Acta Scientiarum 22(2):269-273, 2000.
ISSN 1415-6814.

Chiasma frequency, distribution and terminalization in hexaploid oats (*Avena sativa* L.)

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ABSTRACT. Chiasma counting has been regarded as the most straightforward method of scoring the total number of crossing-over events in the genome. In organisms in which genetic analysis is difficult or impossible to perform, it is a good estimator of the level of genetic recombination. In this paper we evaluated the chiasma frequency and distribution at diakinesis of twelve Brazilian oat varieties (*Avena sativa*), a hexaploid species with cytologically well-defined meiotic chromosomes. There was a high homogeneity of chiasma formation among varieties. The chiasma frequency per microsporocyte ranged from 41.66 to 42.00 among varieties. The frequency of interstitial and terminal chiasmata was more variable. The bivalents may present one, two or three chiasmata, but those with two terminal chiasmata were more frequent. The frequency of univalents was very low at diakinesis but high in metaphase I, suggesting precocious chiasma terminalization. The implications of these findings for oat breeding are discussed.

Key words: chiasma frequency, chiasma distribution, chiasma terminalization, oat.

RESUMO. Freqüência, distribuição e terminalização de quiasmas em aveias hexaplóides (*Avena sativa* L.). A contagem de quiasmas tem sido reconhecida como um dos métodos mais apurados para se avaliar o número total de eventos de permuta genética no genoma. Em organismos em que a análise genética é difícil ou impossível de realizar, ela torna-se um bom índice para a avaliação do nível de recombinação genética. Neste artigo, avaliaram-se a frequência e a distribuição de quiasmas na diacinese em 12 variedades brasileiras de aveia (*Avena sativa*), uma espécie hexaplóide com cromossomos meióticos citologicamente bem definidos. Houve uma alta homogeneidade na formação de quiasmas entre as variedades. A frequência de quiasmas por microsporócitos variou de 41,66 a 42,00 entre as variedades. As frequências de quiasmas terminais e intersticiais foram mais variáveis. Os bivalentes apresentaram um, dois ou três quiasmas, havendo predominância de bivalentes com dois quiasmas terminais. A frequência de univalentes foi baixa na diacinese, mas alta em metáfase sugerindo terminalização precoce de quiasmas. As implicações destes resultados para o melhoramento de aveia são discutidos.

Palavras-chave: frequência de quiasmas, distribuição de quiasmas, terminalização de quiasmas, aveia.

The genetic variability of a population results from the action of a series of genetically controlled factors that are also under the action of natural selection. Particularly significant among the factors is genetic permutation (crossing-over) which involves chromatin exchange between homologous chromosomes with consequent intrachromosomal recombination, giving rise to new combinations of alleles. The sites of genetic exchange are cytologically visible as "crosses" between the arms of chromosome pairs during late prophase I. These crosses were originally described by Janssens (1909) and termed as "chiasmata". The chiasmatype theory, i.e., Janssens's idea that chiasmata are formed at the sites of genetic exchange, sparked a debate that continued for over half a century. The most convincing evidence supporting the chiasmatype theory was provided by Tease and Jones (1978) using techniques for differential sister chromatid labeling.

Chiasma frequency is usually considered a good estimate of the level of genetic recombination in a population, especially in organisms in which genetic analysis is difficult or impossible to perform (Colombo, 1992). In the last century, mean chiasma values have been determined for many species with cytologically well-defined meiotic chromosomes (Nilsson *et al*., 1993). Darlington (1929) proposed an index to estimate genetic recombination in a population by chiasma counting. The recombination index consisted of the sum of the haploid number (n) and the average chiasma number per nucleus (x). The index (n + x) represents the average number of elements that segregate independently, so that the number of possible gametes is equal to $2^{(n + x)}$. However, in recent years, cytogeneticists have proposed a more accurate approach to estimate the recombination index taking into consideration the chromosome length and the exact position of chiasmata (Colombo, 1992).

The cultivated hexaploid oat (*Avena sativa*) presents cytologically well-defined chromosomes that facilitate chiasma counting. Few studies on chiasma evaluation in the genus *Avena* are available (Spier, 1934; Guillin *et al.*, 1995). Due to many factors, the cultivation of *Avena sativa* has increased in Brazil over the last years and seventeen varieties have been recommended for planting in the southern area. In the present study we evaluated the chiasma frequency, location and terminalization of twelve of these varieties.

Material and methods

Among the seventeen oat varieties currently recommended for the southern region, twelve were analyzed. We chose these varieties on the basis of their productivity, i.e., we chose the six most productive ones (UFRGS 16, UPF 16, CTC 3, UFP 17, UFRGS 7 and UFRGS 17) and the six least productive ones (CTC 1, UPF 14, UFRGS 10, UPF 7, UFRGS 15 and UPF 13) as determined in a 1997 trial without fungicide application (Table 1). The varieties were cultivated in the fields of the Cooperativa Agrária Mista de Entre Rios, situated in the district of Entre Rios, municipality of Guarapuava (state of Paraná).

For meiotic analysis the panicles were collected between 8:00 and 10:00 A.M. and fixed in Carnoy (ethanol: acetic acid, 3:1 v/v) for 24 h, 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. Chiasma counts were performed at diakinesis in 20 microsporocytes per plant. Five plants were analyzed per variety. We determined chiasma frequency per microsporocyte and frequency of interstitial and terminal chiasmata. We considered as interstitial chiasmata those that occupied a bivalent position where the chromosome ends could be seen. The frequency of univalent chromosomes was evaluated at diakinesis and metaphase I. Data were analyzed statistically by analysis of variance in a fully randomized design.

Results and discussion

The cytological analysis of microsporocytes revealed that oat bivalents may present one, two or three chiasmata. Bivalents with one or three chiasmata are less frequent. In general, when the bivalent shows only one chiasma, it is terminal; however in bivalents with two chiasmata, they can be terminal or interstitial or a combination of both types. Figure 1 illustrates a diakinesis with 21 bivalents.

Table 1. Chiasma frequency per microsporocyte (CFm), interstitial (CFi) and terminal (CFt) chiasmata frequency accompanied by its coefficient of variation among plants (CV) and the percentage of univalent chromosomes at diakinesis and metaphase I

Variety	CFm		CFi		CFt		Univalent chromosomes(%)	
	M	CV(%)	M	CV(%)	M	CV(%)	Diakinesis	Metaphase I
UFRGS 16	41.68 a	0.43	5.26 c	13.48	36.42 a	1.58	1.40	0.85
UPF 16	41.86 a	0.62	6.47 bc	16.47	35.39 abc	2.39	0.05	1.13
CTC 3	42.00 a	0,00	6.22 bc	26.74	35.78 a b	4.65	0.00	24.38
UPF 17	41.88 a	0.20	5.66 c	20.74	36.22 a	3.21	0.05	1.47
UFRGS 7	41.84 a	0.50	9.69 a	5.10	32.18 d	1.09	0.08	3.11
UFRGS 17	41.96 a	0.13	8.10 ab	15.35	33.80 bcd	3.71	0.02	1.91
CTC 1	41.94 a	0.32	6.90 bc	6.32	35.04 abc	1.04	0.04	1.58
UPF 14	41.80 a	0.41	6.85 bc	8.13	34.94 abc	1.60	0.08	1.55
UFRGS 10	42.00 a	0.17	6.31 bc	13.57	35.91 ab	2.09	0.01	13.77
UPF 7	41.98 a	0.26	8.65 ab	18.90	33.33 cd	4.63	0.02	3.42
UFRGS 15	41.66 a	1.22	6.23 bc	21.28	35.45 abc	3.4	0.13	1.18
UPF 13	41.72 a	0.39	4.69 c	29.41	37.02 a	3.50	0.13	9.41

Means with the same letter were not significantly different by Tukey's test ($p < 0.05$)

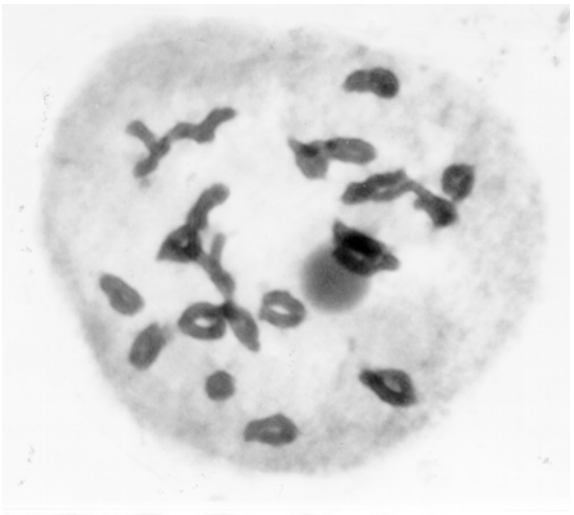

Figure 1. Oat microsporocyte at diakinesis showing 21 bivalents with interstitial and terminal chiasmata

Table 1 shows the mean chiasma frequency per microsporocyte (CFm), interstitial chiasma frequency (CTi) and terminal chiasma frequency (CFt) for the 12 oat varieties. As a measure of the stability of chiasma formation among plants/variety, we calculated the coefficient of variation among plants (CV).

Cytological analysis showed high homogeneity for chiasma formation among varieties. The chiasma frequency per microsporocyte (Table 1) ranged from 41.66 to 42.00 and analysis of variance did not detect differences among varieties. For interstitial and terminal chiasma frequency, analysis of variance showed some heterogeneity among varieties ($p < 0.05$) (Table 1). With respect to the stability of chiasma formation, the coefficient of variation (CV%) calculated among plants (Table 1) showed: (i) very low and homogeneous variation in chiasma frequency per microsporocyte, (ii) low and heterogeneous variation for terminal chiasma frequency, and (iii) high and highly heterogeneous variation of interstitial chiasma frequency.

Although chiasma counting has been regarded as the most straightforward method of scoring the total number of crossing-over events in the genome, it may be insufficient when different populations of the same species need to be compared. In population cytogenetic studies, where the calculation of changes in recombination levels is central to the adaptationist arguments, it is interesting to evaluate the chiasma position. If we consider that genetic exchange is an enormous source of variability, it is necessary to admit that the genetic recombination level promoted by this event depends not only on its frequency, but also on the sites where the exchange occurred. According to Zarchi *et al.* (1972) and Hillel *et al.* (1973), terminal chiasmata have only a physical function for the maintenance of the bivalent structure to ensure perfect chromosome segregation, while interstitial chiasmata are effective in genetic recombination. In this context, Sybenga (1972) postulated that when the chiasmata are strictly terminal, despite their high frequency the organism is free in recombination. However, when the interstitial chiasma frequency is considered as the main event at the genetic recombination level, caution must be taken because of the chiasma terminalization phenomenon. This phenomenon was originally described by Darlington (1929) as an increasing proportion of terminally located chiasmata during the condensation of chromosomes in the late prophase of meiosis. Later, terminalization was defined as the moving of chiasmata to the ends of bivalents, which begins in diplotene and continues up to and includes metaphase I. Nowadays the concept of chiasma terminalization has been widely revised and discussed (Vysotskaya, 1995; Bascom-Slack *et al.*, 1997), because the rate of terminalization may be different for each species, varying from complete terminalization to its complete absence. On the basis of a recent analysis of the phenomenon, Vysotskaya (1995) concluded that meiotic terminalization of chiasmata as postulated by Darlington (1929) does not occur. The chiasmata remain at the sites where they appear until the disjunction of homologous chromosomes in anaphase I. The disjunction of homologues is accounted for the repulsion of the sister chromatids.

In the oat varieties studied here there was a predominance of terminal chiasmata. Despite the low frequency of interstitial chiasmata, there was a considerable variation in this character among varieties suggesting that they could differ at the genetic recombination level. This is interesting because oat is an autogamous species that presents a reduced rate of outcrossing. Yet in the context of genetic variability, it is necessary to consider the variation observed in interstitial chiasma frequency among plants in each variety. The coefficient of variation for interstitial chiasmata among plants revealed that it is possible to find plants with a high frequency of interstitial chiasmata. These differential characteristics among oat varieties might be interesting for breeding purposes.

Despite some opinions (Vysotskaya, 1995) contrary to the old concept of chiasma terminalization as postulated by Darlington (1929), the results obtained for the oat varieties studied here

are very interesting. The frequency of univalents at diakinesis was very low, while the number of cells presenting precocious migration of univalents to the poles in metaphase I was high (Table 1). An increase in the frequency of univalents from diakinesis to metaphase I was also found in the American oat hybrids of *Avena sativa/Avena sterilis* (Mc Mullen *et al.,* 1982) and the authors suggested the occurrence of desynapsis in the bivalent. We believe that the increase in the frequency of univalents in metaphase I in the varieties analyzed may also result from precocious chiasma terminalization because the chiasma frequency per microsporocyte at diakinesis was high in all varieties and most bivalents presented two terminal chiasmata. A hypothesis to explain chiasma terminalization was proposed by Maguire (1974). The "chiasma binder" hypothesis postulates that the presence of a chiasma alone is not sufficient to ensure bivalent structure until the beginning of anaphase I. The chiasma only favors the integrity of the bivalent if the sister chromatids are joined. The chiasma itself appears only because of this junction (Vysotskaya, 1995). Some data reviewed by Bascom-Slack *et al.* (1997) suggest that distal chiasmata may be less able to maintain the association of homologues than more internal ones, such that some terminal chiasmata fail, leaving these homologues to be partioned into univalents.

Some authors (Maguire, 1990, 1993; Suja *et al.,*1992) postulated a force that joins and integrates the sister chromatids. The importance of the junction between sister chromatids for the maintenance of bivalent integrity is especially clear in the case of bivalents with one chiasma. According to Vysotskaya (1995), the analysis of such bivalents reveals that the disjunction of sister chromatids is necessary for the homologues to be able to separate. The nature of the sister chromatid cohesion is not well known. Several proteins have become candidates for promoting cohesion between sister chromatids, among them the topoisomerase, especially topoisomerase II (for review, see Miyazaki and Orr-Weaver, 1994; Bascom-Slack *et al.,* 1997). This enzyme decatenates sister chromatids and allows recombined homologues to segregate. As the homologues separate the chiasma moves toward the telomeres.

Independently of their origin, univalent chromosomes compromise pollen viability because they usually do not suffer regular segregation in the first meiotic division. In general, they migrate precociously to the poles or behave as laggards in anaphase. In both cases, they can originate micronuclei in telophase I and these micronuclei, in general, remain until the tetrad stage (Koduru and Rao, 1981). Because of this common behavior in many plant species, Scoles and Kaltsikes (1974) proposed the evaluation of the frequency of univalents at diakinesis/metaphase I as a standard measure of meiotic disturbance in crop plants.

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Received on April 10, 2000.
Accepted on May 30, 2000.