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PRODUCTION OF ANEUPLOID GAMETES BY MONOSOMICS OF *AVENA SATIVA* L.

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Abstract

Parental monosomics (2n—1=41) derived from three different cultivars of *Avena sativa* were crossed with another cultivar (Sun II) of the same species and their backcross derivatives were compared. On the basis of micronuclei counts of pollen tetrads, it was found that 20—chromosome gametes were produced in disproportionately higher frequency as compared with the normal haploid gametes. Homozygous background results in more deficient gametes.

Introduction

In *Avena sativa* L. (2n=6x=42) and in other plants the meiotic regularity is affected by the level of hybridity. The heterozygous state of any monosomic individual is positively correlated with the higher proportion of pollen mother cells (PMCs) with more than one univalents (Hafiz, 1977; Khan, 1962; Nishiyama *et al.*, 1968; Person, 1956; Sasaki *et al.*, 1963). The behaviour of the univalent during meiosis is of particular interest because it has a bearing on the transmission rate of the monosome. The position of the univalent at prophase, in relation to paired chromosomes, seems to be random. However, at later stages, the univalent shows characteristic retarted movements which first become evident at metaphase I.

While all the bivalents congress at the metaphase plate, the univalent lies away from the plate. When the bivalents start undergoing disjunction, the univalent arrives at the equatorial plate. Usually the longitudinal split of the univalent remains invisible during early anaphase I. The later behaviour of the univalent is quite variable—either it goes to one of the poles undivided, where it may or may not be included in the telophase I nucleus, or it may divide equationally into sister chromatids moving to opposite poles or the same pole, or it may misdivide (Morrison, 1953; Sanchez-Monge & Mac Key, 1948; Sears, 1952). Since the univalent usually undergoes division much later than the separation of the bivalent, the division products lag behind and lie between the poles. They are, sometimes, not included in the telophase I nucleus. The excluded division products of the lagging univalent form micronuclei of their own (Olmo, 1936). During the second division the univalent may divide normally, if it has gone to one of the poles undivided during the the first division of meiosis. However, in those cells where it undergoes equational division or misdivision, its movement is characteristically retarted. The products of meiosis I, consisting of normal chromatids, telocentrics or isochromosomes, reach the metaphase II plate later and lag behind at telophase II. The behaviour of the univalent at telophase II is also variable: i) two of the normal chromatids may pass to one of the poles undivided, consequently two of the four microspores will have

n-chromosomes and the other two n-1, ii) the univalent may divide again at telophase II (Nishiyama, 1931; Sanchez-Monge & Mac Key, 1948; Sanchez-Monge, 1950, 1951; Sears, 1952). As a result of irregular behaviour of the univalent, n-1 spores are produced at a much higher frequency than n spores (Bhowal, 1964; Clausen & Cameron, 1944; Gauthier & McGinnis, 1965; Lafever & Patterson, 1964; McGinnis & Lin, 1966; McGinnis & Taylor, 1961; Morrison, 1953; Morrison & Unrau, 1952; Nishiyama, 1931; Singh & Wallace, 1967). The proportion of n and n-1 gametes produced by a monosomic plant depends upon the frequency of the lagging univalent and its misdivision (Siddiqui, 1972b). In an attempt to extend the range of cytogenetic information on the characteristics of aneuploid gametes in *Avena sativa* the present studies were undertaken. In this study three monosomic lines were backcrossed to Sun II. The monosomic parents, their F_1 monosomic hybrids and 2n-1 individuals of the backcross progenies were investigated in relation to the proportion of deficient male gametes produced.

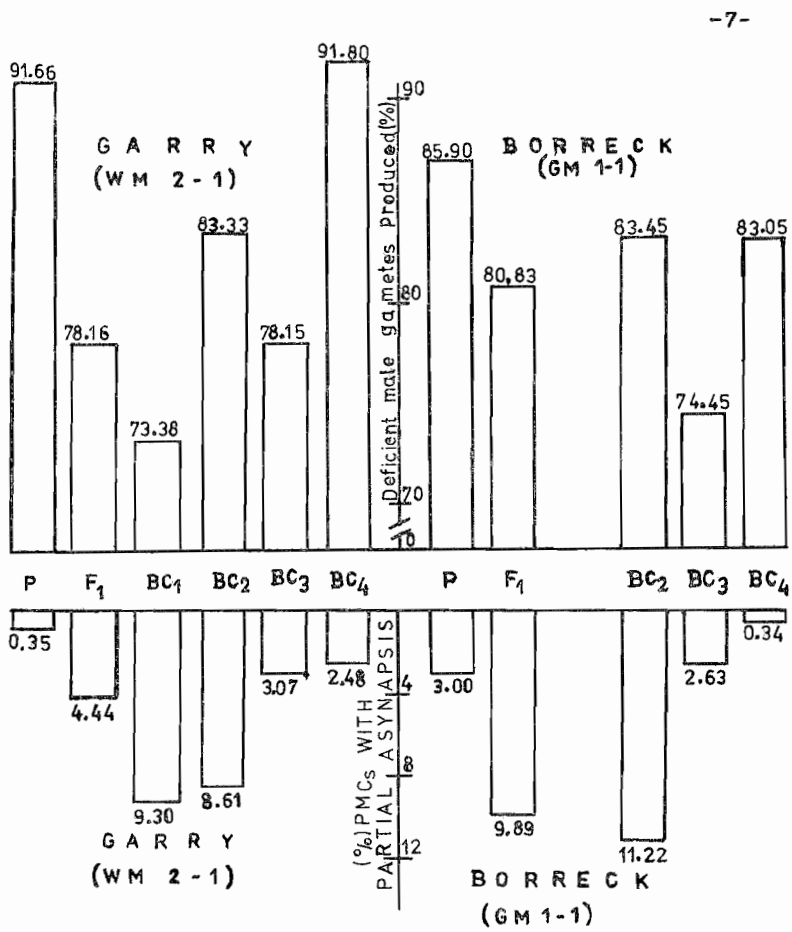

Fig. 1. Relationship between PMCs with partial asynapsis and deficient male gametes produced by monosomic GARRY and BORRECK lines and their F_1 and backcrossed monosomic derivatives.

Materials and Methods

Aneuploid lines isolated from the cultivars Garry (WM 2-1), Borreck (GM 1-1) and Manod (M 56), the derivatives of the common cultivated oat *Avena sativa* L., and the variety Sun II (2n=6x=42) were used in the present investigation. The sources of these cultivars and the hybrids in the backcrossing programme to Sun II involving these monosomic lines have been described earlier (Hafiz, 1977).

The monosomic condition of the plants was established by determining the chromosome number of the root tip cells and further confirmed by analysing the metaphase I or anaphase I of the meiosis.

For scoring the number of micronuclei per tetrad immature panicles were fixed in Carnoy's solution (ethanol: chloroform: acetic acid v/v 6:3:1), mordanted with a saturated solution of ferric chloride in 45% acetic acid and then stored in 70% alcohol. In each case one of the anthers of the floret was examined and when an anther at the right stage of meiosis (telophase II/tetrad stage) was found, the squash preparation was made of the remaining two anthers of the same floret in 1.5-2.0% acetocarmine.

The proportion of the nullisomic (n-1) gametes was calculated by applying the equation $P_{20} = 1 - \frac{2x+y}{4}$ (Hacker, 1965), where

P_{20} = Nullisomic gametes,

x = proportion of PMCs without micronuclei, and

y = proportion of PMCs with one micronucleus each.

Plants were grown in the greenhouse which was heated during the winter months. They were given the supplementary light from Mercury Vapour Lamps.

Results

In the monosomic plants, while the paired chromosomes (20 bivalents) are oriented on the metaphase plate, the univalent fails to do so (Fig. 2A). However, the univalent does come to the equatorial plate after the bivalents have divided and moved towards the poles (Fig. 2B). The univalent then either goes to one of the poles without dividing or it divides longitudinally and each chromatid goes to opposite poles or both the chromatids move to the same pole (Fig. 2C). After reaching the pole, the monosome chromatid may or may not be included in the telophase I nucleus. In the latter case they form micronuclei. All these variations were found during the course of the present investigation, even within the same plant (Fig. 2D).

In PMCs where the chromosome divided into chromatids at the first division, the resulting chromatids often lagged at anaphase II and frequently excluded from the tetrad nuclei, again forming micronuclei. Using the formula $P_{20} = 1 - \frac{2x+y}{4}$ (Hacker, 1965), the proportion of deficient male gametes formed in each monosomic plant was calculated. In this equation,

P_{20} = denotes deficient (n-1) male gametes.

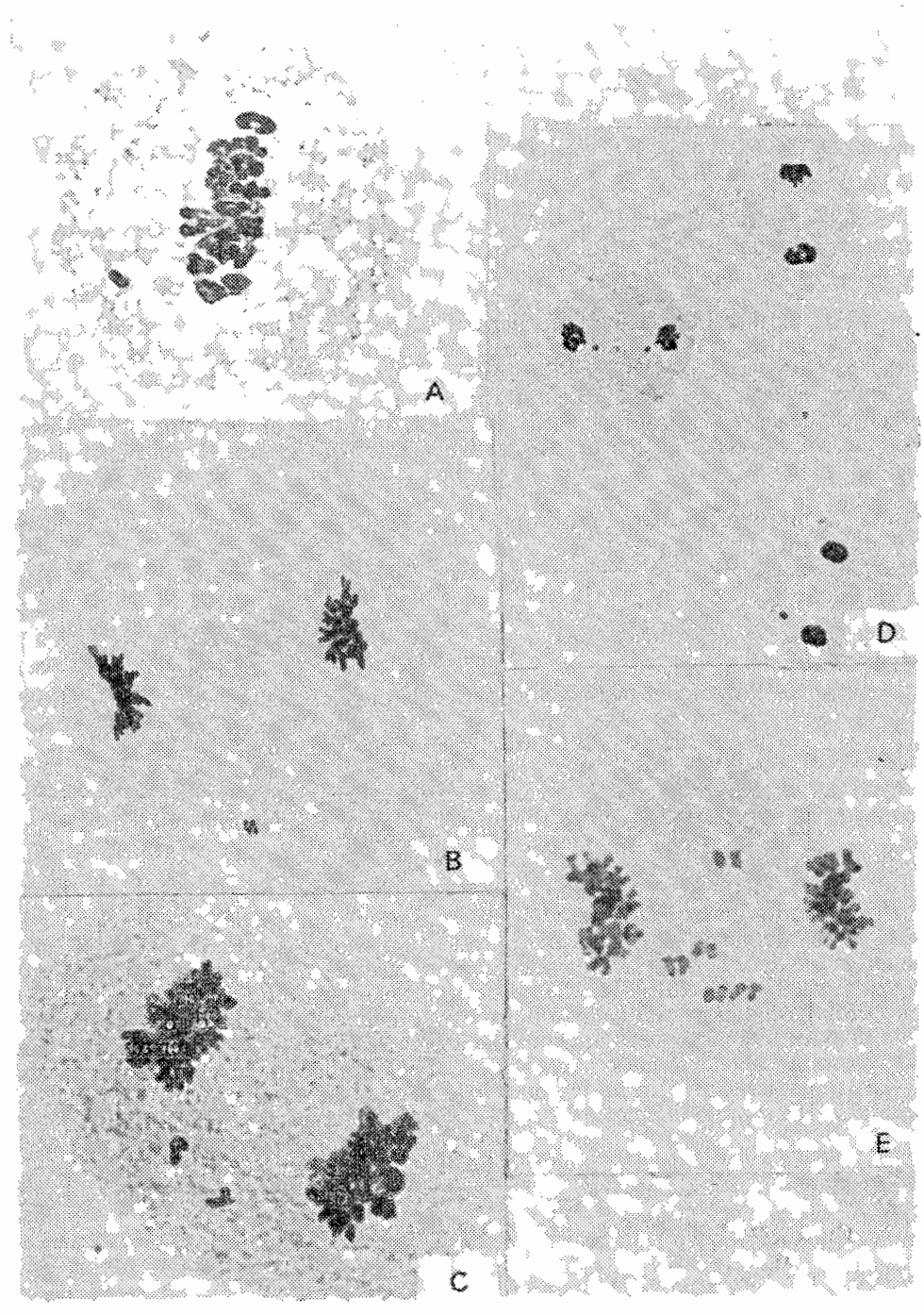

Fig 2. A. Metaphase I. While all the bivalents have assembled at the metaphase plate, the univalent is lagging behind.
B. Anaphase I. The univalent, just after longitudinal division, arrives at the metaphase plate, whilst the sister chromatids of bivalents have moved to opposite poles.
C. Anaphase I. Movement of sister chromatids to opposite poles.
D. Telophase I. Variation in the number of micronuclei within one plant
E. Anaphase I Five dividing univalents

x denotes proportion of pollen tetrads without micronuclei, and

y denotes proportion of pollen tetrads with one micronucleus each.

Some of the PMCs had more than one univalent at anaphase I (Hafiz, 1977). They also divided and showed similar retarded movement (Fig. 2E) and contributed towards the production of deficient male gametes.

The proportions of deficient gametes in the monosomics are presented in Table 1. Unfortunately it was not possible to obtain data for all the monosomics involved. The maximum proportion of deficient male gametes (0.92) was shown

TABLE 1. Proportion of deficient male gametes produced in monosomic parents, monosomic F_1 and 2n-1 individuals of BC_1 —BC_4 (Sun II was used as the recurrent male parent).

Generation Line	Parent	F_1	BC_1	BC_2	BC_3	BC_4
Garry (WM 2-1)	0.92	0.78	0.73	0.83	0.78	0.92
Borreck (GM 1-1)	0.86	0.81	—	0.83	0.75	0.83
Manod (M 56)	—	0.83	0.70	—	0.79	—
Mean	0.89	0.81	0.72	0.83	0.77	0.88

by the WM 2-1 monosomic parent, while the minimum was 0.70 in monosomic BC_1 of Manod origin. The only monosomic line, where the frequency of deficient male gametes was recorded completely through successive backcross generations, was WM 2-1. The lowest frequency of n-1 gametes was found in the BC_1. There was a gradual increase in the frequency of deficient gametes at each successive backcross but the value for BC_4 was identical with that of the original monosomic line, WM 2-1. Similar distribution was recorded in the other monosomic line, Borreck (GM 1-1), and its derivatives.

Since the calculated proportions of the gametes formed having 20 chromosomes was lower in the heterozygous genotypes, it seems that the chromatids of the monosome were excluded from the telophase II nuclei at a lower rate in these genotypes. One of the reasons for this could be that the univalent divided at a lower frequency at anaphase I in these hybrids and moved to one of the poles. Normal separation of the two chromatids of undivided monosome at anaphase II would result in their inclusion in two of the four nuclei of the tetrads. It is apparent (Table 1) that the percentage of deficient male gametes produced was related to the degree of hybridity, as was also found in the case of the asynaptic cells (Hafiz, 1977). However, the relationship between these two characters was negative but not significant (Table 2). In Fig. 1 the percentage of deficient male gametes produced and that of cells with some asynapsis are presented as histograms. From this figure the effect of hybridity on both characters becomes obvious. In F_1 and BC_1 monosomics belonging to the Garry line, where the heterozygosity is expected to be the highest, the values recorded for the deficient male gametes produced were 78.16 and 73.38 per cent respectively

TABLE 2. Relationship between PMCs showing partialasynapsis and deficient male gametes produced by monosomic cultivars, WM 2-1, GM 1-1, and their comparable derivatives (Values are expressed as percentage).

Character	Generation Line	P	F_1	BC_1	BC_2	BC_3	BC_4
PMCs	Garry (MW 2—1)	0.35	4.44	9.30	8.61	3.77	2.48
showing partial	Borreck (GM 1—1)	3.00	9.89	—	11.22	2.63	0.34
asynapsis*	Mean	1.68	7.17	9.30	9.91	2.85	1.41
Deficient	Garry (WM 2—1)	91.66	78.16	73.38	83.33	78.15	91.80
male gametes	Borreck (GM 1—1)	85.90	80.83	—	83.45	74.45	83.05
produced	Mean	88.78	79.50	73.38	83.39	76.30	87.43

*Hafiz (1977).

The latter values were the minimum in the Garry series and they were associated with the maximum per cent of PMCs showing partial asynapsis. Similarly in Borreck the highest per cent, i.e. 9.89 and 11.22 for PMCs with irregular meiosis in F_1 and BC_2 were accompanied by 80.83 and 83.45 per cent respectively for the aneuploid male gametes. The maximum percentage for deficient male gametes produced was recorded in the monosomic parents. In general the production of aneuploid male gametes was appreciably higher than normal gametes and in the homozygous genetic backgrounds more n-1 gametes were produced compared with the heterozygous backgrounds.

DISCUSSION

A natural consequence of meiotic irregularity in any of the individuals would be the production of gametes deficient for the monosome involved. This is because of the retarded movement of the lagging univalent. It may pass to one of the poles undivided where it may or may not be incorporated into the telophase I nucleus, or it may divide equationally and the resulting sister chromatids going to opposite poles at anaphase I. During the second division the univalent may divide normally, if it has passed to one of the poles undivided during the first division. However, in those cells where the monosome undergoes equational division during the first division the normal chromatids may pass to one of the poles undivided or be excluded from the tetrad nuclei and form micronuclei. In some instances the monosome misdivides at anaphase II and the individual arms of the chromosome may be incorporated into the gametes to form telocentrics.

Monosomics in this study produced a higher proportion of deficient gametes when in the homozygous or nearly homozygous genotypes than in heterozygous genotypes. The proportion of gametes deficient for the monosome is a reflection

TABLE 3. Frequency of n-1 pollen grains produced by monosomics of *Avena sativa* and *A. byzantina* as determined on the basis of micronuclei counts.

Species	monosomics	% deficient male gametes produced	Authority
Avena sativa	Mono—C	83.3	Nishiyama (1931).
	Mono—V	93.0	Philp (1935).
	Mono—L	94.0	.. (1938).
	Mono—14	83.4	McGinnis & Taylor (1961).
	Mono—20	91.0	Gauthier & McGinnis (1965).
	Mono—15	84.6	McGinnis & Lin (1966).
	"Clintland—60"	93.7	Lafever & Patterson (1964).
	Mono—VI	88.0	Hacker (1965).
	Mono—VIII	91.0	..
	Mono—IX	87.0	..
	Mono—X	84.0	..
	Mono—XI	86.0	..
Avena	Mono—M3	80.9	Singh & Wallace (1967).
byzantina	Mono—St7	88.3	..
	Mono—SM12	90.7	..
	Mono—St17	78.6	..
	MK. 5 (min.)	72.0	Nishiyama *et al.* (1968).
	MK. 16mx.)	90.0	..
Average		86.6	

of the sequence of chromosome division at meiosis. Any of the following conditions would have an effect on the proportion of deficient gametes formed:

(i) Fewer divisions of the univalent at anaphase I and consequently the normal distribution at anaphase II.

(ii) non-incorporation of the univalent in the telophase II nucleus, and

(iii) chromosomes which fail to pair, other than the monosome involved, would also lag behind.

If the dividing or non-dividing univalents are selectively incorporated in the telophase II nuclei, the proportion of the deficient gametes produced will be reduced considerably.

The genetic background has a significant effect on the behaviour of the monosome (Siddiqui, 1972d). Moreover, the degree of misdivision of the univalent, which is responsible for the production of aneuploid gametes, is influenced greatly by the genetic structure of the variety used (Khush, 1973; Siddiqui, 1972a, b, c). However, the interaction between the genetic background and the monosome involved cannot be excluded.

Previously many workers have, on the basis of micronuclei counts, calculated the frequency of n-1 gametes produced by monosomics of *A. sativa* and *A. byzantina*, and they are summarised in Table 3. These results are in agreement with the present study regarding the production of a higher proportion of deficient gametes. Similar results have also been reported in monosomics of genera other than *Avena*. According to Morrison (1953), the frequency of n-1 gametes was approximately 62% in wheat monosomics, while in *Nicotiana tabacum*, 80 deficient gametes were produced (Clausen & Cameron, 1944).

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