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FUSION OF CENTROMERES AND STAR FORMATIONS AT PACHYTENE OF *CAJANUS* × *ATYLOSIA* HYBRIDS

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SUMMARY

A phenomenon where the centromeres of different bivalents fuse to form typical star-shaped configurations has been observed at the pachytene stage of three hybrids, *Cajanus cajan* × *Atylosia lineata*, *C. cajan* × *A. sericea* and *C. cajan* × *A. scarabaeoides*. The frequency of certain bivalent combinations forming stars was found to be higher than others, thereby, indicating the non-random nature of this phenomenon. Although no hexavalents, octovalents or more than one quadrivalent per cell during diakinesis and metaphase-I were observed, as were to be expected on the basis of the data obtained on the number of arms involved in the star-formations and the number of stars per cell at pachytene, the possibility that these stars are the result of reciprocal translocations cannot be ruled out. A definite relationship between the number of cells showing these star formations at pachytene and the number of cells showing bivalent associations at diakinesis and metaphase I on the one hand, and between the number of arms involved the stars and the number of chromosomes involved in each association on the other, signify that these stars are forerunners of secondary associations.

1. INTRODUCTION

During the course of our investigations on the interrelationships of *Cajanus cajan* (L.) Millsp. (Pigeonpea) and three *Atylosia* W. & A. species, as revealed by pachytene analysis, an interesting phenomenon was observed in all the hybrids, in which the centromeres of the bivalents fused together to form a typical star-shaped configuration during pachytene. Due to the lack of a proper term in the published literature, this configuration of bivalents is termed a "star". Such star formations are rarely reported in the plant kingdom and are analogous to the chromocentres that occur in certain dipteran insects. The present paper deals with the description and frequencies of the occurrence of such stars in the first generation hybrids of *C. cajan* × *A. lineata*, *C. cajan* × *A. sericea* and *C. cajan* × *A. scarabaeoides*. The relationship betweeen the formation of stars at pachytene and the formation of secondary associations at later stages is also indicated.

2. MATERIALS AND METHODS

The flower buds of *C. cajan* (variety T-21) × *A. lineata*, *C. cajan* × *A. sericea*, *C. cajan* × *A. scarabaeoides* and those of all the 4 parents were fixed in propionic acid and ethanol mixture (1:3) with the acid component saturated with ferric acetate. The buds were kept over-night in the fixative and then the anthers were stained in 1 per cent propiono carmine.

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All the eleven pachytene chromosomes of the parents, *C. cajan*, *A. lineata*, *A. sericea* and *A. scarabaeoides* were identified on the basis of their relative length, arm ratio, chromomere pattern and nucleolar associations and a detailed account of pairing behaviour of specific chromosomes of the parents in all the three hybrids have been reported (Reddy, 1981*a*, *b* and *c*). The chromosomes in all the 4 species belong to the differentiated type where the unstained centromeres are clearly flanked by proximal dark staining heterochromatic regions and distal light staining euchromatic regions. No telocentric chromosomes occur in these species.

The roman numeral denotes the chromosome number and the superscripts *C*, *L*, *S* and *Sc* indicate that they belong to *C. cajan*, *A. lineata*, *A. sericea* and *A. scarabaeoides* parents respectively. For example the bivalent V^C–IV^L is the one formed by the pairing of chromosome *V* of *C. cajan* and chromosome *IV* of *A. lineata* in the hybrid.

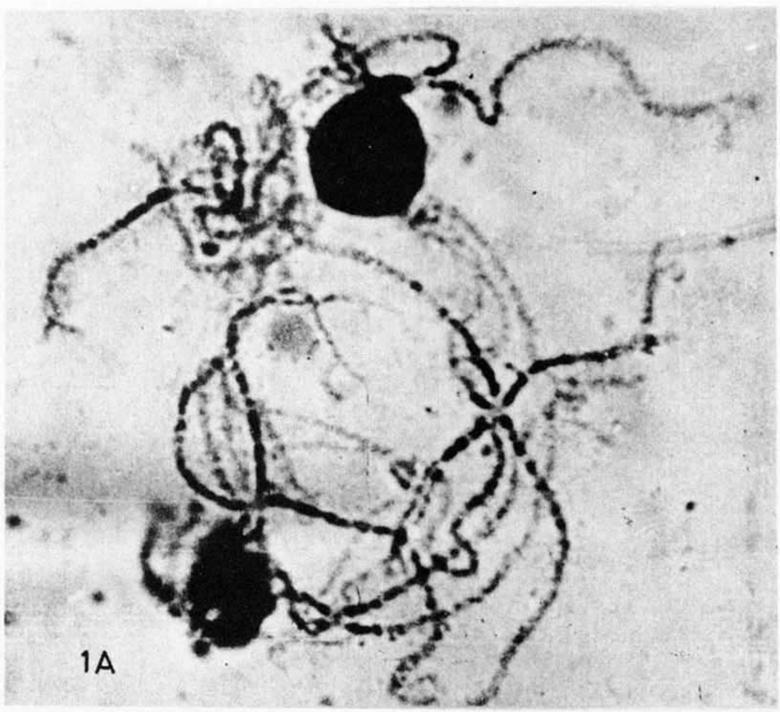
The pollen mother cells were scored for the occurrence of secondary associations based on the number of bivalent groups and the number of bivalents in each group as observed from polar view at diakinesis and metaphase-I. Microphotographs were taken of the cells which were pressed hard to improve the contrast between the cytoplasmic background and the chromosomes.

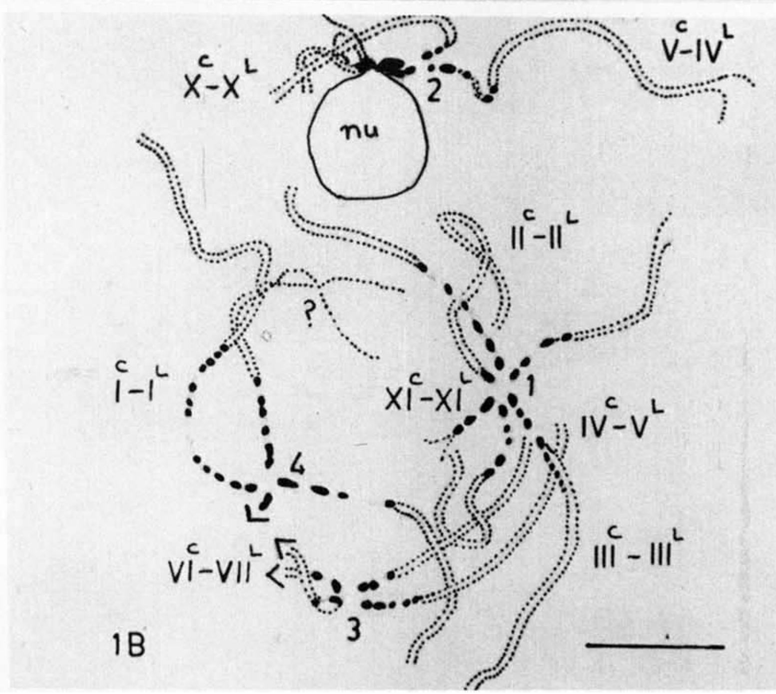
3. Results and discussion

Star formations at pachytene were not observed in any of the four parents although several hundreds of microsporocytes were screened at pachytene for each parent. However, in all the three hybrids *viz.*, *C. cajan* × *A. lineata*, *C. cajan* × *A. sericea* and *C. cajan* × *A. scarabaeoides* they were frequently encountered. A typical star consists of an unstained central region from which radiate arms of the bivalents with proximal heterochromatic segments and distal euchromatic regions. The central unstained region represents the fused centromeres of the bivalents. It is noteworthy that the proximal heterochromatic regions may or may not show pairing. In any hybrid, a single PMC contained one to four stars, and the maximum number of arms involved in a single star were 8 *i.e.*, 4 bivalents. In a given cell, in no case were all the eleven bivalents involved in star formation, the maximum number involved being 9 bivalents. The maximum number of stars observed per cell was four. Although the free bivalents could be identified rather easily, those involved in stars were difficult to identify. This is chiefly due to clumping of the arms. The nature and frequency of stars at pachytene and those of secondary associations at diakinesis and metaphase-I of all the three hybrids are given in table 1.

(i) *C. cajan* × *A. lineata*

At pachytene, 25·9 per cent of the cells that could be analysed exhibited stars. The observed percentages of the 4 arm-, 6 arm- and 8 arm-stars were 14·5, 10·1 and 6·3 respectively (table 2). It was possible to identify the specific chromosomes in a number of stars, a good example of which is given in plates 1(a) and 1(b). This cell contains 4 stars. The biggest star is with 6 arms involving 3 chromosomes namely, bivalents III^C–III^L, II^C–II^L



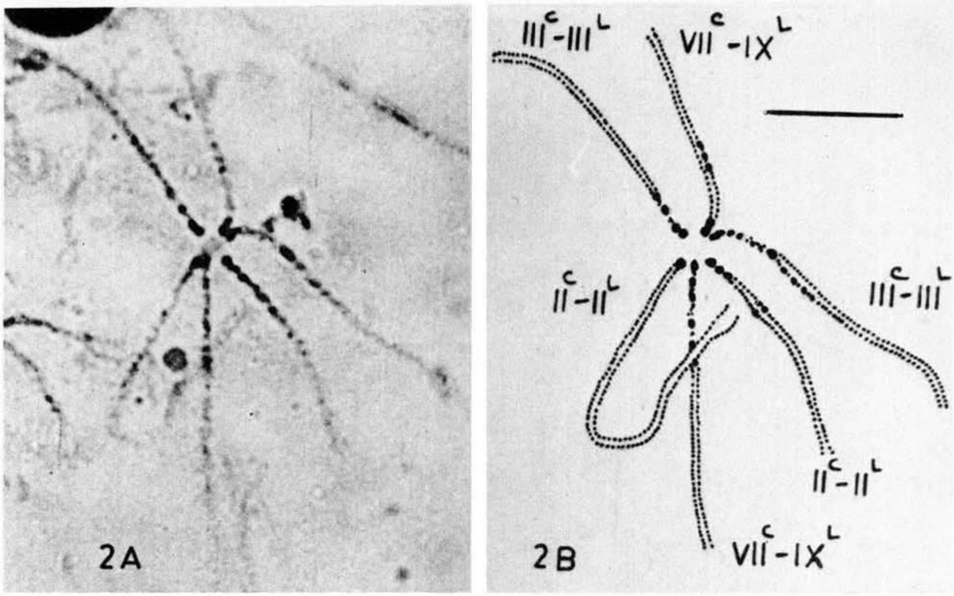


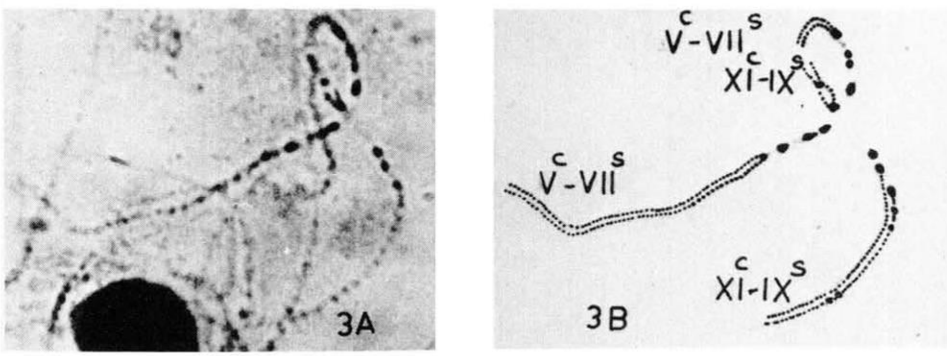
PLATES 2(a) and 2(b). Photograph (a) and drawing (b) of a meiocyte at pachytene stage of *Cajanus cajan* × *A. lineata* hybrid showing a star with 6 arms involving 3 bivalents, III^C–III^L, II^C–II^L and VII^C–IX^L.

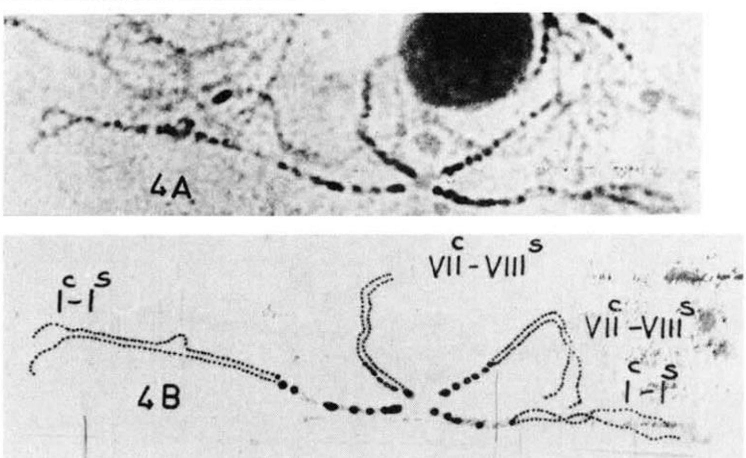
PLATES 3(a) to 4(b). Photographs (a) and drawings (b) of meiocytes of *Cajanus cajan* × *A. sericea* hybrid showing star formations at pachytene.

PLATES 3(a) and 3(b). A star involving the two bivalents, V^C–VII^S and XI^C-IX^S.

PLATES 4(a) and 4(b). A star involving the two bivalents I^C-I^S and VII^C-$VIII^S$. Bar = 10μ.







PLATES 2(a) and 2(b). Photograph (a) and drawing (b) of a meiocyte at pachytene stage of *Cajanus cajan* × *A. lineata* hybrid showing a star with 6 arms involving 3 bivalents, III^C–III^L, II^C–II^L and VII^C–IX^L.

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PLATES 3(a) and 3(b). A star involving the two bivalents, V^C–VII^S and XI^C-IX^S.

PLATES 4(a) and 4(b). A star involving the two bivalents I^C-I^S and VII^C-$VIII^S$. Bar = 10μ.

TABLE 1

Analysis of stars at pachytene and secondary associations at diakinesis and metaphase-I in Cajanus × Atylosia *hybrids*

S. No.	*Configuration	No. of Cells for each type in					
		C. cajan × *A. lineata*		*C. cajan* × *A. sericea*		*C. cajan* × *A. scarabaeoides*	
		A	*B*	*A*	*B*	*A*	*B*
1.	11*F*	117	179	111	192	123	178
2.	9*F*+(2)	11	21	11	16	10	11
	8*F*+(3)	8	15	14	21	13	18
	7*F*+(4)	7	12	8	9	8	13
3.	7*F*+(2)+(2)	4	7	3	4	3	5
	6*F*+(3)+(2)	2	5	6	8	3	8
	5*F*+(4)+(2)	2	2	3	5	2	5
	5*F*+(3)+(3)	2	4	—	2	2	12
	4*F*+(4)+(3)	1	3	—	—	4	9
	3*F*+(4)+(4)	—	—	—	—	—	5
4.	5*F*+(2)+(2)+(2)	1	1	1	—	1	2
	4*F*+(3)+(2)+(2)	—	2	1	3	1	4
	3*F*+(4)+(2)+(2)	—	—	2	—	1	—
	3*F*+(3)+(3)+(2)	1	—	—	—	1	—
	2*F*+(4)+(3)+(2)	—	—	—	—	2	—
5.	3*F*+(2)+(2)+(2)+(2)	—	—	1	—	3	—
	2*F*+(3)+(2)+(2)+(2)	2	—	2	—	2	1
Total No. of Cells analysed		158	251	163	260	179	271

* *F* = Free bivalents. Figures in parentheses indicate number of bivalents involved in the star formation and bivalents involved in secondary associations. *A* = At Pachytene; *B* = At diakinesis and metaphase-I.

and XI^C-XI^L. The other three stars, each with 4 arms involving 2 chromosomes, are made of bivalents V^C-IV^L and X^C-X^L, bivalents IV^C-V^L and VI^C–VII^L and bivalents I^C–I^L and an unidentified one.

In another cell (plates 2(a) and 2(b)) a star with 6 arms involving 3 chromosomes, namely bivalents III^C–III^L, II^C-II^L and VII^C–IX^L was seen. It was noticed that the following three combinations occur more frequently: (i) Bivalents II^C–II^L and VII^C–IX^L, (ii) Bivalents V^C–IV^L and X^C–X^L, (iii) Bivalents I^C–I^L and XI^C–XI^L.

At diakinesis and metaphase-I secondary associations resulting in distinct groups of bivalents were observed in 28·6 per cent of the cells (plate 6).

(ii) *C. cajan* × *A. sericea*

Star formations were seen in 31·9 per cent of the cells that were analysed. The 4 arm-, 6 arm- and 8 arm-stars were observed in 18·4, 14·7 and 6·7 per cent of cells respectively (table 2). The stars with the following bivalent combinations were frequently found in the pachytene of the hybrid: (i)

TABLE 2

Percentage of star formations at pachytene and secondary associations at diakinesis and metaphase-I in Cajanus × Atylosia *hybrids*

S. No.	Description	*C. cajan* × *A. lineata*	*C. cajan* × *A. sericea*	*C. cajan* × *A. scarabaeoides*
1(a)	Cells showing one or more stars	25·9	31·9	31·2
(b)	Cells showing one or more secondary associations	28·6	26·2	34·3
2(a)	Cells with 4 armed stars	14·5	18·4	16·2
(b)	Cells with secondary associations of 2 bivalents	15·1	13·8	13·2
3(a)	Cells with 6 armed stars	10·1	14·7	15·6
(b)	Cells with secondary associations of 3 bivalents	11·5	13·0	19·1
4(a)	Cells with 8 armed stars	6·3	6·7	9·4
(b)	Cells with secondary associations of 4 bivalents	6·7	5·3	11·8
	Correlation coefficient between star formations and secondary associations	0·998**	0·994**	0·957*

** Significant at 1% level; * Significant at 5% level.

Bivalents V^C–VII^S and XI^C-IX^S (plates 3(a) and 3(b)), (ii) Bivalents I^C–I^S and VII^C–$VIII^S$ (plates 4(a) and 4(b)), (iii) Bivalents II^C–II^S and VII^C–VII^S.

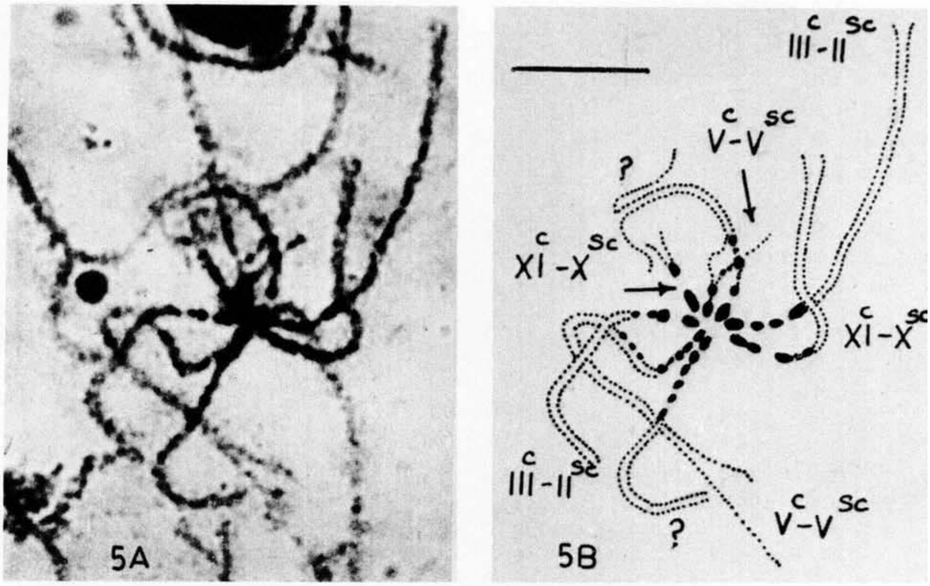
Secondary associations of bivalents were observed in 26·2 per cent of the microsporocytes during diakinesis and metaphase-I (plate 7).

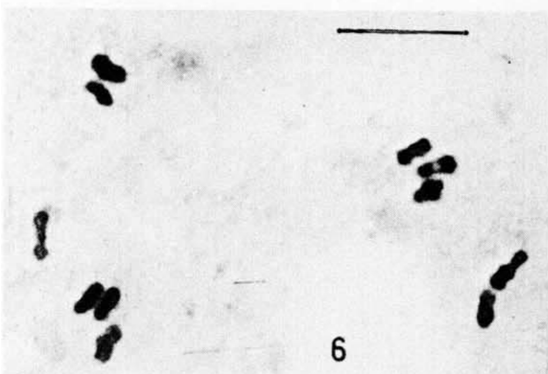
(iii) *C. cajan* × *A. scarabaeoides*

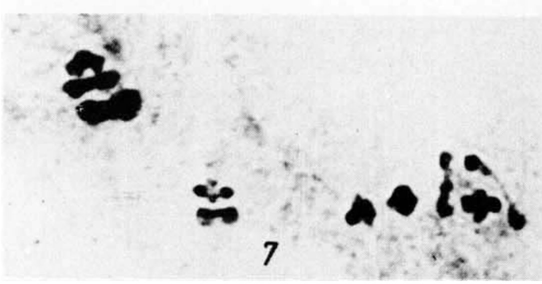
At pachytene 31·2 per cent of the cells analysed showed star formations. The observed percentage of 4 arm-, 6 arm- and 8 arm-stars were 16·2, 15·6 and 9·4 respectively (table 2). It was possible to identify the stars with the following bivalent combinations (i) Bivalents III^C–II^{Sc}, V^C–V^{Sc}, XI^C–X^{Sc} and an unidentified one (plates 5(a) and 5(b)), (ii) Bivalents VI^C-VII^{Sc} and $VIII^C$–$VIII^{Sc}$.

At diakinesis and metaphase-I secondary associations were observed in 34·3 per cent of the cells (plate 8).

The phenomenon of star formation could not be detected in any of the parents, whereas it occurred with a relatively high frequency in the hybrids. The maximum percentage of cells were stars was noticed in *C. cajan* × *A. sericea* hybrid followed by *C. cajan* × *A. scarabaeoides* and *C. cajan* × *A. lineata* hybrid successively. The variations observed in the nature and frequencies of star formations in different hybrids must be attributable to their respective *Atylosia* parents since the *Cajanus* parent (variety T-21) is common to all the three crosses. From the central point of the fused centromeres four to eight arms' radiate giving a typical star shaped configuration. In the salivery gland chromosomes of *Drosophila*, the heterochromatic regions near the centromere fuse together and form a chromocentre







PLATES 5(a) and 5(b). Photograph (a) and a drawing (b) of a meiocyte of *Cajanus cajan* × *A. scarabaeoides* hybrid at pachytene stage showing a star involving 4 bivalents, III^{C}–II^{Sc}, V^{C}–V^{Sc}, XI^{C}–X^{Sc} and an unidentified one (?). Bar = 10μ.

PLATE 6. Metaphase *I* of *C. cajan* × *A. lineata* showing secondary associations of bivalents: $4F+(2)+(3)+(2)$.

PLATE 7. Metaphase *I* of *C. cajan* × *A. sericea* showing secondary associations of bivalents: $5F+(4)+(2)$.

PLATE 8. Metaphase *I* of *C. cajan* × *A. scarabaeoides* showing secondary associations: $5F+(2)+(2)+(2)$. Bar = 10μ.

from which the chromosome arms radiate. This is analogous to what has been found in *Cajanus* × *Atylosia* hybrids. Jakob (1957) reported H-and cross-shaped configurations of four chromosomes (two bivalents) at pachytene of *Ricinus*, which are analogous to the present star formation. He found a correspondence between these interchromosomal associations at pachytene and the secondary associations at diakinesis. He concluded that these secondary associations involving different chromsome pairs were the consequence of the fusion of the chromosomes at centromeres and/or due to the exchange of portions of pairing strands affecting two of the four chromosomes involved in interchromosomal association without the formation of chiasmata in the regions of interchromosomal contact. Although no mention was made of stars by Menzel (1962), one of her illustrations of pachytene nuclei of PMCs of allotetraploid *Lycopersicon esculentum* × *Solanum lycopersicoides* shows such a star with 4 arms. In the present study no hexavalents, octovalents or more than one quadrivalent per cell were observed during diakinesis and metaphase-I, which would be expected on the basis of the data on the number of arms involved in the star formations and the number of stars per each cell at pachytene if these star formations are the result of reciprocal translocations. There are reports in *Phleum* (Muntzing and Prakken, 1940; Nordenskiold, 1961) that when the homology of the chromosomes should permit the formation of multivalents, special mechanisms restrict the resulting configurations to bivalents. In a similar manner it would theoretically possible that the stars in pachytene might appear as bivalents in metaphase-*I*, as a consequence of interference in the crossing over process causing a very restricted number and arrangement of chiasmata.

There is a definite relationship between the number of cells showing stars at pachytene and the number of cells showing secondary associations at later stages on the one hand and the number of arms involved in stars and the number of bivalents involved in the secondary associations on the other (table 2). Correlation coefficients between star formations at pachytene and secondary associations at the later stages were significant in all the three crosses and over all the crosses (0·939**). Hence, it is concluded that the star formations are fore-runners of secondary associations.

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4. References

JAKOB, K. M. 1957. Secondary associations in the castor oil plant. *Cytologia*, *22*, 3–4, 380–392.

MENZEL, M. Y. 1962. Pachytene chromosomes of the intergeneric hybrid *Lycopersicon esculentum* × *Solanum lycopersicoides*. *Amer. J. Bot.*, *49*, 605–615.

MUNTZING, A. AND PRAKKEN, R. 1940. The mode of chromosome pairing in *Phleum* twins with 63 chromosomes and its cytogenetic consequences. *Hereditas*, *26*, 463–501.

NORDENSKIOLD, H. 1961. The occurrence of chlorophyll—deficient seedlings after isolation in two consecutive generations of cultivated timothy (hexaploid *Phleum pratense*). Annals Toral Agricultural College of Sweden 27, 361–383.

REDDY, L. J. 1981*a*. Pachytene analysis in *Cajanus cajan*, *Atylosia lineata* and their hybrid. *Cytologia*, *46*, 397–412.

REDDY, L. J. 1981*b*. Pachytene analysis in *Atylosia sericea* and *Cajanus cajan* × *A. sericea* hybrid. *Cytologia*, *46*, 568–578.

REDDY, L. J. 1981*c*. Pachytene analysis in *Atylosia scarabaeoides* and *Cajanus cajan* × *A. scarbaeoides* hybrid. *Cytologia 46*, 579-589.