BIOCHEMICAL AND CYTOLOGICAL STUDIES OF GENETIC TRANSFER FROM THE M^V GENOME OF *AEGILOPS VENTRICOSA* INTO HEXAPLOID WHEAT. A PROGRESS REPORT

A. DELIBES, R. SANCHEZ-MONGE AND F. GARCIA-OLMEDO

Departamento de Bioquímica
E.T.S.Ingenieros Agrónomos
Madrid-3, Spain

SUMMARY

A double interspecific cross {Triticum turgidum (AB) var. rubroatrum H-1-1 x Ae. ventricosa (DM^V) AP-1} x T.aestivum (ABD) cv. Almatense H-10-15 was carried out in 1950 by M. Alonso Peña (Cuenca, Spain) and 70 lines were derived from it by repeated selfing (lines H-93-1 through 70). Preliminary biochemical evidence indicated genetic transfer from the M^V genome of Ae.ventricosa into some of these lines. A more detailed biochemical and cytological characterization of the H-93- lines was undertaken. A progress report of these studies is presented here.

Fourten biochemical systems, each representing a set of up to 4 homoeologous loci, were investigated in the parental material, in the H-93- lines, and in Ae.squarrosa (DD), Ae. comosa (MM) and Ae. uniaristata (M^uM^u).

Biochemical markers controlled by the A or B genomes of one or both wheat parents were distributed in the H-93- lines as expected if the egcells from the self-sterile $ABDM^V$ hybrid, rescued by the ABD polen, carried the complete A and B genomes from T. turgidum.

The distribution of biochemical markers controlled by the D genomes of one or both D genome parents indicated that most of the eggcells from the $ABDM^V$ hybrid carried most of the D genome, i.e. 3 out of 8 markers of the former type were absent in a few lines each, indicating incomplete homology between the two D genomes, non-homologous transfer or deletion.

Biochemical characters present in Ae. ventricosa (DM^V), Ae. comosa (M), Ae. uniaristata (M^u) and absent in T. aestivum (ABD), Ae. squarrosa (D) and T. turgidum (AB) were selected as M^V genome markers. Two of these markers were not transmited to the H-93- lines, three were transmitted with low frequency and one with a high frequency. Resistance to Erisiphe graminis was determined by Dosba and Doussinault at Rennes and was found to be transmitted with low frequency.

Somatic chromosome numbers of the H-93- lines were counted and all were found to be hexaploid. Meiosis was studied in lines carrying M^V genome markers and in their hybrids with the T. aestivum parent, to determine the maximum number of alien chromosomes present in each line.

The joint consideration of the biochemical and the cytological evidence seems to indicate that the genetic transfer has taken place by chromosome substitution and by recombination.

INTRODUCTION

The genetic transfer of characters from *Aegilops ventricosa* (genomes $DDM^V M^V$) to hexaploid wheat, *Triticum aestivum* (AABBDD), has been recognized of practical interest for a long time (1). Such transfer has been attempted in three different ways: i) by crossing a synthetic amphiploid ($AABBDDM^VM^V$ or $AADDM^VM^V$)

with *T.aestivum* (2-4), ii) by direct hybridization between *Ae. ventricosa* and *T.aestivum* (5), or iii) by crossing an ABDM^{V} hybrid with *T. aestivum* (1,6).

The first method has been successfully applied to the transfer of genes involved in resistance to the eyespot disease, caused by *Cercosporella herpotrichoides* (Fron) (2,4). It appears that, in these cases, only genes located in the D genome of *Ae. ventricosa* were transferred to the hexaploid lines that were finally obtained.

The direct *T. aestivum* x *Ae. ventricosa* hybrid has been repeatedly reported as unsuccessful (see ref. 4). Recently, Dosba and Cauderon (5) obtained one such pollen sterile hybrid, which yielded 3 seeds by free pollination and 12 seeds by backcrossing with the wheat parent.

In 1952, Simonet (1) first reported a meiotic study of (*Ae. ventricosa* x *T. durum*) x *T. aestivum* and (*Ae. ventricosa* x *T. dicoccum*) x *T.aestivum* hybrids, and proposed this type of double cross as a possible method for genetic transfer. In 1950, M. Alonso Peña (unpublished) carried out a similar cross, (*T. turgidum* x *Ae.ventricosa*) x *T.aestivum*, and derived from it a series of lines by repeated selfing. Delibes and García-Olmedo (6) presented preliminary biochemical evidence of gene transfer from the M^{V} genome of *Ae. ventricosa* into some of these lines. A more complete biochemical and cytological study of these lines was subsequently undertaken and a progress report in presented here.

MATERIALS AND METHODS

Biological material

Seventy F_{20} lines (H-93-1 through 70) derived from a double cross {*T.turgidum* (AABB) var. rubroatrum H-1-1 x *Ae. ventricosa* (DDM^{V}M^{V}) AP-1} x *T. aestivum* (AABBDD) var. Almatense H-10-15, the parental material, and samples of *Ae. squarrosa* (DD), *Ae. comosa* (MM) and *Ae. uniaristata* (M^{U}M^{U}) were kindly given by M. Alonso Peña (Cuenca, Spain). Hybrids between the H-10-15 parent and the appropriate H-93- lines were obtained by us.

Biochemical methods

A total of fourteen biochemical systems, each representing a set of chemically or genetically related markers, were investigated. These are listed in Table 1. References concerning their analysis and genetic control are the following: CM Proteins, low MW components of the chloroform:methanol extract (7-11); NGE Proteins, non-gliadin components of the 70% ethanol extract controlled by different groups of homoeologous chromosomes (9,11); U Proteins, low MW components extracted with 2M Urea (6), gliadins (6, 12), athins (6,12), purothionins (14,15), sterol esters (16-20), alkaline phosphatase isozymes (21), peroxidase isozymes (22), aryl esterase isozymes (23), alcohol dehydrogenase isozymes (24).

Nitrogen and aminoacid analysis, Farinograph curves and protein fractionation were carried out by standard procedures as described by Delibes (25).

Cytological observations

Chromosome staining for somatic chromosome counts and for meiotic studies was carried out by Feulgen reaction as described by Sharma and Sharma (26).

RESULTS AND DISCUSSION

Possible chromosomal locations in the genitors of the H-93- lines of different types of genetic markers are represented in Fig. 1: *a*) represents a gene-

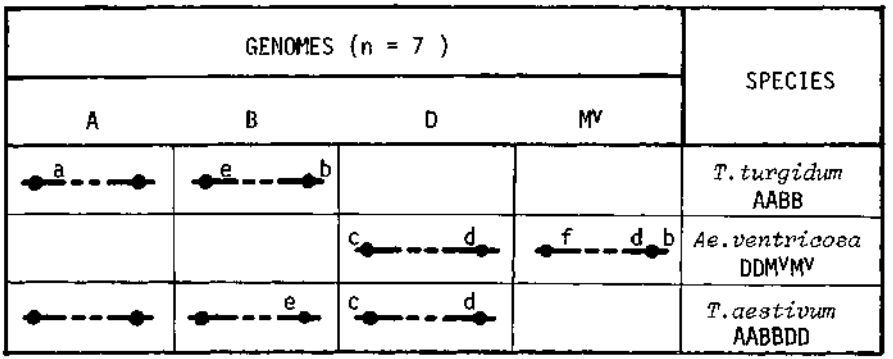

FIGURE 1.

tic variant that is present in the A, B, or D genomes of only one of the genitors; *b*) idem, except that the marker is also present in the M^V genome; *c*) represents a locus which is occupied by the same allele in the A, B, or D genomes of two genitors; *d*) idem, except that the marker is also associated with the M^V genome; *e*) represents a marker that is present in more than one genitor, but in non homologous loci; and *f*) is a genetic marker that is only associated with the M^V genome of *Ae.ventricosa*.

If eggcells from the ABDM^V hybrid carried the complete A, B and D genomes, and only homologous transfers would have taken place, the expected frequencies of the different markers in the H-93 lines (42 chromosomes) would be as follows: 50% for types *a* and *b*, 100% for types *c* and *d*, 75% for type *e*, and 0% for type *f*.

The presence of a type *f* marker in a line would unequivocally indicate genetic transfer from the M^V genome. Absence of a type *c* or *d* marker from a line could be due to deletion, to incomplete homology between D genome chromosomes in *Ae. ventricosa* and *T. aestivum*, or to genetic transfer from the M^V genome. Markers of types *a*, *b* or *e* can be useful in the characterization of the genetic make up of a particular line, but deviations from the expected frequencies are meaningless in our case because lines H-93-1 through -70 do not represent an unbiased sample of the progeny (M. Alonso Peña, private communication).

Fourteen biochemical systems, each representing a set of chemically or genetically related markers, were investigated. Their distribution in the H-93 lines and in the parental material is summarized in Table 1.

Biochemical markers controlled by the A or B genomes of one or both the wheat parents (types *a*, *c*, and *d*) are distributed in the H-93 lines as expected if the eggcells from the self-sterile ABDM^V hybrid, rescued by the ABD polen, carried the complete A and B genomes from *T. turgidum*. However, the distribution of D genome markers is only compatible with the presence of most of the D genome in most of the eggcells from the ABDM^V hybrid: three out of eight markers of the *c* or *d* type were present in the H-93 lines with frequencies close to but lower than the expected 100%, indicating incomplete homology between the two D genomes, non-homologous transfer or deletion.

Biochemical characters present in *Ae. ventricosa* (DM^V), *Ae. comosa* (M), and *Ae. uniaristata* M^U, and absent in *T. aestivum* (ABD), *Ae. squarrosa* (D) and *T. turgidum* (AB) were selected as M^V genome markers. Two of these markers were not transmitted to the H-93 lines, three were transmitted with low frequency

TABLE 1. Distribution, type and genome assignment of biochemical markers

Biochemical system	Marker symbol	Distribution in genitors*						Marker type**	Genome assignment	Frequency (%) in H-93-lines
		ABD	AB	D	$M^{V}D$	M	M^{u}			
CM Proteins	CM1	+	-	+	+	+	+	d	D & M	93
	CM2	+	+	-	-	-	-	c	AB	100
NGE Proteins, 16, 17, 17v	NGE-16	+	+	-	-	-	-	c	AB	100
	NGE-17	+	-	+	-	-	-	a	D aest.	69
	NGE-17v	-	-	-	+	+	+	f	M^{V}	31
NGE Proteins, 5,6-7,14-15	NGE-5	+	-	+	+			c	D	98.5
	NGE-6-7	+	+	-	-			c	AB	100
	NGE-14-15	+	+	-	-			c	AB	100
NGE Proteins,1	NGE-1	+	-	+	+			c	D	100
NGE Proteins,11	NGE-11	+	-	+	+			c	D	98.5
U Proteins	U-1	-	-	-	+	+	+	f	M^{V}	4
	U-2	+	-	-	-	-	-	a	AB aest.	59
	U-3	-	-	-	+	+	+	f	M^{V}	0
Purothionins	α	+	+	+	+	-	-	c	B & D	α/β 2:1
	β	+	+	-	-	(+)	(+)	c	A	100 %
Gliadins	Gl-1	-	+	-	-	-	-	a	AB turg.	32
	Gl-2	+	-	-	+	-	-	c	D	75
	Gl-3	+	-	-	-	-	-	a	ABD aest.	40
Athins	Ath-1-2	+	-	-	-	-	-	a	ABD aest.	72
Sterol esters	PL	+	-	+	+	+	+	d	D & M	100
Alkaline phosphatase	Aph-1-2	+	+	-	+	+	+	d	AB & M	100
	Aph-3	-	-	-	+	+	+	f	M^{V}	3
	Aph-5-6	+	-	+	+	+	+		D & M	100
Peroxidase	Px-a	+	-	+	+	-	-	c	D	100
	Px-m	-	-	-	+	+	+	f	M^{V}	0
	Px-d	+	+	-	-	-	-	c	AB	100
Esterase	Es-dv	-	-	+	+	-	-	a	D aest.	33
Alcohol dehydrogenase***	Adh-μ	-	-	-	+			f	M^{V}	1.5

* ABD, *T. aestivum*, H-10-15; AB, *T. turgidum*, H-1-1; DM^{V}, *Ae. ventricosa*, D, *Ae. squarrosa*; M, *Ae. comosa*; M^{u}, *Ae. uniaristata*

** See Fig. 1

*** A description of other markers included in these systems is postponed for technical reasons. These markers appear alternatively.

and one with high frequency. The latter is NGE-17v, a marker which appears alternating with protein NGE-17, which is controlled by chromosome 4D in *T. aestivum* cv. Chinese Spring. This means either that the gene(s) for NGE-17v must have been located in the D genome of *Ae. ventricosa* prior to the performance of the hybridization under study, or, less probably, that the transfer took place during the meiosis of the ABDM^{V} hybrid.

Resistance to *Erisiphe graminis* was determined by Dosba and Doussinault at Rennes (France) and was found to be transmitted with low frequency. Furthermore, the three resistant lines are among the four lines carrying M^{V} genome markers (Fig. 2).

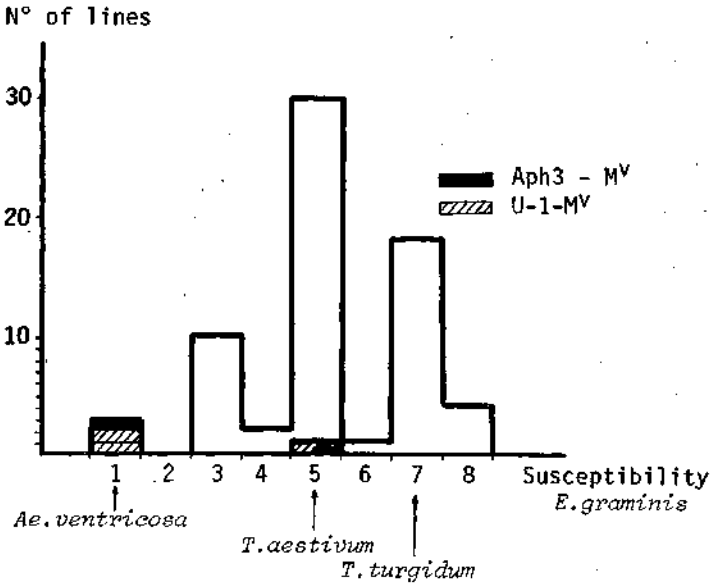

FIGURE 2. Susceptibility of lines H-93-1 through 70 to *Erisiphe graminis*.

Somatic chromosome numbers were counted in the root tips from germinating seeds. All H-93 lines showed 42 chromosomes, except H-93-1. This line was originally suspected of being an ABDM^{V} alloploid, because some cells had 56 chromosomes (6), but a more detailed study demonstrated mosaic formation. Cells with 42 chromosomes were the most frequent, but cells with 21, 28, 35, and 56 chromosomes were also observed. However, meiosis of line H-93-1 was regular and showed 42 chromosomes at metaphase I.

In order to further characterize the form of integration of the alien material in the H-93 lines, meiosis was studied in the following stocks: lines H-93-1, -8, -33, and -35, which carry M^{V} genome markers; lines H-93-10 and -51, which lack marker CM1; hybrids between all these lines and the *T.aestivum* H-10-15 genitor. Also included in the study was line H-93-5 and its hybrid, which was not suspected of carrying non homologous transfers but was the line with the highest protein content. Meiotic observations are summarized in Table 2. Meiosis of all lines, except H-93-33, was rather regular and only the number of open bivalents was significantly higher than in the H-10-15 wheat. Line H-93-33 was significantly more irregular than H-10-15 at the three meiotic phases stu-

TABLE 2. Meiosis of select H-93- lines and their hybrids with *T.aestivum* (H-10-15)

Material	Metaphase I*		Anaphase I	%
	Univs/cell	Open bivs/cell	Laggards/cell	Micronuclei
10-15	0.04±0.04 (0-2)	2.52±0.25 (0-7)	0.16±0.10 (0-4)	0.01±0.01 (0-1)
93-1	0.28±0.10 (0-2)	4.28±0.31 (1-9)	0.14±0.06 (0-2)	0.02±0.01 (0-1)
93-1x10-15	2.92±0.27 (0-8)	5.82±0.40 (1-14)	1.70±0.30 (0-8)	0.58±0.08 (0-4)
93-5	0.00	2.70±0.54 (0-6)	0.39±0.07 (0-4)	0.55±0.08 (0-4)
93-5x10-15	0.42±0.09 (0-4)	5.14±0.38 (0-12)	0.58±0.09 (0-4)	0.43±0.13 (0-8)
93-8	0.28±0.10 (0-2)	5.60±0.35 (1-11)	0.20±0.11 (0-4)	0.01±0.01 (0-1)
93-8x10-15	4.32±0.19 (2-8)	4.12±0.29 (0-9)	2.98±0.27 (0-8)	1.01±0.09 (0-3)
93-35	0.00	4.64±0.33 (1-12)	0.10±0.06 (0-2)	0.04±0.02 (0-2)
93-35x10-15	2.56±0.22 (0-8)	4.12±0.32 (0-10)	1.10±0.17 (0-4)	0.94±0.07 (0-3)
93-10	0.56±0.15 (0-4)	3.46±0.32 (0-9)	0.32±0.08 (0-5)	0.11±0.04 (0-3)
93-10x10-15	1.86±0.15 (0-5)	4.40±0.30 (0-10)	2.19±0.15 (0-6)	0.78±0.08 (0-3)
93-33	1.00±0.18 (0-4)	4.98±0.31 (0-12)	0.59±0.09 (0-5)	0.12±0.03 (0-2)
93-33x10-15	3.74±0.21 (2-8)	6.17±0.33 (0-11)	2.90±0.17 (0-8)	0.69±0.07 (0-3)
93-51	0.00	2.72±0.28 (0-8)	0.26±0.07 (0-4)	0.02±0.01 (0-1)
93-51x10-15	1.6 ±0.18 (0-4)	3.32±0.27 (0-8)	1.8 ±0.12 (0-6)	0.54±0.06 (0-2)

* One cell with one trivalent was observed in H-93-10 x H-10-15 and one cell with one quadrivalent in H-93-5 x H-10-15. No associations above bivalent was otherwise observed.

died. Meiosis of the hybrids involving the selected lines were significantly more irregular (2-4 univalents) than those of the corresponding genitors, except in the case of H-93-5 x H-10-15, which was quite regular.

The distribution of biochemical markers and the maximum number of chromosomes from the M^V genome in the selected lines are registered in Table 3. The latter is deduced from the number of univalents at MI observed in the hybrid and represent an overestimation for two reasons: i) because it has been repeatedly observed that there is incomplete homology between the D genomes of *T.aestivum* and *Ae. ventricosa*, and ii) because the transfer of a terminal segment of

TABLE 3. Distribution of genetic markers and maximum number of chromosomes from the M^V genome in selected H-93 lines

Genomes	Markers	Lines H-93-					
		1	8	10	33	35	51
M^V	Aph-3	+	-	-	+	-	-
	U-1	+	+	-	-	+	-
	Pm*	-	+	-	+	+	-
	Adh-μ	-	-	-	+	-	-
D^V/D^a	NGE-3 y -4	-	-	-	+	+	-
	NGE-11	-	+	+	+	+	+
D^V	NGE-17v	+	+	+	+	-	+
	Es-dv	+	+	+	-	-	-
D^A	NGE-17	-	-	-	-	+	-
	Adh-δ	+	-	+	-	-	+
	Ath-1 y -2	-	+	-	-	+	-
Maximum number of M^V chromosomes		1	2	1	2	1	1

* resistance to *E. graminis*

a chromosome from the M^V genome to a wheat chromosome would suffice to drastically reduce its meiotic pairing with the original wheat chromosome. It can be concluded, from the joint consideration of the biochemical and the cytological data, that the genetic transfer from the M^V genome of *Ae. ventricosa* to hexaploid wheat has taken place not only by chromosome substitution but also by recombination. In other words, lines carrying, at most, one whole M^V genome chromosome also carry two independently inherited M^V genome markers.

The H-93 lines were screened for outstanding quality related characters. Protein contents (Kjeldahl) were in the range 11.1-17.2% none of the lines reaching the 18.3% content of *Ae. ventricosa* AP-1. Sequential protein extraction

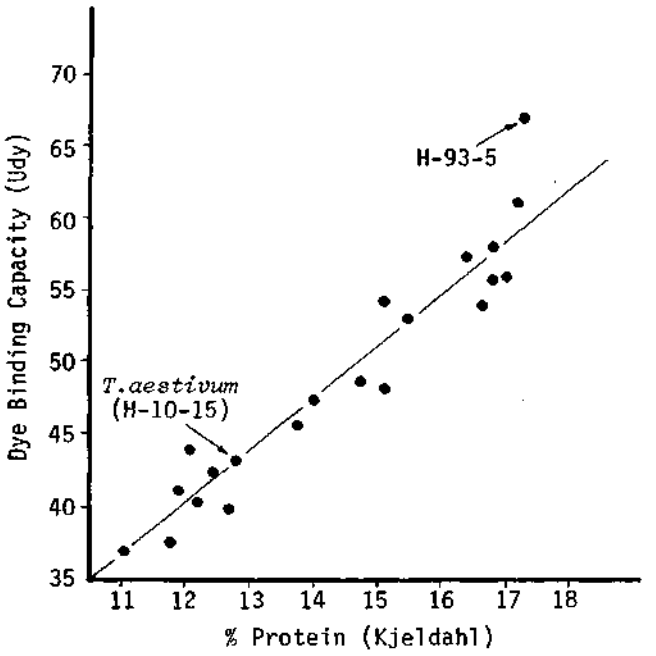

FIGURE 3.

TABLE 4. Nutritional and technological quality of line H-93-5

Determination	H-1-1	H-10-15	H-93-5
Protein (%)	15.8±0.1	12.6±0.1	17.2±0.1
Gliadins (% of protein)	35.0	30.5	36.0
Lysine (9/16 gN)	3.06	3.66	3.25
Zeleny index	11	17	26
Pelskenke index	42	62	38
Brabender valorimetric number	30	38	44

of flour from each line was carried and no extreme variant in the proportion of gliadins was found. In Figure 3, dye binding capacity (DBC, acylan orange G) of selected lines is plotted versus Kjeldahl protein. Only H-93-5, which is also the line with the highest protein content, had a higher than expected DBC and was subjected to further tests. These are summarized in Table 4. Although H-93-5 shows some interesting features, none of them is clearly outstanding.

AKNOWLEDGEMENTS

We gratefully acknowledge biological material given by M. Alonso Peña. The

work was supported in part by a grant from the Juan March Foundation.

REFERENCES

1. M. Simonet, Inst. Rech. Agron., Paris, Ser. B. Ann. Amel. Pl., 2 (1952) 175.
2. N. Maia, C.R. Acad. Agric. Fr., 53 (1967) 149.
3. G. Kimber, J. Agric. Camb., 68 (1967) 373.
4. K.A. Siddiki and J.K. Jones, Can. J. Genet. Cytol., 9 (1967) 776.
5. F. Dosba and I. Cauderon, Wheat Inform. Ser., 35 (1972) 22.
6. A. Delibes and F. García-Olmedo, Proc. 4th Int. Wheat Genet. Symp., Columbia Mo. (1973) 161.
7. F. García-Olmedo and P. Carbonero, Phytochemistry, 9 (1970) 1495.
8. C. Aragoncillo, M.A. Rodríguez-Loperena, P. Carbonero and F. García-Olmedo, Anal. Biochem., 63 (1975) 603.
9. M.A. Rodríguez-Loperena, C. Aragoncillo, P. Carbonero and F. García-Olmedo, Phytochemistry, 14 (1975) 1219.
10. C. Aragoncillo, M.A. Rodríguez-Loperena, P. Carbonero and F. García-Olmedo, Theoret. Appl. Genetics, 45 (1975) 322.
11. M.A. Rodríguez-Loperena, C. Aragoncillo, P. Carbonero and F. García-Olmedo, Plant Sci. Lett., 5 (1975) 387.
12. K.W. Shepherd, Proc. Third Int. Wheat Genet Symp., (1968) 86.
13. P. Carbonero and F. García-Olmedo, Experientia, 25 (1969) 1110.
14. R. Fernández de Caleya, C. Hernández-Lucas, P. Carbonero and F. García-Olmedo, Genetics, 83 (1976) 687.
15. C. Hernández-Lucas, R. Fernández de Caleya, P. Carbonero and F. García-Olmedo, Genetics, 85 (1977) 521.
16. F. García-Olmedo, Nature, 220 (1968) 1144.
17. J.V. Torres and F. García-Olmedo, Plant Sci. Lett., 3 (1974) 213.
18. J.V. Torres and F. García-Olmedo, Biochim. Biophys. Acta, 409 (1975) 367.
19. P. Carbonero, J.V. Torres and F. García-Olmedo, Febs Lett., 56 (1975) 198.
20. J.V. Torres, P. Carbonero and F. García-Olmedo, Phytochemistry, 15 (1976) 667.
21. G.J. Brewer, C.F. Sing and E.R. Sears, Proc. Nat. Acad. Sci. US, 64 (1969) 1224.
22. T. MacDonald and H.H. Smith, Genetics, 72 (1972) 77.
23. Y. Nakai and K. Tsunewaki, Jap. J. Genet., 46 (1971) 321.
24. G.E. Hart, Proc. Nat. Acad. Sci. US, 66 (1970) 1136.
25. A. Delibes, Ph. D. Thesis, Madrid Complutense Univ. (1976)
26. A.K. Sharma and A. Sharma, Chromosome Techniques, Butteworth & Co. (Publishers) (1965) 116.