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Chromosome pairing in tall fescue haploids derived by anther-panicle culture

ABSTRACT: Meiotic chromosome pairing relationships and female fertility were examined in androgenic haploids of tall fescue (*Festuca arundinacea* Schreb., $2n = 6x = 42$). Chromosome associations observed at metaphase I in six haploid plants ranged from 15.98 to 20.40 univalents, 0.30 to 2.56 bivalents, and 0.00 to 0.18 trivalents. A low level of female fertility was demonstrated by the production of euploid, monosomic, and double monosomic progeny from the haploid plants after open-pollination by adjacent tall fescue. Our observations do not support a previously proposed hemizygous ineffective mechanism of chromosome pairing, which predicts high levels of pairing in haploids. However, our observations do support the hypothesis that chromosome pairing in tall fescue is controlled by a number of genes and/or structural differences that affect pairing. Better understanding of chromosome pairing will benefit basic genetic studies of tall fescue and aid in interspecific and intergeneric hybridization.

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TALL FESCUE (*Festuca arundinacea* Schreb., $2n = 6x = 42$) is an economically important pasture and turf grass. It is classified as both an allohexaploid[5] and an auto-allohexaploid[9] based on the mode of chromosome pairing in euploids, hybrids, and haploids. Intergenomic chromosome pairing has been observed in hybrids between ryegrass (*Lolium multiflorum* Lam. and *L. perenne* L., $2n = 2x = 14$) and tall fescue[16]. Also, the only previously reported haploid of tall fescue had an average of four bivalents per cell[10]. Based on these data and the observation that euploid tall fescue formed only bivalents, Jauhar[4,5] proposed that a hemizygous-ineffective pairing mechanism functioned in tall fescue. The present paper deals with meiotic pairing in tall fescue haploids.

Haploid plants obtained via twin seedlings, although very low in frequency, have been used for investigation of genome relationships in some polyploid species[11,13]. The classic example is bread wheat (*Triticum aestivum* L., $2n = 6x = 42$), in which an average of 1.3 to 1.7 bivalents was found in the euhaploid. A 20-chromosome nullihaploid had an average of 4.2 bivalents and 0.8 trivalents per cell[12]. This nullihaploid was missing chromosome 5B, which carries the major gene that suppresses pairing of homoeologous chromosomes[12,14]. Monosomics of wheat have been obtained by pollinating haploids with fertile pollen[13]. Haploids and monosomics have been useful in a number of basic studies, but a more rapid means to obtain large numbers of haploids and their derivatives should enhance basic genetic studies and improvement of forage grasses.

Androgenic haploid plants of tall fescue were derived from anther-panicle cultures[7]. Somatic metaphase counts of the plantlets showed that 22 of the 23 examined were haploids, $n = 21$. The 22 haploid lines were studied for physical and chemical characteristics under field conditions[6], and potential usefulness of the haploids and their derivatives is under study. Better understanding of genome homology in these materials is needed. Objectives of the present study were to investigate genomic relationships as follows: examine meiotic pairing in tall fescue haploids; study implications of chromosome pairing in the haploids; and observe chromosome pairing in progeny of haploids that were open-pollinated with fertile pollen from tall fescue plants.

Materials and Methods

Androgenic haploids that were derived by anther-panicle cultures from field-grown tall

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fescue (*Festuca arundinacea* Schreb. cv. Kentucky 31)[7] were studied. The haploid plantlets were increased through tillers. They were established in pots in a greenhouse and then transplanted to a field plot. After undergoing vernalization in the field, representative plants of six of the haploid lines were randomly selected and brought into a greenhouse to develop inflorescenses for meiotic analyses. Some of the plants remained in the field plot near a tall fescue nursery to study effects of open pollination of the haploids.

Panicles were fixed in Carnoy's A (6 parts ethanol: 3 parts acetic acid: 1 part chloroform, v/v/v) with a trace of iron chloride added. For meiotic analyses, the anthers were removed, stained with propionic carmine, squashed and observed under a light microscope. Chromosome pairing configurations at metaphase I were recorded. The computer model of Alonso and Kimber[1] was used to analyse these data for genome relationships.

Haploid plants that flowered in the field plot were open-pollinated by tall fescue plants growing in a nearby nursery. Panicles were harvested after they dried naturally on the plants. Most of the panicles were seedless, but a low percentage had some seed. The seed were sown on sterilized soil in July 1980. A few seedlings developed from five of the lines. These were grown in a protected nursery (lath house) until the autumn of 1981 when they were transplanted to a field plot to undergo vernalization. Panicles were collected from these plants in 1982 and 1983. They were fixed in Carnoy's A. Microspore mother cells were observed under a light microscope to determine chromosome numbers and amount of pairing.

Results and Discussion

Morphology

All of the haploids had smaller panicles and shorter, narrower leaves than the tall fescue plant from which they were derived. Some variation existed in leaf and panicle characteristics among the 22 haploid lines[6]. Representative panicles are shown in Figure 1.

FIGURE 1 Representative panicles from a Kentucky 31 plant (left) and an androgenic haploid from Kentucky 31 (right).

Meiotic analyses

At metaphase I, the haploid plants had from 9 to 21 univalents, 0 to 6 bivalents, and 0 to 1 trivalents (Table I). Haploids 4, 7, and 22 had more chromosome pairing than haploids 1, 3, and 9. In the haploids with more pairing, the average number of univalents ranged from 15.98 to 18.10, bivalents from 1.33 to 2.36, and trivalents from 0.00 to 0.10. A cell with high pairing (5 bivalents and 11 univalents) is shown in Figure 2*A*. In haploids with less pairing, the average number of univalents ranged from 20.38 to 20.66 and bivalents from 0.17 to 0.31. A cell with no pairing (21 univalents) is shown in Figure 2*B*. Also, pseudo-bivalents (Figure 2*C*), which exhibited end-to-end pairing and other secondary associations, were observed at metaphase I. (Pseudo-bivalents display a "stickiness" between two chromosomes, which may indicate some homology.) Some bridges and fragments were observed at anaphase I (Figure 2*D*), suggesting that there were some structural differences between paired chromosomes. Binucleate and multinucleate cells as well as a wider than usual range of meiotic stages were observed at all stages of meiosis in a given anther (Figure 2*E*).

Tall fescue ($2n = 6x = 42$) has been classified as both an allohexaploid[5] and an autoallohexaploid[9]. In our haploids, the low frequency of bivalents and trivalents at metaphase I suggests that the paired chromosomes are all homoeologous rather than some being homologous. Homoeology was confirmed using the computer model described by Alonso and Kimber[1]. The model was designed to determine relatedness of two species by using chromosome pairing observed at metaphase I in hybrids derived from the two species as an indication of chiasmata formation and thus homology. Similarly, the amount of chromosome pairing in a haploid plant derived from a polyploid species can be used to determine relationships of the genomes in an allopolyploid.

The Alonso-Kimber model was originally developed for allohexaploid bread wheat and its hybrids, but has been expanded to other species. Pairing observed in a wheat haploid averaged 18.05 univalents + 1.38 bivalents + 0.07 trivalents[11], which is similar to the overall average pairing observed in our tall fescue haploids (18.75 univalents + 1.04 bivalents + 0.04 trivalents), supporting the hypothesis that

Table I. Chromosome pairing at metaphase I in six androgenic haploids of tall fescue

Haploid	No. pollen mother cells	Chromosome pairing I (range)	II (range)	III (range)
No. 1	50	20.40 (13-21)	0.30 (0-4)	0.00
No. 3	65	20.38 (13-21)	0.31 (0-4)	0.00
No. 4	49	18.10 (11-21)	1.33 (0-5)	0.08 (0-1)
No. 7	50	16.96 (11-21)	2.02 (0-5)	0.00
No. 9	53	20.66 (19-21)	0.17 (0-1)	0.00
No. 22	50	15.98 (9-21)	2.36 (0-6)	0.10 (0-1)

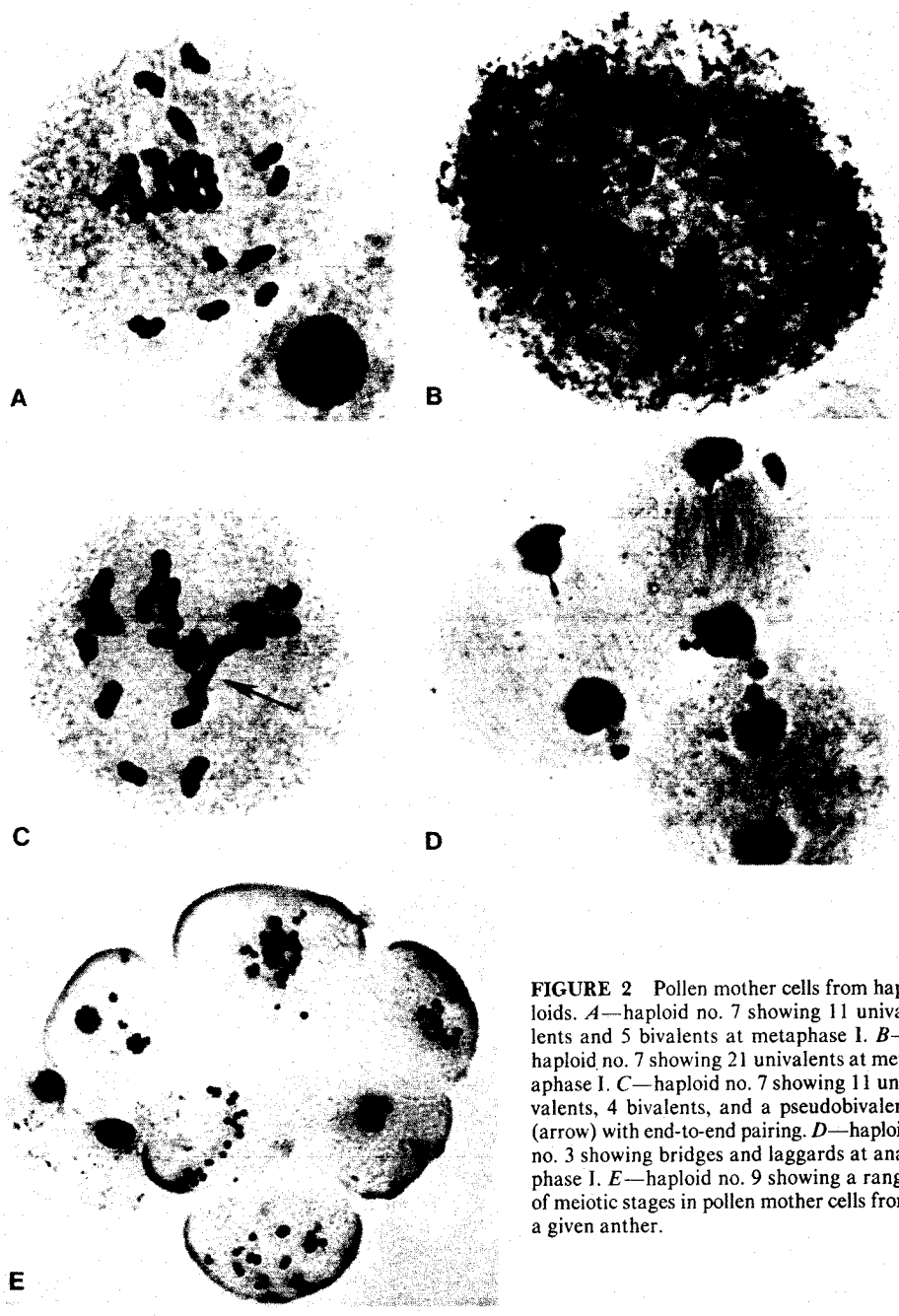

FIGURE 2 Pollen mother cells from haploids. *A*—haploid no. 7 showing 11 univalents and 5 bivalents at metaphase I. *B*—haploid no. 7 showing 21 univalents at metaphase I. *C*—haploid no. 7 showing 11 univalents, 4 bivalents, and a pseudobivalent (arrow) with end-to-end pairing. *D*—haploid no. 3 showing bridges and laggards at anaphase I. *E*—haploid no. 9 showing a range of meiotic stages in pollen mother cells from a given anther.

tall fescue is an allohexaploid with some homoeologous pairing. These observations suggest the genomic relationship AABBCC[5], rather than that of an autoallohexaploid AABBB′B′ [9].

Jauhar[4,5] proposed that a hemizygous-ineffective pairing mechanism functioned in tall fescue. The mechanism assumes that when the gene(s) for regulation of pairing is present in one dose, it is not effective in prevention of pairing between homoeologues. On the other hand, mainly bivalents are formed when a complete set of chromosomes is present and there are two doses of the gene(s) for regulation of pairing. The hypothesis was based on the observation that intergeneric as well as intergenomic pairing occurs in annual (and perennial) ryegrass × tall fescue hybrids. This is indicated by the presence of more than seven bivalents in the hybrids; for example, 3.10 univalents + 9.52 bivalents + 1.60 multivalents (trivalents and quadrivalents). Also, the only previously reported haploid of tall fescue[10] had an average of 12.96 univalents + 4.01 bivalents. That haploid, derived from a twin seedling, had a higher frequency of pairing than the haploids described in this paper. The lower chromosome pairing found in our haploids does not support a hemizygous-ineffective mechanism for controlling chromosome pairing.

An alternative hypothesis is that chromosome pairing in tall fescue is controlled by a number of genes similar to what has been reported for hexaploid wheat. Wheat has one major gene and a number of secondary genes controlling chromosome pairing[15]. If a number of genes controlled chromosome pairing, there would be a diffuse control. Some of the observed differences could be accounted for by segregation of the alleles at these loci in the microspores. Thus, the combined strength of the alleles that control pairing would determine the amount of chromosome pairing observed in the haploid (or hybrid) as the case may be.

The amount of chromosome pairing in tall fescue also might be influenced by structural differences, as suggested by bridges and fragments during anaphases I and II in the haploids. The fact that structural differences exist between different tall fescue accessions and cultivars has been shown by crossing the accessions and cultivars with each other and observing various univalents and multivalents at metaphase I, and bridges and fragments at anaphases I and II[3]. Also, Malik[8] observed structural differences at pachytene when he studied a tall fescue × tall fescue hybrid. Structural differences may have accounted for the different levels of pairing observed among our haploids (Table I and Figure 2*A–E*). Endrizzi[2] hypothesized such a mechanism in cotton whereby structural differences accounted for the lack of chromosome pairing.

Another argument supporting the structural difference hypothesis is the variation observed in chromosome pairing at metaphase I in our six haploid plants. Although our haploid plants were derived from the same panicle, pairing differences may have been a consequence of chromosomal segregation during meiosis. This assumes that different microspores received chromosomes with different structural changes during meiosis I. This might occur in Kentucky 31, from which our haploids originated, because it is a broadly adapted open-pollinated cultivar with greater variability than most other tall fescues.

In summary, the pairing mechanism in tall fescue seems to be more complex than previously proposed. Chromosome pairing probably is controlled by a number of different loci that are influenced by structural differences. Better understanding of the regulation of chromosome pairing will aid in the interspecific and intergeneric hybridization of forage grasses, especially for the incorporation of desirable characteristics from related species.

Open-pollinated progeny of haploids

Twenty plants were obtained following open-pollination of cytologically verified an-

Table II. Chromosome numbers and pairing behavior at metaphase I in open-pollinated progeny from five androgenic haploids of tall fescue

Haploid parent	No. progeny plants	Chromosome number	Chromosome pairing (II) (I)
No. 3	9	41	20 + 1
	1	40	19 + 2
No. 5	1	42	21
	1	41	20 + 1
	1	40	19 + 2
No. 6	1	42	21
	2	41	20 + 1
No. 7	1	42	21
	2	41	20 + 1
No. 8	1	42	21

drogenic haploid plants (Table II). The parent haploid plants had been grown in a nursery adjacent to other tall fescue plants, the presumed pollen source. After ripening in the field, the panicles were collected from eight of the haploid lines. A very low percentage of the seed germinated, and seedlings developed from five of the haploids. The progeny that survived to the flowering stage the following year ranged in chromosome number from 40 to 42 (Table II). Euploid (21 bivalents), monosomic (20 bivalents + 1 univalent), and double monosomic (19 bivalents + 2 univalents) plants were among the progeny. We hypothesize that these tall fescue plants resulted from fertilization of 21-, 20- or 19-chromosome ova with 21-chromosome pollen.

Derivation of some potentially useful euploid and monosomic plants by pollinating androgenic haploids with normal pollen indicates a possible direct use of haploids in a plant improvement program. The procedure would involve production of a large number of androgenic haploids from a heterozygous source, screening them under various stress situations, cytologically verifying haploidy, exposing selected haploids to normal pollen from a desired pollen source, cytologically verifying ploidy of the hybrids, and evaluating the plants under field situations. The number of progeny derived by this procedure would be extremely low, but it would be possible to use the haploids sooner than if they were doubled before they were used.

Further studies are in progress to double the haploids. Since tall fescue is out-crossing, doubled haploids will be valuable for forage grass breeders and geneticists because the doubled haploids should be both fertile and homozygous.

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