cross between the asynaptic and a normal plant. The F_1 was normal and in the F_2 out of the 7 plants analysed for male meiosis, 5 were normal and 2 were asynaptics (Figs. 28–30). These observations indicated that the asynapsis is controlled by a recessive gene.

Discussion

To ascertain whether the plant is asynaptic or desynaptic is rather difficult. Complete univalent formation at post-pachytene stages does not necessarily mean that the plant is asynaptic. The only reliable evidence comes from analysis of pachytene. It is worth bearing in mind that some cases of supposed asynaptics have in fact turned out to be desynaptics, after pachytene studies (Celarier, 1955).

At no stage did we observe any chiasmata in a bivalent. The association was always terminal (Figs. 5-7, 9-20) and even at diplotene/diakinesis, no clearly observable exchange points were noticed (Figs. 5–7). The only evidence that they are true bivalents comes from their arrangement at the equatorial region. However, the same behaviour has been reported for quasi-bivalents (Östergren & Vigfusson, 1953). The present observation makes it difficult to discriminate between ordinary and quasi-bivalents. As no arguments for the opposite can be presented, the bivalents are regarded as ordinary bivalents.

Levan (1940), Vig & Mehrotra (1965) and Bozzini & Martini (1971) attribute asynapsis to partial failure of synapsis. Similarly, in the present investigation, there is some reason to believe that the mutated gene does not govern chiasma formation but chromosome pairing. The pairing would be interrupted as soon as it starts at the ends of the chromosomes, with the result that we observed rod bivalents with terminal chiasmata even at early stages or univalents when chiasmata were not formed. The meiotic behaviour in the present material is thus considered to be due to (partial) asynapsis rather than desynapsis.

Univalents are often found to be randomly distributed at metaphase I (John & Lewis, 1965; Singh *et al.*, 1977; Dhesi *et al.*, 1973). During the present investigation it was observed that the orientation of univalents was either polar or they remained in the equatorial region, depending on the number of bivalents present in the cell. Similarly, Östergren & Vigfusson (1953), and Henderson (1962) also found that the distribution of univalents at metaphase I may be polar when there are few bivalents and equatorial when the bivalent number is higher. However, in the present material it was observed that when the number of bivalents is four (half of the zygotic number), the orientation of univalents was not influenced by the bivalents present in the cell. They may be polar or equatorial or both, in a cell. If polar movement of univalents in cells with low numbers of bivalents would result primarily from precocious separation of the cooriented chromosomes of bivalents, the number of bivalents should be lower at metaphase than at earlier stages. In fact, the number of chiasmata is significantly higher at metaphase than at diplotene-diakinesis. The reason for this is unknown, but it shows that polar movement is an autonomous function of the univalents. Such movement has been attributed to the concerted action of the centromere and one or more weaker neo-centromeres at or near the chromosome ends (Sybenga, 1981). It is not clear, however, whether in this material neo-centromeres actually operate.

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