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Cytogenetic studies of aneuploidy in the varieties "Senatore Capelli" and "Bidi 17" of Triticum durum Desf.

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CYTOGENETIC STUDIES OF ANEUPLOIDY IN THE VARIETIES « SENATORE CAPELLI » AND « BIDI 17 » OF TRITICUM DURUM DESF.

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Introduction.

Obtention of aneuploids has a great interest both from the theoretical and practical points of view. Sears (1954) was the pioneer of aneuploidy in common wheat.

In spite of being an allotetraploid species, monosomics are very difficult to obtain in *durum* wheat. Efforts have been made in order to obtain them by conversion technique (Mochizuki, 1968 a and b) or by mutagenic treatments (Bozzini et al., 1966, 1969; Martini e Bozzini, 1967).

In the present paper recently initiated investigations in order to obtain aneuploids (hypo- and hyperaneuploids) of *durum* wheat are reported.

Material and methods.

Because of their importance as crops in Spain, the varieties used are Senatore Capelli, a very well known Italian variety, and Bidi 17, an Argelian variety improved by the INRA in France.

Seeds of both varieties were irradiated with gamma-radiation (Cs 137) in the Campo de Radiaciones Gamma del Encín, INIA. Doses of 10 Kr, 20 Kr and 30 Kr were applied. After the treatment seeds were germinated on petri dishes at 20 °C, transplanted to pots and growing to maturity.

Cytological controls were made on root tips after being pretreated with monobromonaphtalene, fixed in glacial acetic and stained with fuchsin.

Meiotic behaviour was analyzed in PMCs. Anthers were fixed in acetic alcohol 1:3 and PMCs stained with fuchsin. Observations were made on permanent slides. M_1, M_2 and M_3 generations have been obtained by selfing under bag.

RESULTS.

Table 1 shows the effects of the different doses of radiation (10 Kr, 20 Kr and 30 Kr) on root tip cells of M_0 seedlings.

These results are graphically represented in Figure 1, in which linear regressions are found when the percentages of normal cells and absolute values of chromosome fusions per cell are plotted against doses of radiation.

M_0 plant fertility measured as percentage of seed setting and the proportion of chromosome aberrant M_1 plants of the Senatore Capelli variety are shown in Table 2. Among the aberrant offspring a plant with $2n = 26 + 1$ dicentric was found. This plant showed mosaicism in the meristematic tissue of root tips (26 + 1 dic./26 + 2 telos) Figures (2, 3 and 4) and in the ear ($2n = 26 + 1$ telo long arm). Meiotic pairing at Metaphase I is summarized in Table 4 (see figures 5 to 7). The origin of mosaicism in root tips is represented in figure 8.

An M_2 trisomic plant arising from a 28-chromosome M_1 plant produced trisomic and tetrasomic M_3 offspring (Figure 9).

In the Bidi 17 variety, the meiotic behaviour of an M_1 plant with $2n = 27 +$ telo (Figures 10 to 12) was analyzed. This plant showed mosaicism in the ears; one of them had a $2n = 27$ constitution and the other one maintained the $2n = 27 +$ telo (see Table 4). Among its offspring two monosomic plants (Figure 13) (which died at the 2-3 leaves stage) and one 27 + telo plant were found.

In the same variety M_2 trisomics have been obtained from M_1 structural heterozygous plants for reciprocal translocations.

In order to obtain monosomics, haploid plants of both varieties were crossed as female parents with normal plants. Haploids were detected as members of twin seedlings from polyembryonic seeds. No one monosomic plant was obtained from the crosses (Table 5).

DISCUSSION.

On analyzing the effects produced by the γ-irradiation on seeds (Table 1) a higher proportion of dicentric than tricentric chromosomes has been found. It is quite logical because three breaks and two fusions must take place to produce the tricentric chromosomes while only two breaks and one fusion are necessary in the case of dicentrics. The positive correlation found between the number of fusions per cell and doses and, likewise, the negative correlation between the proportion (%) of normal cells and doses are normal facts. More interesting are the linear regressions observed in both cases.

The lower proportion of aberrant plants among the M_1 offspring corresponding to the higher doses of radiation (Table 2) seems to be pa-

Table 1. — DOSAGE EFFECT γ-IRRADIATION (Cs 137) ON SEEDS OF *Triticum durum* DESF. VAR. SENATORE CAPELLI. SOMATIC METAPHASE CONTROLS OF ROOT TIPS OF Mo SEEDLINGS.

			Dose		
			10 Kr	20 Kr	30 Kr
Cells observed			114	120	56
Normal metaphases			54 (47.4)	28 (23.3)	7 (12.5)
Abnormal metaphases	hypo-aneuploid cells	2n=27	8 (7.0)	—	—
		2n=27+frag.	4 (3.5)	—	—
		2n=27+telo	4 (3.5) *	1 (.8) **	—
	Dicentrics	1	28 (24.6)	51 (42.5)	16 (28.6)
		2	8 (7.0)	23 (19.2)	13 (23.2)
		3	2 (1.8)	9 (7.5)	12 (21.4)
		4	—	2 (1.7)	4 (7.1)
		5	—	1 (.8)	—
	1 Tricentic		3 (2.6)	1 (.8)	—
	Dic. + Tric.				
	1 + 1		3 (2.6)	3 (2.5)	1 (1.8)
	2 + 1		—	1 (.8)	—
	2 + 2		—	—	1 (1.8)
	4 + 1		—	—	1 (1.8)
	5 + 1		—	—	1 (1.8)
	Total number of fusions		65	152	116
	Fusions per cell		1.48	1.67	2.37

( ) percentages * 1 cell had 27 + 2 telos; ** 27 + 2 telos.

radoxical, but the reason is clear: the higher cytological effect on M_0 seedlings the lower transmission of the abnormalities to the offspring.

The mosaicism 26 + 1 dic./26 + 2 telos (Table 3, Figures 2, 3, 4 and 8) observed in the meristematic tissue of root tips of a Senatore Capelli plant can be explained as the consequence of the anaphasic segregation of the dicentric chromosome when the four centromeric halves are crossly inserted on spindle fibers. The opposite attraction forces to the poles produce the break-down of the chromatids in any point intermediate between the two centromeres. This is the reason why the resulting telocentric chromosomes are of different types (telo- and subtelocentrics) with the short arm of distinct sizes (Figure 8).

Table 2. — M_0 PLANT FERTILITY AND PROPORTION OF CYTOLOGICALLY ABERRANT M_1 OFFSPRING IN THE VARIETY SENATORE CAPELLI

Dose	M_0 Generation					M_1 Generation	
	Plant	Number of ears	Florets	Seeds obtained	Seed set %	Seeds controlled	Aberrant plants
10 Kr	1	1	24	16	66.7	8	2
	3	3	66	8	12.1	1	1
	4	4	84	12	14.3	7	0
	5	4	88	17	19.3	11	1
	6	4	68	20	29.4	15	1
	7	2	42	33	78.6	19	2
	8	5	118	36	30.6	27	2
	9	1	24	17	70.8	10	1
	10	1	20	11	55.0	6	0
	12	4	93	18	19.4	11	2
		Total	627	188	30.0	115	12 (10.4%)
20 Kr	1	1	18	0	0.0	—	—
	3	3	72	3	4.2	1	0
	6	3	68	3	4.4	3	0
	7	3	62	0	0.0	—	—
	8	2	46	0	0.0	—	—
	10	7	156	13	8.3	8	0
	13	2	42	20	47.6	12	1
	14	3	62	16	25.8	8	1
	17	2	44	9	20.5	4	0
	18	3	70	23	32.9	16	1
		Total	640	87	13.6	52	3 (5.8%)

Table 3. — CYTOLOGICAL CONTROL OF M_1 SEEDLINGS MADE ON ROOT TIPS. VARIETY SENATORE CAPELLI

Doses	Plants controlled	Chromosome constitutions				
		2n = 28	2n = 28 (1 hetero-brachial)	2n = 28 + 1 telo	2n = 27 + 1 dic.	2n = 26 + 1 dic.
10 K	115	103	2	1	8	1*
20 K	52	49	—	—	3	—

* This plant showed a 26 + 1 dic/26 + 2 telos mosaicism.

Another particularity was observed in this plant: the chromosome constitution of PMCs of an ear was $2n = 26 +$ telo (Table 4). It is known that each ear is represented by only one or few cells in the embryo (D'Amato et al., 1962). So, if the dicentric chromosome is broken giving rise to two telocentrics by the mechanism above mentioned, it is possible that one of them becomes unstable being lost in the early divisions of the zygote. Identical reasoning can be made to explain the mosaicism found in another M_1 plant of Bidi 17 (Table 4).

The trivalents observed in the $2n = 26 +$ telo PMCs of Senatore Capelli (Figures 6 and 7) seem to indicate that the dicentric chromosome was formed by fusion of two non-homologous chromosomes.

Polyembryony can be an effective source to detect the presence of haploids in plants (Kimber and Riley, 1963). Both the frequency of twin seedlings and the occurrence of haploidy in any of them ($2n - n$ or $n - n$) is different between species and, even, between genotypes for a given species. In *Triticum aestivum*, Wilson and Ross (1961) found the following percentages of polyembryony .035, .030 and .018 in the varieties Ottawa, C.I. 13285 and Wichita, respectively. They also recorded a 5.2% of haploids among the twin seddlings. These results are in agreement with those observed in our material (Table 5). Polyembryony in *durum* wheat there was previously reported by other authors (Kihara, 1936; Müntzing, 1938; Kasparayan, 1938; etc.).

A certain number of lines of the monosomic series in the Chinese Spring variety obtained by Sears (1939, 1944) arose from crosses between haploid and normal plants. In *durum* wheat the situation does not seem the same, at least in our material. It is striking that no monosomics were found among the 81 plants which constituted the offspring of ♀ haploid x ♂ diploid crosses. The probability that a gamete with *h* chromatids originates from a meiotic process depends on the Anaphase I segregation assuming a completely asynaptic Metaphase I. This probability, after the general expression of the binomial distribution $(1/2 + 1/2)$, is

$$p = \frac{1}{2^x} \binom{x}{h}$$

being x the number of chromosomes of the haploid. It is considered that every chromosome has the probability of 0.5 of emigrating to a given pole of the cell.

So, in our case, $x = 14$ and $h = 14$; then $p = \frac{1}{2^{14}}$ According to this theoretical model, among the 585 crosses made in the Senatore Capelli variety, the probability of the 14 chromatid eggs would be $585 \times \frac{1}{2^{14}} = .05$ and $368 \times \frac{1}{2^{14}} = .03$ in the case of Bidi 17. For this reason, it cannot be explained as a normal phenomenon the obten-

Table 4. — Meiotic pairing at Metaphase I in PMCs of M_1 plants of *Triticum durum* Desf. varieties Senatore Capelli and Bidi-17

Variety	2n in root tips	2n in PMCs	PMCs observed	Univalents	Bivalents		Triva-lents	Chiamata per cell	Chiasmata per paired chromosome
					Ring	Rod			
Senatore Capelli	26 + 1 dic.	26 + telo long arm	49	.45 ± .10	10.81 ± .15	1.39 ± .16	.71 ± .06	24.41 ± .20	.98 ± .01
Bidi - 17	27 + 1 telo	Ear No. 1: 2n = 27	44	1	12.41 ± .30	.30 ± .07	—	25.14 ± .59	.97 ± .02
		Ear No. 2: 27 + telo	104	1.96 ± .03	12.48 ± .07	.53 ± .07	—	25.49 ± .07	.98 ± .001

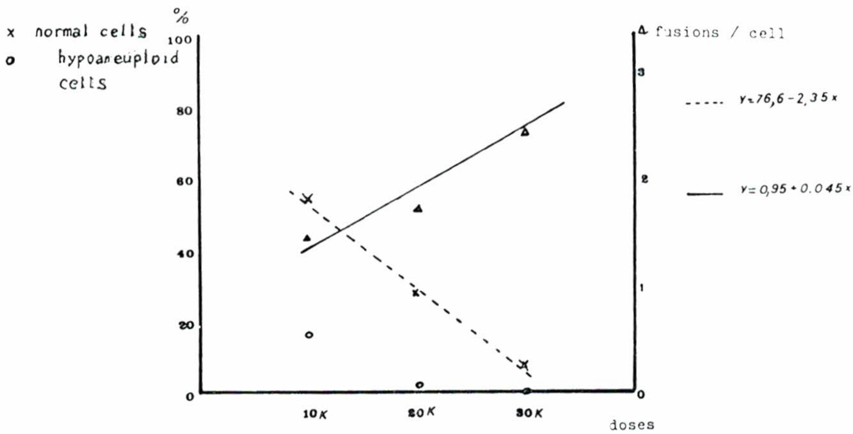


Fig. 1 - Dosage-effect relationships in the Senatore Cappelli variaty.

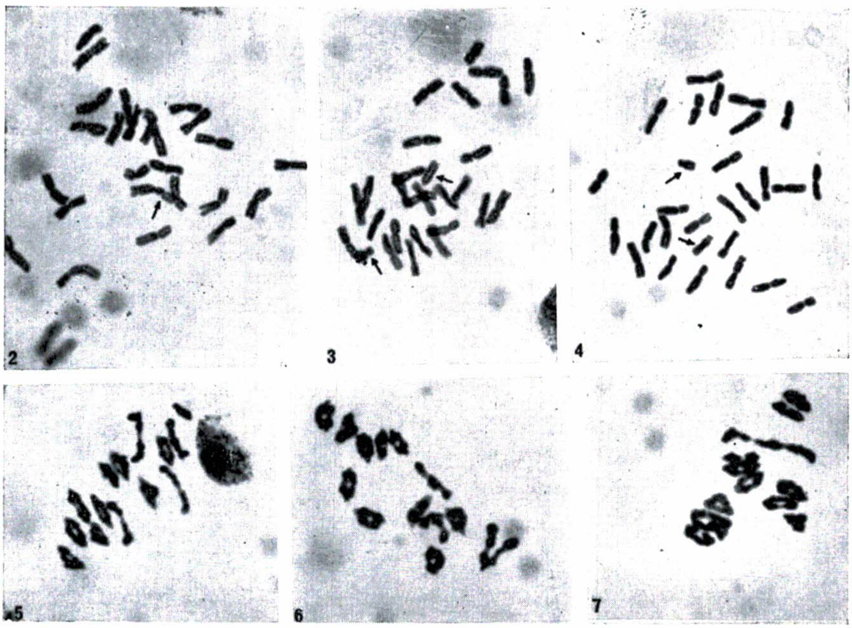
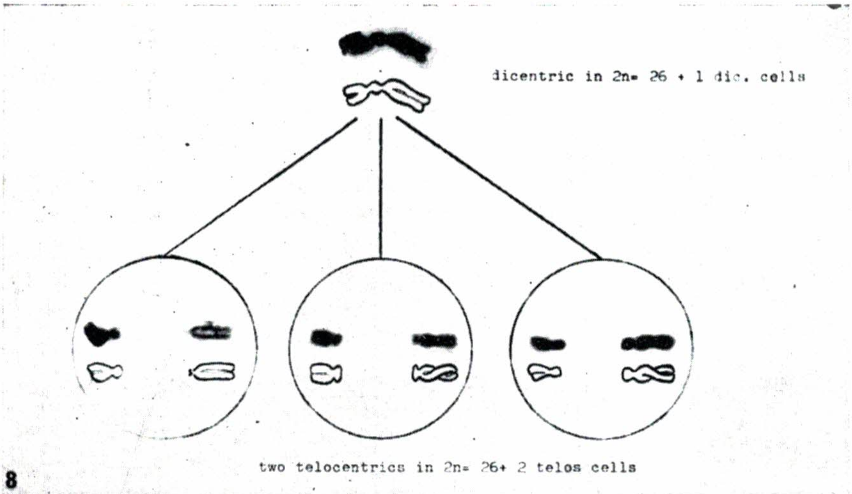


Fig. 2 - Mosaicism 26 plus 1 dicentric/26 plus 2 telos in the rootips of an M_1 Senatore Cappelli plant. Somatic metaphase 2n = 26 plus 1 dicentric.
Fig. 3 - Idem. Somatic metaphase 2n = 26 plus 2 telos.
Fig. 4 - Idem. Somatic metaphase 2n = 26 plus 2 telor.
Fig. 5 - The same plant showed root tip/ear mosaicism. The observed PMCs had 2n = 26 plus 1 telo. MI showing 13 bivalents plus 1 univalent.
Fig. 6 - Idem - idem. MI showing 12 bivalents plus 1 trivalent.
Fig. 7 - Idem - idem. MI showing 12 bivalents plus 1 trivalent.



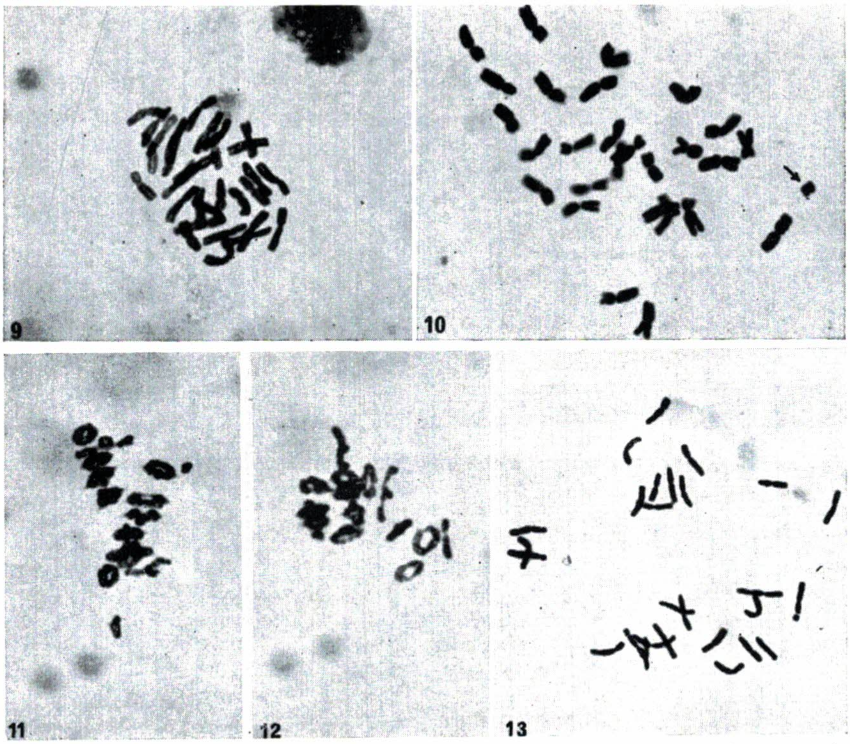


Fig. 8 - Types of telo - and subtelocentric chromosomes found in root tip cells of the M_1 Senatore Cappelli plant which showed mosaicism (26 plus 1 dic./26 plus 2 telos).
Fig. 9 - M_3 tetrasomic plant of Senatore Cappelli. - Somatic metaphase $2n=30$.
Fig. 10 - M_1 plant of Bidi 17. Somatic metaphase $2n=27$ plus 1 telo.
Fig. 11 - Idem. MI showing 13 bivalents plus 2 univalents.
Fig. 12 - Idem. MI showing 14 bivalents, one of them heteromorphic.
Fig. 13 - M_2 monosomic plant of Bidi 17. Somatic metaphase $2n=27$.

Table 5. — Haploidy in polyembryonic seeds in *Triticum durum* Desf. varieties Senatore Capelli and Bidi-17

Variety	Seeds germinated	Twin embryos	Polyembryony in per cent	Haploids	Florets pollinated with pollen from normal plants	Offspring	
						2n = 28	2n = 29
Senatore Capelli	78,922	29	.037	3 (10.3 %)	585	20	1
Bidi - 17	77,182	36	.047	2 (5.6 %)	368	59	1

tion of 20 and 59 disomic plants in the Senatore Capelli and Bidi 17 varieties, respectively. A similar situation there was been found previously by one of us (Lacadena, unpublished).

Although a non-random segregation of the chromosomes in first-anaphase in the microsporogenesis of a haploid Senatore Capelli plant was described by Lacadena and Ramos (1968) the modal class corresponded to the 8-6 segregation; even Kihara (1936) in haploid *durum* wheat reported a random Anaphase I segregation. Therefore another rare event must be assumed. This could be the transformation in embryo sac of any nucellus or integument cell; that is to say, a phenomenon of apospory has taken place. This last possibility is now being tested.

It is well known that the obtention and maintainance of monosomics lines in *durum* wheat are difficult (Mochizuki, 1968a and b; Bozzini et al., 1969). So, it is not rare the death of the two monosomic plants obtained in the variety Bidi 17. In spite of the many difficulties in obtaining a monosomic series in *durum* wheat, its potential value both basic and applied genetics must stimulate to prosecute the investigations.

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ABSTRACT

Seeds of the varieties « Senatore Cappelli » and « Bidi 17 » were irradiated with gamma-radiation (Cs 137) at doses of 10 Kr, 20 Kr and 30 Kr.

In the variety *Senatore Capelli* the M_0 was cytologically analyzed. A doses-effect correlation was found when the effect is measured as the numbers of chromosome fusions per cell. The fusions being calculated according to the kinds and number of polycentric chromosomes observed. Fertility percentages were also recorded.

An M_1 plant with $2n = 26 + 1$ dicentric showed somatic mosaicism ($26 + 1$ dicentric/$26 + 2$ telocentrics) which origin is discussed. Its meiotic behaviour was also analyzed.

An M_2 trisomic plant descendant from a 28-chromosome M_1 plant originated M_3 trisomic and tetrasomic offspring.

In the *Bidi* 17 variety. the meiotic behaviour of an M_1 plant with $2n = 27 +$ telo was studied. This plant produced hypoaneuploid offspring ($2n = 27$, two plants which died, and $2n = 27 +$ telo). M_2 trisomics have been found among the offspring of M_1 structural heterozygous for reciprocal translocations.

Haploid plants have been found in polyembrionic seeds of the two varieties. Haploid x diploid crosses produced disomic offspring but two trisomic plants.

RIASSUNTO

STUDI CITOGENETICI SULL'ANEUPLOIDIA NELLE VARIETA' DI FRUMENTO DURO "SENATORE CAPPELLI" E "BIDI 17"

Semi delle varietà « Senatore Cappelli » e « Bidi 17 » sono stati irraggiati con raggi gamma (Cs-137) alle dosi di 10 kr, 20 kr e 30 kr.

Nella varietà « Senatore Cappelli » la M_0 è stata analizzata citologicamente, riscontrando una correlazione fra dose e effetto, inteso come numero di fusioni cromosomiche per cellula calcolate in base al tipo e numero di cromosomi policentrici osservati. Sono state rilevate anche le percentuali di fertilità. Viene discussa l'origine di una pianta M_1 con $2n = 26 + 1$ dicentrico, che mostrava mosaicismo somatico ($26 + 1$ dicentrico/$26 + 2$ telocentrici). È stato analizzato anche il suo comportamento meiotico. Inoltre, una pianta trisomica M_2 discendente da una pianta M_1 con 28 cromosomi ha dato origine ad una progenie M_3 trisomica e tetrasomica.

Nella varietà « Bidi 17 » è stato studiato il comportamento meiotico di una pianta M_1 con $2n = 27 +$ telo. Questa pianta ha dato origine ad una progenie ipoaneuploide (due piante $2n = 27$ che in seguito sono morte, e $2n = 27 +$ telo). Trisomici M_2 sono stati rinvenuti nella discendenza di piante M_1 eterozigoti strutturali per traslocazioni reciproche.

Piante aploidi sono state infine trovate in semi poliembrionici delle due varietà. L'incrocio tra piante aploidi e diploidi ha dato discendenza disomica con due piante trisomiche.