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POLYSPERMY VERSUS UNREDUCED MALE GAMETES AS THE ORIGIN OF NONAPLOIDS COMMON WHEAT PLANTS*

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SUMMARY

Four nonaploid plants (2n=9x-63)of common wheat found amongst approximately 9000 cytologically analyzed plants, constitute the subject of this article. Two nonaploid plants derived from hand-crosses and showing telocentrics in their karyotypes were exhaustively analyzed to establish their probable origin. One of them (2n=63t) seems likely to be the result of a fertilization of the oösphere carried out by two normal male gametes (polyspermy) instead of a single unreduced male gamete. In the second case (2n=64tt), similar conclusion could be reached, again fertilization carried out by two different normal male gametes seems the most likely explanation, given the simultaneous occurrence of two very uncommon phenomena in wheat, i.e. twin (2n=43tt) and nonaploid (2n=64tt) plants production. Two additional cases (2n=63) derived by selfing euploid plants were recorded only to establish the frequency of spontaneous nonaploidy.

KEY WORDS: Triticum aestivum - nonaploids common wheat - unreduced gametes - Polyspermy - polyploids origin.

INTRODUCTION

The occurrence of plants derived from unreduced gametes has been mentioned in several taxa (Mok and Peloquin, 1975a; Rammana, 1979; Harlan and De Wet, 1975). In *Triticum* cases have been reported involving interspecific or intergeneric hybrids (Harlan and De Wet, 1975). The occurrence of nonaploid plants with a triplicated 63 chromosome complement (2n = 9x = 63) in common wheat (*T. aestivum*) 2n = 6x = 42) presumably derived from unreduced male gametes has been indicated in virus infected plants by Linde-Laursen and Siddiqui (1974) and in supposedly uninfected plants by Worland (personal communication). Also in barley, the presence of triploid plants in both previous situations (Sandfaer, 1973, 1975) has been indicated. A less common origin of polyploids is polyspermy, or the fertilization of an egg by two male nuclei (Grant, 1981). This process has been reported in *Listea ovata* and some other orchids (Hagerup, 1947). Although one pollen tube to an embryo sac may thus be considered as the usual conditions, the entry of accesory tubes is not unknown, and several examples were cited by Maheshuari (1950). The entry of this additional pollen tube naturally results in the release of supernumerary male gametes inside the embryo sac. Rarely, one and the same pollen tube maybe also carry more than two sperms (Maheshuari, *l.c.*). On the other hand, polyembryony is known in grasses inducing heteroploid ones, diploid-triploid twins have been reported in *Triticum vulgare* (Yamamoto, 1936) and *Secale cereale* (Kostoff, 1939).

This article deals with the occurrence of four cases of nonaploid plants of wheat arising spontaneously among approximately 9000 seedlings cytologically analyzed during 1984, 1985 and 1986.

MATERIAL AND METHODS

Of the four nonaploid plants arising spontaneously at our laboratory from normal morphology seeds, two were derived from hand-crosses, as part of routine backcrossing programs. Telocentrics present in their karyotypes enabled analysis of their origin. The other two plants were originated by natural pollination in two different semi-dwarf wheat cultivars. No chromosomal markers were involved in these karyotypes. The chromosome analysis of the last two cultivars were performed in order to establish spontaneous aneuploid frequency as they were known to show karyo-phenotype instability.

Root tips were pretreated for 4,30 hs. in saturated solution of -
-bromenaftalene; fixed in glacial acetic acid and stained in Feulgen after 15 min of hydrolysis in 1N HCl at 60 C°. To study meiosis the anthers were fixed in 3:1 ethylic alcohol-acetic acid and stained with the same methods used for root tips.

RESULTS

The first plant analyzed carried 63 chromosomes, one of which was a telocentric (63t, Fig. 1A). It was derived from the 5th backcross between Chinese Spring monotelosomic 5D (CS Mt 5D, 2n = 41t) and a substitution line of Sinvalocho M.A., monosomic for the same chromosome (CS $5D^4$/Sin. , 2n = 41). Four other plants from the same backcross carried 2n = 41 or 2n = 42t chromosomes as expected. The chromosomal number of the parental substitution line was subsequently confirmed by progeny testing.

The second case involving telosomics was a plant carrying 64 chromosomes, 2 of which were telocentric (Figs. 1 B, C and D), this plant has a twin with 2n = 43tt (Fig. 38). These were derived from a backcross of Sinvalocho M.A. first backcross onto the F_1 (Fig. 3A). Several other plants belonging to the same backcross were 2n = 42 or 2n = 43tt as expected.

Two other plants carried 63 chromosomes (Figs. 1E and 1F) and are considered only in order to calculate the frequency of spontaneous nonaploid plant occurrence in the wheat material analyzed.

DISCUSSION

From the 63t chromosome number of the first nonaploid plant observed and the chromosome number of both parents (female 2n = 41t and male 2n = 41), it can be deduced that the female gamete giving rise to the nonaploid plant should be n = 21t (Fig. 2A). An unreduced (aposporic) embryo sac arising by first division restitution (FDR) or second division restitution (SDR) would give 62 t or less probably 63 tt chromosome progeny, respectively (Fig. 2A). If a somatic nucleus (Nucellar tissue) is considered as a possible female "gamete", then only 62t progeny would be obtained.

From the previous considerations only a male origin seems to explain the obtained result. If a male unreduced gamete is postulated as the origin of the nonaploid plant, then as the male plant was a monosomic (2n = 41), no FDR could account for it, because it would give 62t chromosome progeny.

If SDR is proposed, another complication arises since in monosomics most frequently the univalent undergoes equational centromere division at anaphase I, so by SDR in this case only 41 chromosome gametes and a final number of 2n = 62t should be expected. Thus the only possibility for the occurrence of a male gamete of 42 chromosome by SDR is, if at meiosis I, the univalent is included in a telophasic nucleus. This occurrence is known to be very unfrequent. In wheat, pollen grains having different number than "n" are generally selected against, even in the case that only one chromosome is absent or repeated. It seems quite unlikely that a male gamete of 42 chromosomes would compete with normal gametes (Sears, 1954, Law and Worland, 1973)

It therefore seems likely that the origin of this nonaploid plant would be a polyspermy(simultaneous fertilization with two normal male gametes, n=21, Fig. 2A).

Analysis of the 64 chromosome plant carrying 2 telocentrics (64tt) could not establish whether its origin was through (Fig. 2B):

a) an unreduced (aposporic) embryo sac, produced only by FDR. SDR can be eliminated as only 63 or 65 tttt chromosome plants would be expected;

b) an unreduced male gamete;

c) the fertilization by two male gametes simultaneously (polyspermy).

However the fact that this nonaploid plant was a twin to another being 2n = = 43tt and its spike phenotype was more similar to that of the nonaploid plant than the corresponding to the F_1 female parent (Fig. 3B), it indicates that at least one fertilization by supernumerary male gametes took place. A somatic origin without fertilization in order to explain the presence of this twin plant (2n = 43tt) should be discarded because of its spike phenotype. The nonaploid origin through an unreduced embryo sac produced by FDR should also be discarded because the B_1 phenotypic twin 2n = 43tt. This situation could lead to the supposition that four male gametes (n = 21) were able to reach in the same embryo sac, giving two embryos, the polyspermic (2n = 64tt), the twin (2n = 43tt) and a putative endosperm (2n = 65tttt). Also in this way the simultaneous occurrence of two very uncommon phenomena, i.e. nonaploid and twin plants, can be explained. If both events are thought to be independent, the probability of occurrence should be approximately 4×10^{-7}; twin plants 1/1000 (frequency obtained in the material analyzed) and nonaploids 4/9000.

One way of establishing almost unequivocally the origin of nonaploid plants in wheat would be crossing female euploid plants with male ditemonosomics 2n = 42tt (monosomic carrying telocentric corresponding to a single chromosome showing meiotic configuration $20'' + 1' + 1'^{t}$). Any derived plant carrying 65 tttt chromosomes should be considered as derived from simultaneous fertilization by two male gametes, since if an unreduced gamete was to be stipulated as the origin in this case, the following premises shoud be fulfilled:

a) Male origin
b) SDR
c) No equational centromere division occurring to both telocentrics (very infrequent)
d) Both telocentrics being included in the same nucleous.
e) No certation or very reduced influence of it.

When analyzing the origin of the polyploids species, simultaneous fertilization by two gametes (polyspermy) shoud be considered as another mechanism involved in it, since it seems to be a recurrent event in nature.

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FIG. 1: Different nonaploid wheat plants.

A-B and E-F = Mitotic metaphases., C = Metaphase I and D = Anaphase I.

A = 63t. B = 64tt. C and D = 64 tt

E and D = 63. B-F with the same enlargements. The scales represent 10 um

FIG. 2: Different fertilization possibilities for both crosses involving telocentrics

FIG. 3: Spike phenotype of:

A: F_1 (CSDtD6B x Sinvalocho M.A.) 2n = 43tt

B: B_1 Twin plant, 2n = 43tt

C: B_1 Nonaploid (twin) plant 2n = 64tt

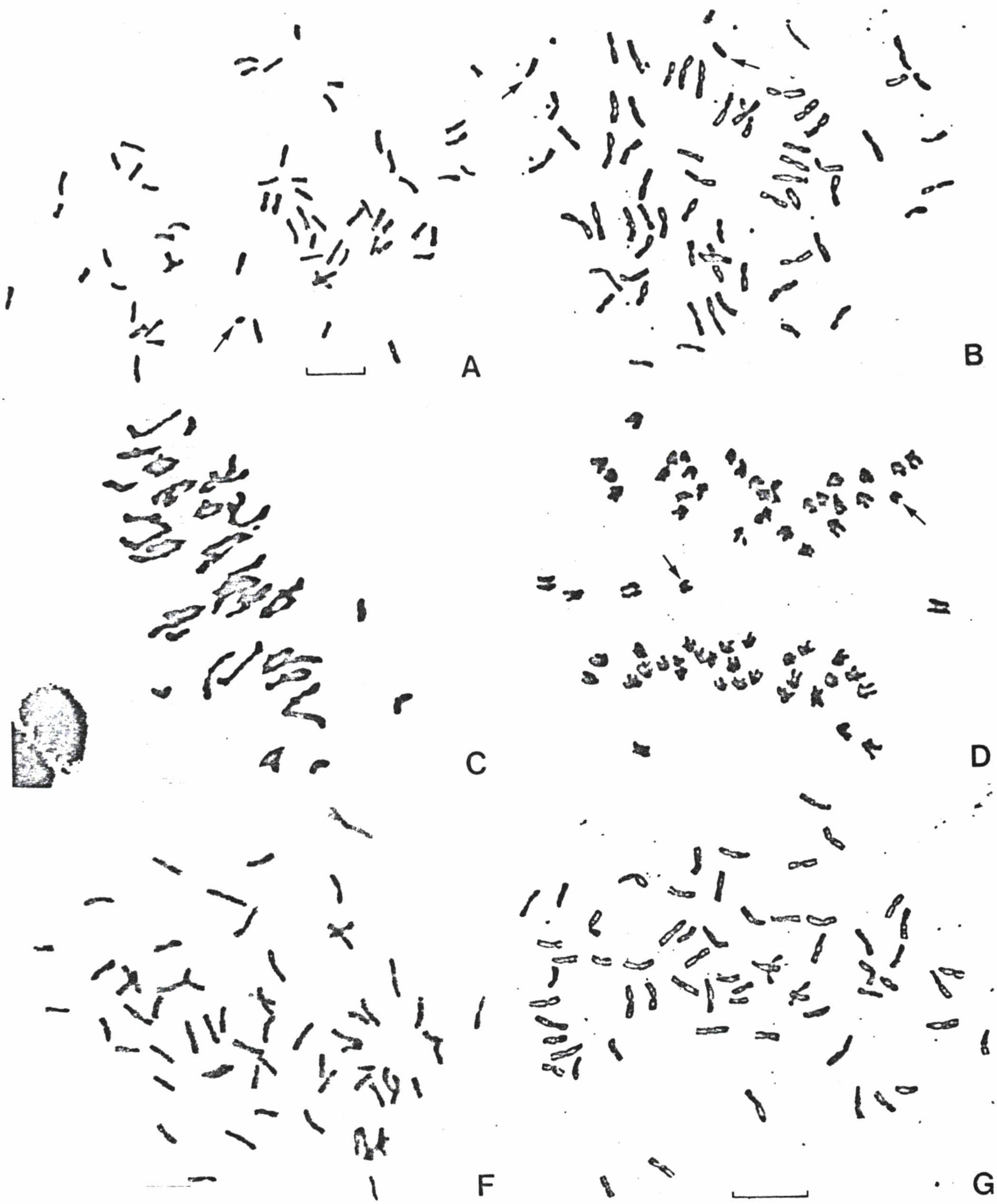

Figure 1

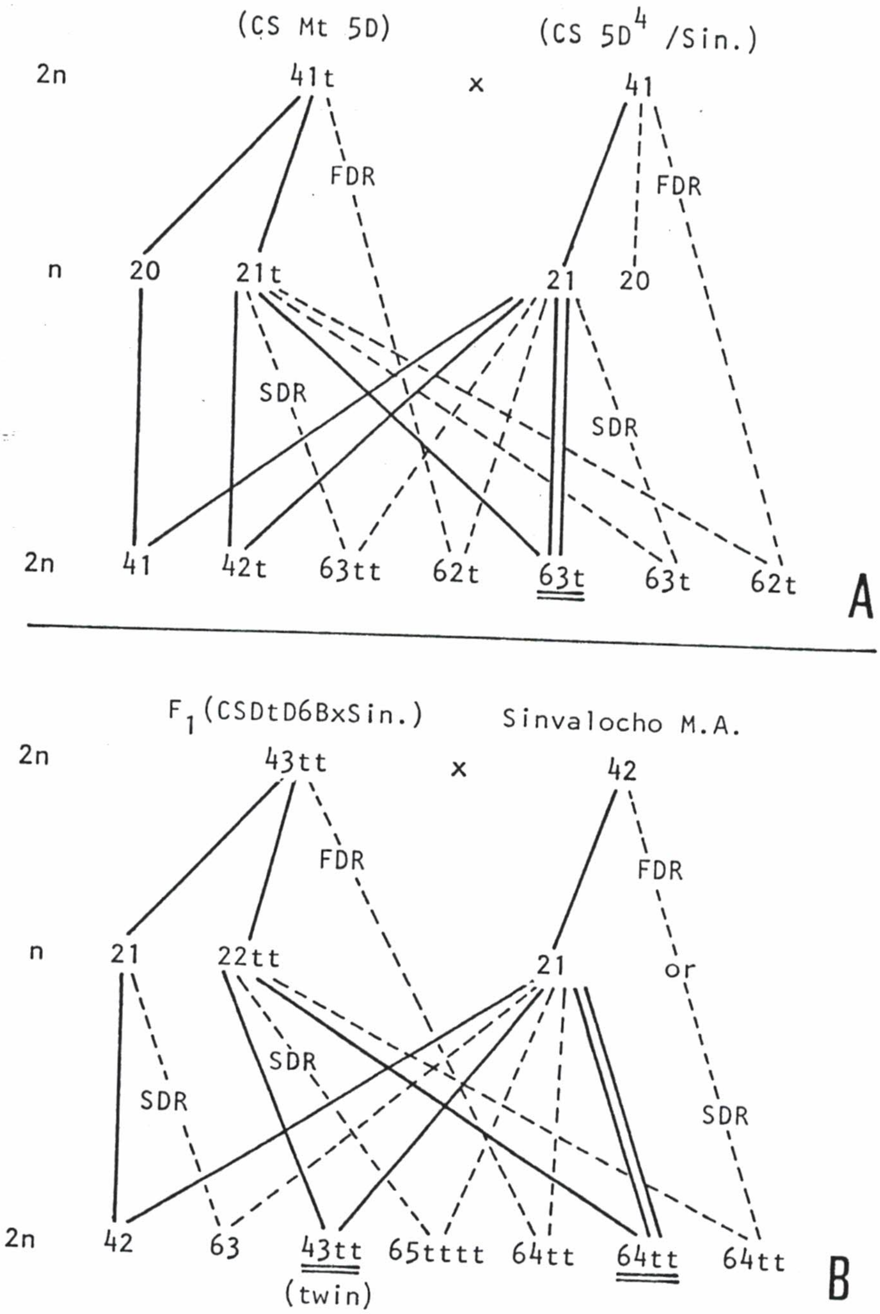
(CS Mt 5D)
(CS 5D4 /Sin.)
2n
41t
x
41
FDR
FDR
n
20
21t
21
20
SDR
SDR
2n
41
42t
63tt
62t
63t
63t
62t
A
F1 (CSDtD6BxSin.)
Sinvalocho M.A.
2n
43tt
x
42
FDR
FDR
n
21
22tt
21
or
SDR
SDR
SDR
2n
42
63
43tt
(twin)
65tttt
64tt
64tt
64tt
B

Figure 2

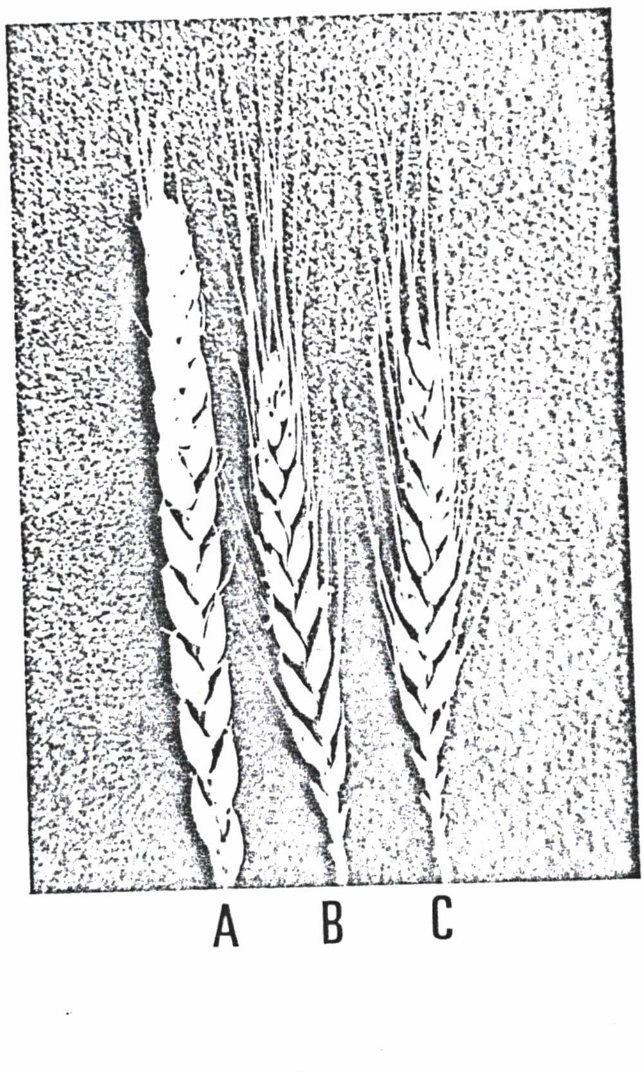

Figure 3