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CYTOGENETIC STUDY OF VARIOUS TYPES OF TEA (*CAMELLIA SINENSIS*) CULTIVARS IN IRAN *

M. SHEIDAI[1**] H. JAHANBAKHT[2]
AND P. SOFI-SIYAVASH[3]

[1] Biology Department, Shahid Beheshti University, Tehran, I. R. of Iran
[2] Genetic Department, Azad University, Tehran, I. R. of Iran
[3] Tea Research Institute, Lahijan, I. R. of Iran

Abstract – Tea (Camellia sinensis) is one of the most economically important crops and is considered to be the national drink of Iran. Although there has been an intensive cytogenetic study of tea in different regions of the world, there has been no report from Iran. Therefore, the present investigation was carried out in order to present the basic cytogenetical features of various tea cultivators grown in the country. The meiotic analysis of the tea cultivators studied showed variation in the prophase sub-stage of meiosis-I, due to the occurrence of a synezetic knot and post pachytene diffuse sub-stage. All tea cultivators studied were diploid, with the chromosome number n=15. Although the major feature of chromosome association was bivalent formation, quadrivalents were formed in the cultivator 3013, possibly due to structural hybridity. The ANOVA test showed the presence of a significant difference among the cultivators for terminal, intercalary and total chiasmata, as well as ring bivalents indicating their genomic differences. Cluster analysis and PCA ordination of cultivators which are based on meiotic characteristics, group those cultivators which show cytogenetic similarities.

Keywords – Tea, cytogenetic, chiasma frequency, cluster analysis

1. INTRODUCTION

Tea (Camellia sinensis) is one of the most economically important crops and is considered to be the national drink in Iran. It belongs to the genus Camellia (Family Theaceae), which possesses about 82 species distributed mainly in Eastern Himalaya, Nepal, Bhutan, Bangladesh and East Africa.

Although there has been intensive cytogenetic study of tea in different regions of the world [1-4], there has been no report from Iran. The occurrence of natural polyploids, including triploid and tetraploid and pentaploid among tea cultivars/ populations [5, 6], adds to the importance of cytogenetical investigation of the cultivars available in Iran. Moreover, the cytogenetic studies performed on tea cultivars are mainly confined to karytypic analysis, while the present study considers the meiotic analysis and chromosome pairing.

Therefore, the present investigation was carried out to present the basic cytogenetical features of some tea cultivars grown in the country with a look at the chromosome number, meiotic behavior and any other cytogenetical peculiarities present.

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**Corresponding author

2. MATERIALS AND METHODS

The cultivars studied were grown in the agricultural fields of the Tea Research Center, Lahijan, Iran. For cytogenetical studies, suitable flower buds were obtained from six cultivars (Table 1), including cultivars 3020, 3013, B275 and DG39 (imported from Seri Lanka), cultivar 100 (cultivated in Iran) and the hybrid cultivar X, (hybrid of Chinese and Cambodian cultivars now cultivated in Iran).

Table 1. Tea cultivars and their meiotic characteristics

Cultivar	TX	IX	TOX	RB	ROB	TXN	IXN	TOXN	RBN	ROBN
3013	15.41	2.82	18.23	8.00	7.36	1.03	0.19	1.22	0.53	0.49
3020	17.63	2.25	19.88	8.00	6.00	1.18	0.15	1.33	0.53	0.40
B275	15.05	3.50	18.45	9.60	5.40	1.00	0.23	1.23	0.64	0.36
100	17.87	1.87	19.73	11.50	4.50	1.19	0.12	1.32	0.77	0.30
X	20.60	2.00	22.60	7.80	7.20	1.37	0.13	1.51	0.52	0.48
DG39	17.50	4.50	22.00	9.25	5.75	1.17	0.30	1.47	0.62	0.38

The young flower buds were collected from 8 to 11 A.M. and fixed for 24 hrs in glacial acetic acid: ethanol 70 % (1:3), then washed and preserved in ethanol 75 % until used [7, 8]. The Squash technique was used for cytological preparations with 2 % acetocarmine as the stain.

The cytogenetic characteristics studied include the chromosome number and ploidy level, chromosome pairing (i.e. bivalent, univalent and quadrivalent formation), chiasma frequency and distribution, as well as chromosome segregation. Chromosome pairing and chiasma frequency were determined from a minimum of 100 meiocytes showing diakinesis/ metaphase-I stages, while chromosome segregation was studied in a minimum of 500 anaphase-I and II stages.

Some variation was observed in the meiosis-I prophase substages which were also studied in detail. For determining the sequence of these substages, a flower sized gradient was used.

Pollen stainability as a measure of fertility was determined by staining a minimum of 1000 pollen grains with 2 % acetocarmine: 50 % glycerin (1:1) for about ½ hr. Round/ complete pollens which were stained were taken as fertile, while incomplete/ shrunken grains with no stain were considered as infertile [8].

In order to determine whether the cultivars differ significantly in their cytogenetic characteristics, an analysis of variance (ANOVA) test was performed using a completely randomized design (CRD) followed by the least significant difference test [8].

UPGMA (unweighted paired group mean average) cluster analysis, as well as ordination based on principal components analysis (PCA), was performed to identify the cultivars showing similarities in their meiotic characteristics [15]. For cluster and principal components analysis, standard values (mean = 0, variance = 1) were used. Euclidean distance was used as a measure of similarity in cluster analysis [8].

In those cultivars possessing B-chromosomes, a t-test analysis was performed to determine the difference in meiotic characteristics among the cells possessing B-chromosomes and those devoid of Bs [9]. Analysis was performed on at least 50 meiocytes collected randomly from 50 different flower buds.

In order to determine any effect of meiotic characters on pollen fertility, the Pearson coefficient of correlation was determined [8]. Statistical analyses used SPSS ver.9 (1998, SPSS Inc.) software.

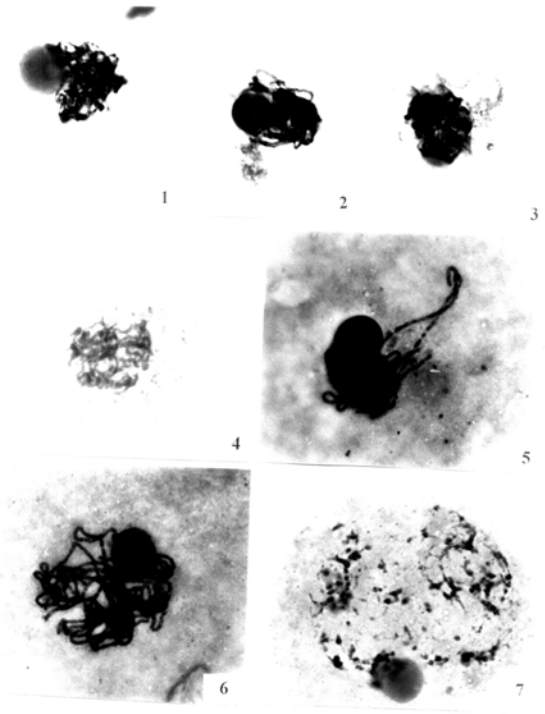
3. RESULTS AND DISCUSSION

a) Sub-stages of prophase in meiosis-I

The meiotic analysis of the tea cultivars studied showed variation in the prophase sub-stages of meiosis-I (Figs. 1-7). The first sub-stage was the occurrence of a synezetic knot stage instead of leptotene and zygotene stages. In the early synezetic knot stage, thin chromatin strands surround the nucleolus and eventually covering it completely (Figs. 1-4). Later on, paired chromosomes (now thick strands) unraveled from the knot (Fig. 5), entering the pachytene stage (Fig. 6). End-to-end attachment of chromosomes in pachytene is a feature reported in those taxa showing a synezetic knot stage [8].

Despiralization of the chromosomes occurs after pachytene, commencing with the diffuse stage (Fig.7). The occurrence of the diffuse stage has been reported in several plant species [10]. It may be of the complete type in which whole chromosomes undergo decondensation, or it may be partial, where some part of the genome undergoes decondensation. The present study showed the occurrence of an almost complete diffuse stage in *Camellia sinensis*.

A variety of reasons have been suggested for the occurrence of the diffuse stage: high synthetic activity analogous to the lampbrush stage in amphibian oocytes; shedding of the lateral elements of the synaptonemal complex; the post pachytene elimination or modification of histone proteins; meiotic arrest to withstand adverse environmental conditions [8]. The exact reason for the occurrence of a diffuse stage in tea cultivars is not known.



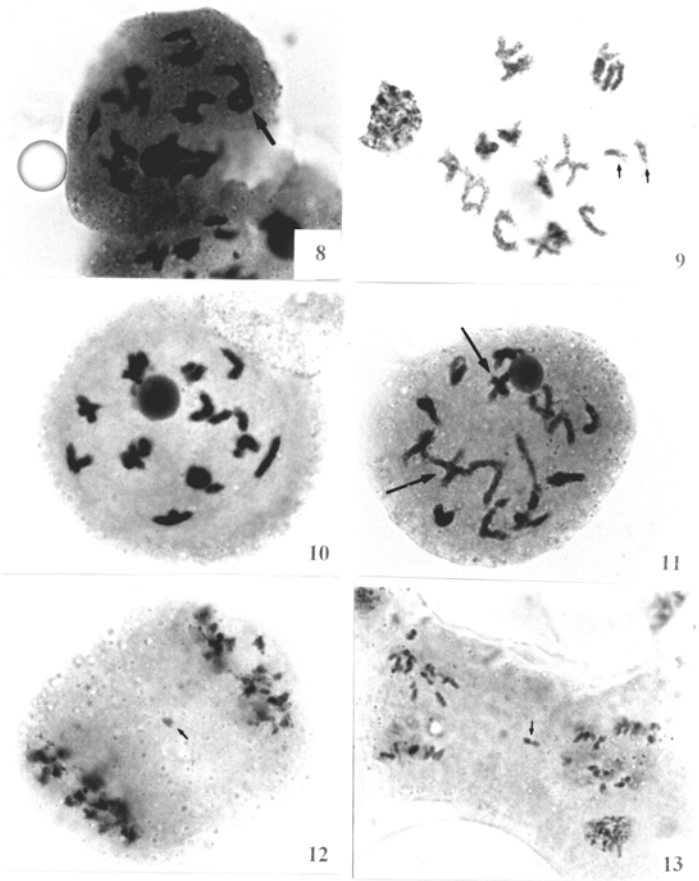
Figs. 1-7: 1-4 = Early to complete synezetic knot stage in cultivar 3020
5 = Unraveling of paired chromosomes from the knot in cultivar 3020
6 = Pachytene stage in cultivar 3020
7 = Diffuse stage in hybrid cultivar Cambodia × Chinese

b) Chromosome pairing and chiasma frequency

Data on meiotic chromosome number and chiasma frequency, as well as chromosome pairing, are presented in Table 1 (Figs. 8-11). All cultivars were diploid, with the chromosome number n = 15. The hybrid cultivar Cambodia × Chinese possessed the highest values of the total (22.60), terminal (20.60) and intercalary chiasmata (2.00), while cultivars 3013 and B275 possessed the lowest values of the total (18.23 and 18.45, respectively) and terminal chiasmata (15.41 and 15.05, respectively). The highest value of intercalary chiasmata occurred in DG39 (4.50), followed by B275 (3.50).

Although the major feature of chromosome association was bivalent formation, quadrivalents were formed in the cultivar 3013, possibly due to structural hybridity. Irregular separation of these quadrivalents could explain the reduced pollen fertility of this cultivar.

Laggard chromosomes were occasionally observed in anaphase-I and II, as well as telophase-I and II stages, leading to micronuclei formation (Figs. 12 & 13). Such laggard chromosomes may have played a role in the reduction of pollen fertility in the cultivars studied.



Figs. 8-12: 8 = Meiocyte showing a single quadrivalent (arrow) in cultivar 3013
9 = Meiocyte showing bivalents and univalents (arrows) in 3020
10 = Meiocyte showing 15 bivalents in 3013
11= Meiocyte showing bivalents with intercalary chiasma (arrow) in 3013
12 = Telophase-I laggard chromosome in hybrid cultivar Cambodia × Chinese
13 = Anaphase-I laggard chromosome in cultivar DG39

The ANOVA test, followed by LSD performed for meiotic characteristics, showed the presence of a significant difference among the cultivars ($p < 0.01$) for terminal, intercalary and total chiasmata, as well as ring bivalents (Table 2).

Table 2. ANOVA test for meiotic characters among the tea cultivars

		Sum of Squares	df	Mean Squares	F	Sig.
TX	Between Groups	53.419	5	10.684	285.792	.001
	Within Groups	.449	12	3.738E-02		
	Total	53.868	17			
IX	Between Groups	14.886	5	2.977	212.907	.001
	Within Groups	.168	12	1.398E-02		
	Total	15.054	17			
TOX	Between Groups	44.492	5	8.898	145.729	.001
	Within Groups	.733	12	6.106E-02		
	Total	45.224	17			
RB	Between Groups	42.549	5	8.510	1.849	.178
	Within Groups	55.235	12	4.603		
	Total	97.784	17			
ROB	Between Groups	17.831	5	3.566	1506.819	.001
	Within Groups	2.840E-02	12	2.367E-03		
	Total	17.859	17			

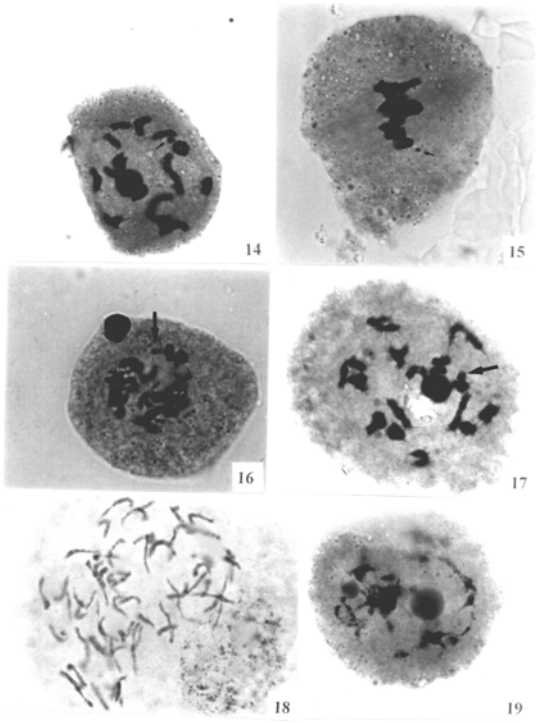
A significant difference among the cultivars for rod bivalents was not shown, but the LSD test did show a significant difference between cultivars 3013 and 3020.

Variation in chiasma frequency and localization is genetically controlled [11, 12] and has been reported in several plant species as well as in crop plant varieties [8, 13, 14]. Such a variation in the species/ populations with the same chromosome number is considered as a means for generating new forms of recombination, influencing the variability within natural populations in an adaptive way [14]. Differences observed in chiasma frequency and distribution in the studied tea cultivars constitute an aspect of their genomic differences, as these plants were grown under uniform conditions in the experimental field. Such genomic variations, if combined with the other characteristics, can be used for genetic and breeding purposes. It should also be added that the cultivars studied all possessed characters of a standard tea including tanin contents, total ash content, percentage of water extract, etc.

c) *B-chromosomes*

One to 2 B-chromosomes (Bs) were observed in the cultivars 3020, 3013, DG39 and hybrid Cambodia × Chinese (Figs. 14-17). These were smaller than the A-chromosomes and did not form any meiotic association with them, although they were able to arrange themselves along with the A-chromosomes on the equatorial plane of the spindle and move to the poles during anaphase.

B-chromosomes are accessory chromosomes reported in many plant and animal species. They show numerical polymorphism and when present in high number, negatively affect the growth and vigor of the plants [18], while in low number they may benefit the plant possessing them [19]. The Bs may affect the frequency and distribution of chiasmata as well as chromosome association, either directly or by affecting the genes present on the A-chromosomes that control meiosis [20].



Figs. 14-17 = B-chromosomes (arrow) in cultivars 3013 (14 & 15), DG39 and 3020, respectively
18- Desynsptic cell in hybrid cultivar Cambodia × Chinese
19 = Meiocyte showing two nucleoli in hybrid cultivar Cambodia × Chinese

d) Accessory nucleoli and desynapsis in hybrid cultivar Cambodia × Chinese

Desynaptic cells were also observed in the cultivar Cambodia × Chinese (Fig.18). Such cells did not possess any bivalents and only univalents were formed. Desynapsis is known to be controlled by DS genes affecting chiasma formation [17]. The hybrid nature of cultivar Cambodia × Chinese may have activated these genes, forming desynaptic cells. However the frequency of such cells was low and the reason for this is not known.

One of the interesting cytogenetic features observed in the hybrid cultivar Cambodia × Chinese was the occurrence of two nucleoli in its meiocytes, which differed in size (Fig. 19). The number of nucleoli in meiocytes is considered to be constant in a particular species and corresponds to the number of secondary constrictions present in the haploid chromosome complement [10]. In a majority of plant and animal cells only one nucleolus is present, but in a few instances more than one has been reported [15].

Different reasons have been provided for the occurrence of extra nucleoli in meiocytes, including the breakage of the nucleolar organizing region by a translocation, followed by independent activity of the broken parts and hybrid nature of the organism [10]. The hybrid nature of cultivar Cambodia × Chinese seems to be the reason for the occurrence of accessory nucleoli in this cultivar. Possibly the SAT-chromosomes of the parent plants of this hybrid are active and function independently, each to produce a nucleolus in the hybrid plant.

Miller and Brown [16] have shown that the difference in the size of accessory nuclei corresponds to the difference in the size of the secondary constrictions and the number of ribosomal RNA genes

per genome. Therefore, we may suggest that the parental genomes of cultivar Cambodia × Chinese also differ in the size of their secondary constrictions.

Cluster analysis and PCA ordination of the cultivars based on meiotic characteristics produced similar results (Figs. 20-22), separating the cultivars from each other due to their difference in meiotic characters, thus supporting the ANOVA test.

Factor analysis of meiotic data revealed that the first three PCA components comprise about 98 % of total variance. In the first component, which comprises about 38% of total variance, mean number of rod bivalents possessed the highest positive correlation (>0.90), while in the second component, with about 36 % of total variance, total chiasmata and terminal chiasmata possessed the highest correlation (>0.90). Therefore, these meiotic characters may be considered as the most variable characters among the tea cultivars. The plot of the first two PCA components separates all the cultivars from each other, supporting their cytogenetical distinctness (Fig. 20). In the third component, which comprises about 23 % of total variance, intercalary chiasma frequency possessed the highest correlation (>0.99). This meiotic character separates cultivar DG39 from the others due to its difference in intercalary chiasmata (Fig. 21).

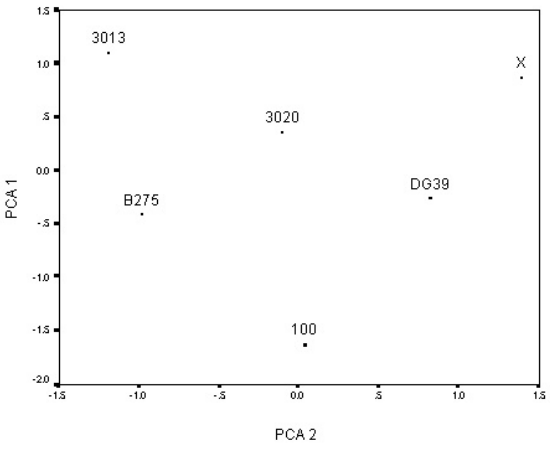


Fig. 20

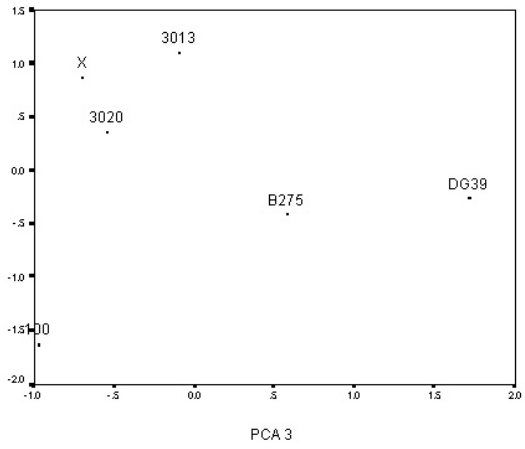
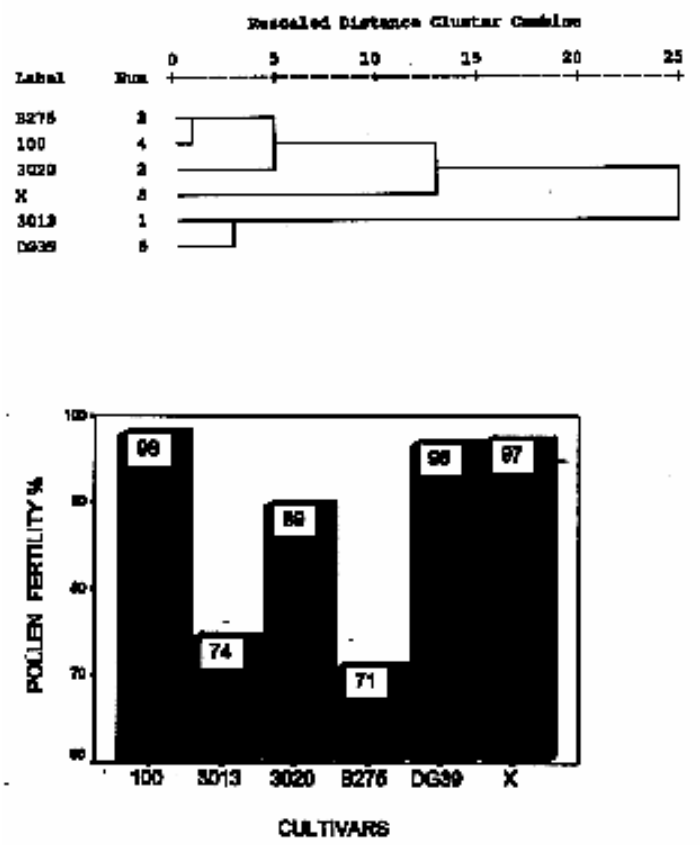


Fig. 21

Figs. 20 & 21: PCA ordination of tea cultivars

The percentage of pollen fertility in the studied plants is presented in Fig. 23. The cultivars 100, DG39 and hybrid Cambodia × Chinese showed a high pollen fertility, while cultivars 3013 and B275 showed a lower pollen fertility. Coefficient of correlation determined among cytogenetical characters and pollen fertility revealed a positive significant correlation between terminal and total chiasma and pollen fertility (Table 3). Therefore, with an increase in terminal and total chiasma, an increase in pollen fertility occurs in tea cultivars which may be considered in the planning of selection and breeding programs. From the present data we may suggest crossing between the cultivars 100, DG39 and hybrid Cambodia × Chinese, due to their high pollen fertility and high mean frequencies of terminal as well as total chiasmata. They also possess standard chemical properties explained before (unpublished data).



Figs. 22 & 23: UPGMA cluster analysis and pollen fertility in tea cultivars

Table 3. Correlation among pollen fertility (PF) and meiotic characters

	TX	IX	TOX	RB	ROB	TXN	IXN	TOXN	RBN	ROBN	PF
IX	-.45										
TOX	.86*	.54									
RB	-.16	.01	-.18								
ROB	.16	-.07	.15	-.89*							
TXN	1.00*	-.45	.86*	-.16	.16						
IXN	-.45	1.00	.05	.01	-.07	-.45					
TOXN	.86*	.05	1.00	-.18	.15	.86*	.05				
RBN	-.16	.01	-.18	1.00	-.89*	-.16	.01	-.18			
ROBN	.16	-.07	.15	-.89*	1.00	.16	-.07	.15	.59		
PF	.84*	-.23	.81*	.20	-.21	.84*	-.23	.81*	-.69	-.21	

* = Significant at 0.05 level

The effect of B-chromosomes was studied by comparing the meiotic characteristics of cells possessing Bs and those devoid of Bs in the cultivars 3020 and 3013 in which many meiocytes with B- chromosomes were obtained. T-test analysis showed a significant reduction in total and intercalary chiasmata, as well as ring and rod bivalents in these cultivars (Fig. 24). Transmission of B-chromosomes and their effect on tea cultivars will be investigated in further studies following crossing among cultivars with and without B-chromosomes.

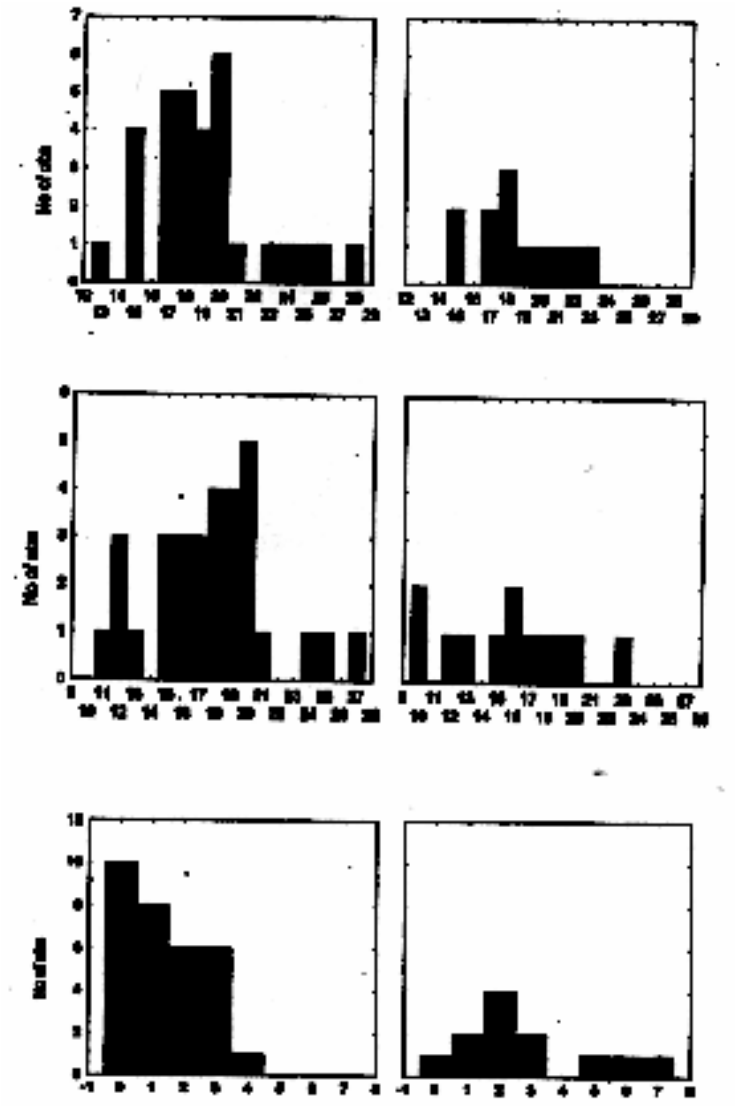


Fig. 24. T-test histograms comparing total, terminal and intercalary chiasma frequency (top to bottom) between meiocytes possessing B-chromosomes (right side) and devoid of Bs (left side) in cultivar 3020

NOMENCLATURE

TX	Terminal chiasmata
IX	Intercalary chiasma
TOX	Total chiasma
RB	Rod bivalent
ROB	Rod bivalent
TXN	Terminal chiasma/bivalent
IXN	Intercalary chiasma/bivalent
TOXN	Total chiasma/bivalent
RBN	Rod bivalent/haploid chromosome number
ROBN	Rod bivalent/ haploid chromosome number

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