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Short Communication

Elimination of micronuclei from microspores in a Brazilian oat (*Avena sativa* L.) variety

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Abstract

Chromosome elimination in interspecific hybrids is a powerful tool in breeding programs. Of 12 Brazilian oat varieties recommended for cultivation in southern Brazil, one variety, UFRGS 15, presented a new type of chromosome elimination never described in any other species. Chromosomes that produced micronuclei during meiosis were eliminated from microspores as microcytes. The micronucleus reached the microspore wall and formed a kind of bud, separated from the microspore. The eliminated microcytes gave origin to small and sterile pollen grains. The possible applications of this finding for oat breeding are discussed.

INTRODUCTION

The genus *Avena* L. (Poaceae) has a basic chromosome number x = 7 and contains diploid, tetraploid and hexaploid species. *Avena sativa*, the cultivated and most important commercial oat, is a hexaploid species (2n = 6x = 42) with an AACCDD genomic constitution. In general, A-type genomes of diploid species show metacentric chromosomes, whereas in C-type genomes most chromosomes are subterminal. It is generally believed that hexaploid oats originated by tetraploidization of *A. maroccana* and *A. murphyi*, both with AC-type genomes (Leggett, 1984), while the origin of the D-type genome in hexaploid oats remains unknown.

Unlike the large number of cytogenetic studies that have been dedicated to elucidating the origin and evolution of the genus *Avena*, few studies are available on the evaluation of meiotic behavior. McMullen *et al*. (1982) evaluated the meiotic process in American hexaploid oats (*A. sativa* and *A. sterilis*), while Guillin *et al*. (1995) investigated these processes in *A. sativa* lines and hybrids from Argentina. In both of these studies the authors found meiotic irregularities, particularly those types related to chromosome segregation that produced micronuclei in tetrads, although these papers did not discuss the fate of these micronuclei. In an extensive evaluation of meiosis in 12 of the 17 Brazilian oat varieties recommended for planting in southern Brazil we found numerous types of meiotic abnormalities, some of which gave rise to micronuclei that remained until the tetrad stage. In one variety, UFRGS 15, we observed a different pattern, never before described in any other species, with microspore micronuclei being eliminated as microcytes. This phenomenon and its importance for breeding will be discussed in this study.

MATERIAL AND METHODS

The varieties were chosen on the basis of their productivity as determined in a 1997 trial without fungicide application, the strains being UFRGS 16, UPF 16, CTC 3, UPF 17, UFRGS 7 and UFRGS 17 (the 6 most productive) and CTC 1, UPF 14, UFRGS 10, UPF 7, UFRGS 15 and UPF 13 (the 6 least productive). The varieties were cultivated in fields belonging to the Cooperativa Agrária Mista de Entre Rios (Guarapuava, Entre Rios district, PR, Brazil).

Panicles were collected for meiotic analysis between 8:00 and 10:00 a.m. and fixed for 24 h in Carnoy solution (ethanol:acetic acid, 3:1 v/v), after which they were transferred to 70% alcohol and stored at 4ºC. Pollen mother cells (PMCs) were prepared by the squash technique and stained with 1% propionic carmine. Five plants per variety were analyzed. The same procedures were employed to test pollen fertility.

RESULTS AND DISCUSSION

The main meiotic abnormalities observed in the 12 varieties were related to chromosome segregation. During diakinesis there was a low frequency of univalent chromosomes, but during metaphase I the number of cells with univalents presenting precocious migration to the poles was high. McMullen *et al*. (1982) also found an increase in the frequency of univalents from diakinesis to metaphase I in American oat hybrids (*A. sativa*/*A. sterilis*), and suggested

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that desynapsis occurred in the bivalent. We believe that the increase in the frequency of univalents in metaphase I in the varieties analyzed can also result from precocious chiasma terminalization, because the chiasma frequency per microsporocyte was high in all varieties (mean = 41.66 to 42.00), and the majority of bivalents presented two terminal chiasmata.

Because univalents usually do not suffer regular segregation in the first division, the frequency of univalents in diakinesis/metaphase I has been used as a standard measure of meiotic disturbances in other crop species (Scoles and Kaltsikes, 1974). In general, univalents migrate precociously to the poles or behave as laggards in anaphase, but in both cases they can produce micronuclei in telophase I which normally remain until the tetrad stage (Koduru and Rao, 1981). Although chromosome not included in the nucleus during the first or second division can produce micronuclei in the tetrad, the fate of these micronuclei in microspores and pollen grains has not been discussed in the literature. In 11 of the oat varieties used in our experiments, a micronucleus remained in the microspore after dissolution of the callose wall of the tetrad and reached the pollen grain stage (Figure 1a-d). However, Figure 2 shows that in one variety, UFRGS 15, after microspore formation micronuclei were eliminated by a curious mechanism in which one or more micronuclei approached the microspore wall (Figure 2a) and formed a kind of bud (Figure 2b) which separated from the microspore and was eliminated as a microcyte (Figure 2c) that gave rise to small, sterile, pollen grains (Figure 2d).

The elimination of a micronucleus from the microspore is a kind of chromosome elimination, a common phenomenon in interspecific and intergeneric hybrid embryos produced as a result of crosses between certain plant species. Well-known examples being crosses between *Hordeum vulgare* and *H. bulbosum* (Davies, 1974), crosses between other *Hordeum* species (Jorgensen and Bothmer, 1988) and interspecific hybrids in the genera *Nicotiana* (Gupta, 1969) and *Solanum* (Pijnacker *et al.*, 1989). The combination of two distinct genomes in hybrids frequently results in aberrant mitotic and meiotic divisions, and sometimes leads to chromosome elimination, and a positive correlation between chromosome elimination and genetic distance between species has been found in Brassicaceae (Sundberg and Glimelius, 1991). In our experiments, the micronucleus elimination observed is surprising because *Avena sativa*, although being a allohexaploid species, is meiotically stable, with chromosomes pairing as 21 bivalents.

Several examples in the literature show that chro-

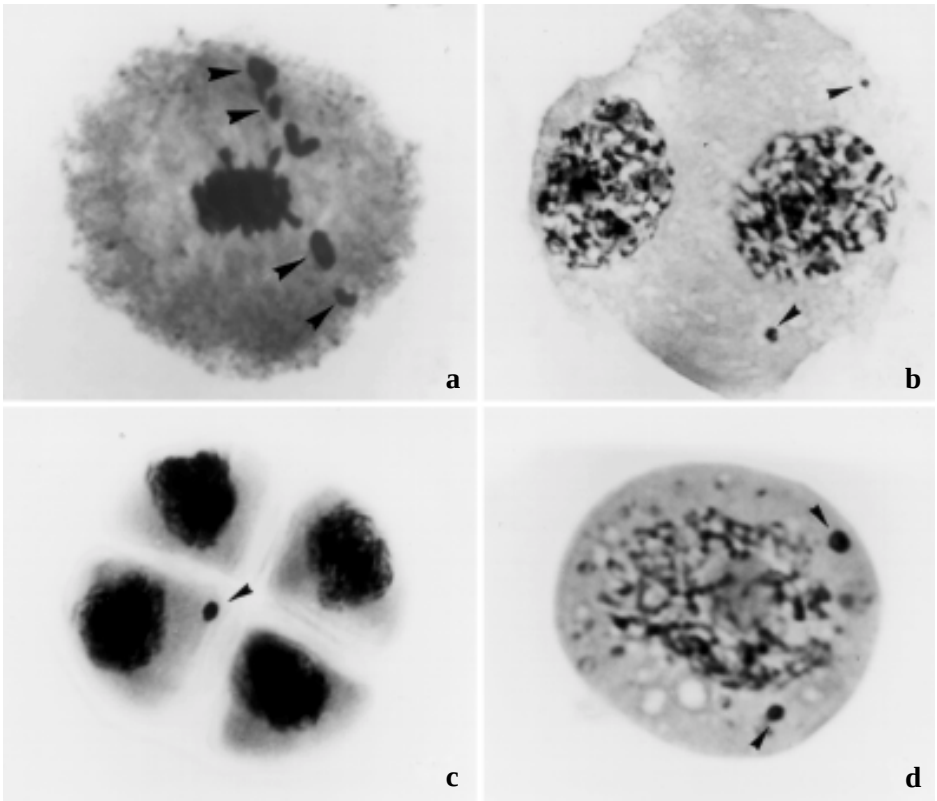

Figure 1 - The origin and general behavior of micronuclei in oat varieties. Arrowheads show: **a**) metaphase I with non-oriented bivalents at the equatorial plate and univalent chromosomes migrating precociously to the poles, **b**) telophase I with different sized micronuclei, **c**) tetrad showing a micronucleus in one microspore and **d**) microspore showing two micronuclei.

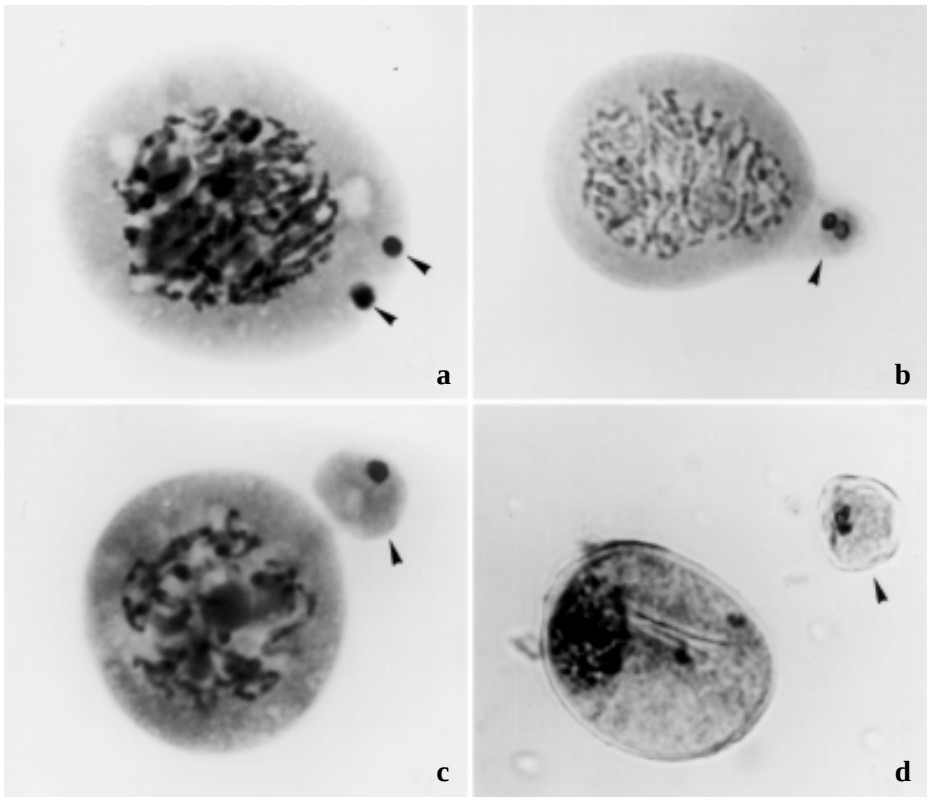

Figure 2 - The process of micronucleus elimination in the oat variety UFRGS 15. Arrowheads show: **a**) microspore showing two peripheral micronuclei, **b**) microspore showing bud formation with the micronuclei inside, **c**) elimination of the bud from the microspore as a microcyte containing the micronucleus and **d**) small, sterile, pollen grain produced from the microcyte.

mosome elimination can be partial or total for one parental genome (Adamowski *et al*., 1998) and in some cases such elimination is tissue-dependent and affected by the physiological conditions of the cells. Elimination of micronuclei in the microspore stage, as found in this oat variety, has not been reported for any species.

Many mechanisms of chromosome elimination have been described, including chromosome fragmentation, micronucleus formation, chromatin degradation, non-oriented chromosomes at metaphase and laggards in anaphase (Singh, 1993). Several hypotheses have been suggested in an attempt to explain the phenomenon, including inactivation of chromosomes by nuclease, formation of multipolar spindles, asynchrony in nucleoprotein synthesis, genome ratios, spatial separation of genomes, suppression of centromere function in the eliminated chromosomes, asynchronous cell cycle phases, and asynchronous mitotic and meiotic rhythms (Adamowski *et al*., 1998), but the exact mechanism is still obscure. Chromosome elimination in interspecific hybrids is a powerful tool in breeding programs, with differential chromosome elimination having facilitated the production of addition and substitution lines, while total elimination of one genome has permitted the formation of haploid lines. Interploidy gene transfer in *Avena* using monosomic alien substitution lines has been applied in oat breeding (Forsberg, 1990) and molecular cytogenetic techniques (Katsiotis *et al*., 1997) are being used for chromosome recognition in the genus. The elimination of micronuclei in microspores, accompanied by accurate recognition of the eliminated chromosome might also be useful in oat breeding.

RESUMO

Eliminação de cromossomos em híbridos interespecíficos é uma ferramenta poderosa em programas de melhoramento. Entre 12 variedades brasileiras de aveia recomendadas para cultivo na região Sul, uma (UFRGS 15) apresentou um tipo de eliminação cromossômica nunca antes descrita em outra espécie. Cromossomos que deram origem a micronúcleos durante a meiose foram eliminados do micrósporo como micrócito. O micronúcleo aproximava-se da parede do micrósporo, formava um tipo de brotamento e sofria separação do micrósporo. Os micrócitos eliminados deram origem a grãos de pólen pequenos e estéreis. As possíveis aplicações deste achado para o melhoramento de aveia são discutidas.

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