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Characterization of 'Sun II' oat monosomics through C-banding and identification of eight new 'Sun II' monosomics

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Abstract Monosomics are a powerful tool for genetic mapping in allopolyploid plant species such as oat (*Avena sativa* L., 2n = 6*x* = 42). A C-banded karyotype of the oat cultivar Sun II was compared with previously described oat karyotypes and was used to identify the missing chromosome in each line of Sun II aneuploids. These included new aneuploids, isolated among derivatives of oat haploids obtained from Sun II oat × maize crosses, along with the original Sun II aneuploid set which had been obtained by cytological screening of a Sun II population for spontaneous aneuploids. Eight new Sun II monosomics were identified among the derivatives of haploids from the oat × maize crosses, to give a total of 18 unique Sun II monosomic/nullisomic lines. All seven C-genome chromosomes are represented by Sun II monosomics. Chromosomes 13, 14 and 17 are not represented by Sun II aneuploids but are found in the Kanota monosomic series. Therefore, monosomics of some form are now available for all 21 oat chromosomes. A reciprocal translocation involving chromosomes 3C and 14, found in a portion of the original set of Sun II monosomic lines, was also described. No new translocations were detected in the Sun II × maize crosses.

Key words Oat (*Avena sativa* L.) · Monosomics · C-banding · Oat × maize wide hybridization · Translocations

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Introduction

Cytogenetic stocks, such as monosomics, nullisomics, nulli-tetrasomics, telosomics, and deletion lines, are useful for assigning genetic markers to chromosomes and chromosome segments (Hart 1979; Sharp et al. 1989; Anderson et al. 1992; Werner et al. 1992). In hexaploid oat (*Avena sativa* L.) only a small number of aneuploid lines in various genetic backgrounds were available for many years, and they had been used to assign only a few morphological and isozyme markers to chromosomes (Rajhathy and Thomas 1974; Price and Kahler 1983; Marshall and Shaner 1992). Also, in many of the cited oat studies, identification of the missing monosome or nullisome pair was not possible due to similarities in chromosome morphology. Recently, C-banding was demonstrated to be an effective method for discriminating among all 21 pairs of somatic oat chromosomes (Jellen et al. 1993 a). Using this method, Linares et al. (1992) and Jellen et al. (1993 b) identified the missing chromosomes in a set of monosomic lines developed by Morikawa (1985) in a 'Kanota' genetic background. Jellen et al. (1993 b) reported that among this set of lines, monosomics for 12 oat chromosomes were represented, with several lines being duplicates of one another. The partial Kanota

aneuploid series was subsequently used to assign RFLP markers to oat chromosomes (Rooney et al. 1994), which confirmed the occurrence of duplicates. The marker assignments identified two sets of potentially homoeologous chromosomes, but the remaining five homoeologous chromosome groups were not identifiable due to the incompleteness of the monosomic set and an apparently high frequency of intergenomic, non-homoeologous translocations (Rajhathy and Thomas 1974; Chen and Armstrong 1994; Jellen et al. 1994). Difficulties in clearly distinguishing dosage levels in DNA hybridization-blot autoradiograms also hindered the assignment of RFLP bands to monosomes (Rooney et al. 1994).

The other substantial set of oat aneuploids, in the Sun II genetic background (Hacker and Riley 1965), probably consists of 11 unique monosomic lines, as demonstrated by genomic in situ hybridization (GISH) (Leggett and Markhand 1995). Nullisomics are more readily obtained from the selfed monosomic lines in this genetic background than in the Kanota genetic background (Hacker and Riley 1965; Morikawa 1985). These Sun II nullisomics have proven useful in assigning molecular-marker loci to chromosomes (Mendu et al. 1993; Chen and Armstrong 1995; Kianian et al. 1997). For this reason, Sun II aneuploids are preferred over the Kanota monosomics for genetic mapping. A concerted effort has been made to extract new monosomic lines in the Sun II genetic background using derivatives of haploids generated by the oat × maize crossing technique (Rines and Dahleen 1990; Rines et al. 1997).

Previous oat cytogenetic studies employing C-banding, GISH, and in situ hybridization (ISH) with a cloned repetitive-sequence probe distinguished putative C-genome chromosomes or chromosome segments from those of the A and/or D genomes. In oat C-banding analysis, C-genome chromosomes stain more darkly than A/D-genome chromosomes, presumably due to their being more highly heterochromatic (Linares et al. 1992; Jellen et al. 1993 a). In GISH analysis, hybridization with labeled genomic DNA from either an A-genome *Avena* diploid (Chen and Armstrong 1994; Jellen et al. 1994) or a C-genome diploid (Leggett and Markhand 1995) enabled the visualization of chromatin of putative C-genome origin. Similarly, ISH using the labeled C-genome heterochromatin-specific repetitive-sequence probe pAm1 resulted in hybridization patterns in hexaploid oat similar to the C+ bands obtained by C-banding on chromosomes 1C–7C (Fominaya et al. 1995). None of these techniques enabled assignment of the 14 remaining pairs of chromosomes to the A or D genomes. All three techniques revealed the existence of multiple translocations between A/D- and C-genome chromosomes in various hexaploid oat cultivars. In addition, the presence of a large A/D-C intergenomic reciprocal translocation in 15 of the original 18 Sun II monosomic lines was detected using GISH (Chen and Armstrong 1994; Leggett and Markhand 1995). C-banding was employed to identify the breakpoints of what is presumed to be the same reciprocal translocation (or interchange) between terminal segments of the short arm of chromosome 3C and the long arm of chromosome 14 (Phillips et al. 1995).

The objective of the present study was to develop a C-banding karyotype for the cultivar Sun II which could then be used to determine the missing chromosome in each line of the Sun II aneuploid series, including new aneuploids isolated among the progeny of haploids obtained from Sun II oat × maize crosses.

Materials and methods

The oat × maize crossing method used to generate oat haploids and to derive the new Sun II aneuploids in the present study has been previously described (Rines and Dahleen 1990; Rines et al. 1997). A group of previously isolated Sun II aneuploid lines was kindly provided by H. Thomas and J. M. Leggett, Institute of Grassland and Environmental Research, Aberystwyth, UK. This set of lines had been isolated by screening large populations of Sun II for spontaneous aneuploids. The lines had been given Roman numerical designations based on the order in which they were identified as monosomic (Hacker and Riley 1963, 1965; Leggett and Markhand 1995). Selections of these lines and the new Sun II aneuploids were given 'AVA' ('*Avena* aneuploid') numerical designations at the University of Minnesota. Chromosome preparations and the C-banding technique were performed according to Jellen et al. (1993 a, 1994). Identification of monosomes was based on observations in at least three C-banded monosomic cells per line.

Results

A C-banded karyotype of the cultivar Sun II (Fig. 1) was generated to complement and compare with karyotypes previously described for cvs Kanota and Ogle (Jellen et al. 1993a). This karyotype was then used to identify the missing chromosomes in both the original and the newly recovered Sun II sets of monosomics and to determine their relationship to those described in the Kanota monosomic series (Jellen et al. 1993b). The C-banding analysis revealed 11 unique monosomics among the original 18 lines that had been isolated as spontaneous aneuploids in Sun II population cytological screenings (Table 1). The other seven were duplicates of some of the 11. The presence of duplicates had also been detected by Leggett and Markhand (1995) in GISH analysis and by Mendu et al. (1993) and Kianian et al. (1997) in molecular-marker analyses of this set. C-banding analysis of the 28 Sun II aneuploids, recently recovered among progeny of Sun II haploids derived from oat × maize crosses, identified monosomics for eight chromosomes for which there were no monosomics in the original Sun II set (Table 1).

Altogether 18 of the 21 chromosomes in the Sun II karyotype are now represented among the monosomic

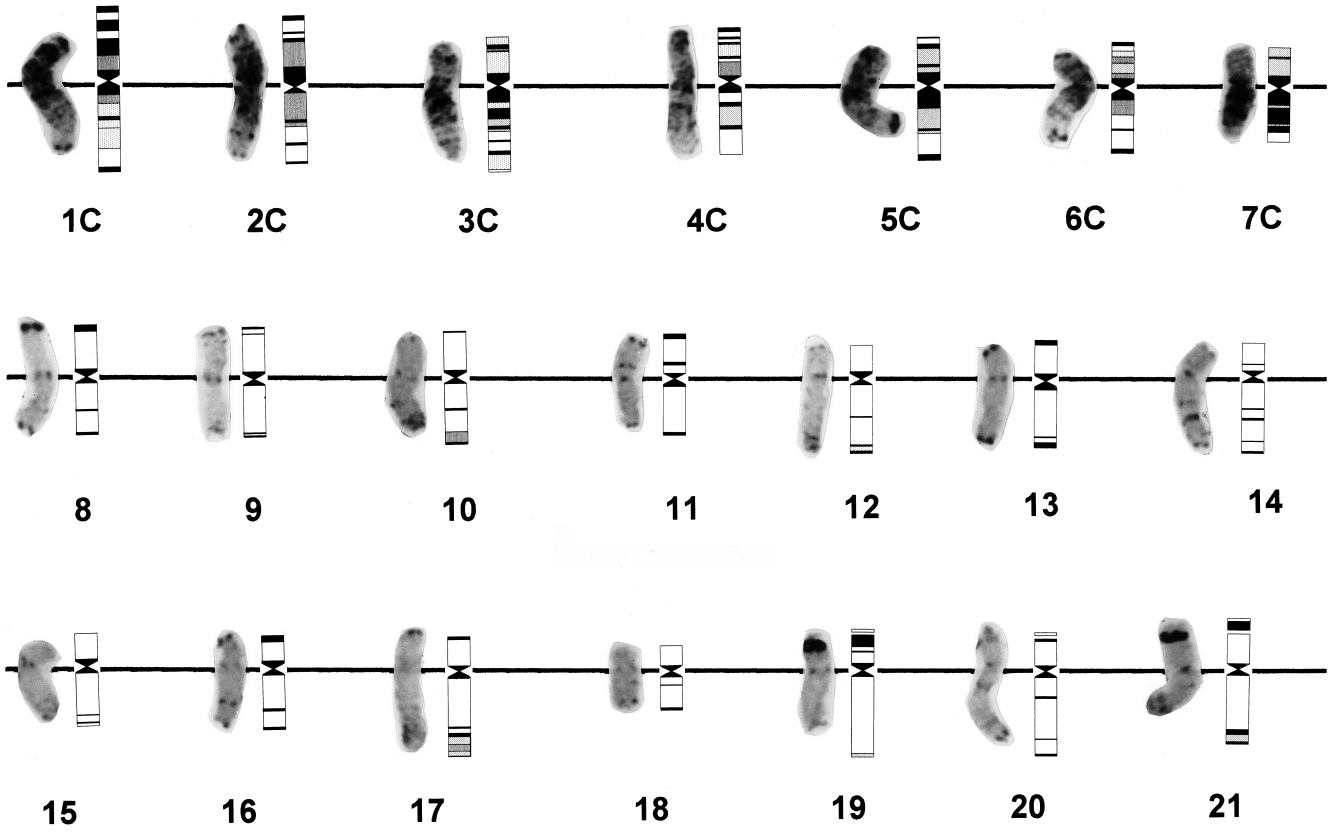

Fig. 1 Karyotype and idiogram of C-banded *A. sativa*cv. Sun II. C-genome chromosomes are arranged along the top row. *Areas of differing shading* on the idiogram reflect staining intensity differences observed in treated cells of at least ten separate preparations. The chromosomes are *numbered* according to the convention established in Jellen et al. (1993a, b) and Rooney et al. (1994)

lines (Table 1), excluding chromosomes 13, 14 and 17. Examples of C-banded chromosomes of Sun II in aneuploids are presented in Fig. 2. Our results using C-banding are in agreement with those of Leggett and Markhand (1995) who used GISH to identify C-genome monosomes of Sun II. These authors also noted that certain monosomic lines carried a large intergenomic reciprocal translocation. By C-banding, we determined that this interchange involves chromosomes 3C and 14 (Fig. 2). The 3C-14 interchange was present in 15 of the 18 original Sun II monosomics, indicating that the population from which this original set was isolated must have been heterogeneous for the translocation. One of the original Sun II monosomic lines, XI, is monosomic for the $3C^{14}$ translocation chromosome (Table 1). None of the new Sun II monosomic lines contained this translocation; nor did the Sun II re-selected line used as a parent in the oat × maize crosses. In the original set the only Sun II C-genome monosomics available were for chromosomes 5C (Mono IV) and 7C (Mono VII, XIV), as well as the translocation chromosome indicated above in Mono XI. The results of the present study indicate that each of the C-genome chromosomes in Sun II is represented by a new monosomic line. Furthermore, other new C-genome aneuploid lines have been recovered including Trisomic 5C (AVA 146), Monotelo 2C (AVA 558), and Nulli 2C/Mono 6C (AVA 152).

Discussion

Monosomics are now available in a Sun II background for 18 of the 21 oat chromosomes. Monosomics for three chromosomes, 13, 14 and 17, are available in Kanota but not Sun II. Among these, chromosome 13 appears to be morphologically identical in terms of its C-banding pattern in Kanota and Sun II. We therefore believe there are no major detectable chromosomal rearrangements involving 13 that would differentiate this chromosome in the two cultivars. Fox et al. (unpublished results) in assigning molecular markers to monosomes identified a potential interchange between Kanota and Sun II involving the long arm of chromosome 14 and chromosome 8. This interchange was not detected using C-banding, as chromosomes 14 and 8 have similar C-banding patterns in the two cultivars.

Banding polymorphisms were observed between C-banded Sun II and Kanota for chromosomes 7C and 17. Chromosome 7C has an arm-ratio difference in the two cultivars, being submetacentric in Sun II and

Table 1 Sun II monosomic/nullisomic line designations and the chromosome missing in each of them. The presence or absence of the 3C-14 reciprocal translocation is also given, along with the corresponding Kanota aneuploid line. Roman numerals refer to Sun II aneuploids isolated previously (Hacker and Riley 1965; Leggett and Markhand 1995). AVA numbers were assigned at the University of Minnesota. Chromosomes are numbered as in Jellen et al. (1993 a)

Aneuploid chromosome	Source of monosomic lines		
	Kanota	Sun II	Sun II/maize haploids
Monosome/Nullisome			
1C	K1, K2		AVA 555
2C	K3, K4		AVA 554, AVA 563
			AVA 152 (Nulli 2C, Mono 6C)
3C			AVA 557
4C			AVA 125, AVA 136
5C	K8	SIV (AVA 228)	AVA 585
6C			AVA 126, AVA 583
			AVA 152 (Nulli 2C, Mono 6C)
7C		SVII (AVA 76)[a]	AVA 131, AVA 157, AVA 159
		SXIV (AVA 97)[a]	
8	K6	SXVI (AVA 181)[a]	
		SXVII (AVA 104)[ab]	AVA 118, AVA 581
		SXVIII (AVA 107)[b]	
9			AVA 133, AVA 149, AVA 559
10			AVA 116
11	K19	SI (AVA 59)[a]	AVA 580
12		SV (AVA 69)	AVA 120
13	K9, K16		
14	K7		
15	K5, K10, K15, K20	SXV (AVA 99)[a]	
16	K18	SVI (AVA 72)[a]	
17	K11, K17		
18	K21	SII (AVA 62)[a]	AVA 578
		SIII (AVA 225)[a]	
		SXIII (AVA 94)[a]	
19	K12, K14	SXII (AVA 91)[a]	
20			AVA 572
21		SVIII (AVA 77)[a]	AVA 586
		SIX (AVA 83)[a]	
		SX (AVA 85)[a]	
Deficient segments			
$3C^{14}$		SXI (AVA 89)[a]	
14^{7C}	K13	2CL	AVA 558 (Monotelo 2CS)
21S			AVA 724 (Ditelo 21L)
Trisomic chromosome			
5C			AVA 146 (Trisomic 5C)

[a] Lines having the 3C-14 interchange
[b] Indicates a discrepancy with Leggett and Markhand (1995) regarding presence of 3C-14 interchange chromosomes

metacentric in Kanota (Jellen et al. 1993 b; Chen and Armstrong 1994; Phillips et al. 1995). Chromosome 17L is longer in Sun II than in Kanota due to the presence of a large C-genome translocation segment comprising approximately one-third of the distal long arm in Sun II (Chen and Armstrong 1994; Jellen et al. 1994; Phillips et al. 1995). Fox et al. (unpublished results) and O'Donoughue et al. (1995) generated RFLP mapping data which indicated that differences between Sun II and Kanota for these two chromosomes are most likely the result of an interchange. Furthermore, we now know from RFLP marker-chromosome assignments (Fox et al., unpublished results) that our previous labeling of chromosome 17 as chromosome 15 in Ogle (Jellen et al. 1993 a, 1994) was erroneous. The Ogle chromosome we previously labeled as 17 was therefore chromosome 15 (Jellen et al. 1993a). The C-banded karyotype of Ogle is essentially identical to that of Sun II (Fig. 1). A $3C^{14}$ translocation chromosome detected in some of the original Sun II monosomic lines was not present in Ogle, nor in any other of the hexaploid oat lines we have examined using C-banding (Jellen et al. 1993 b and unpublished results), indicating that this interchange was probably novel in the particular Sun II source of these monosomics.

Although we now have monosomics for chromosome 15 in Kanota and Sun II, this chromosome is heteromorphic between the two cultivars. Chromosome 15 has a slightly shorter long arm in Sun II than

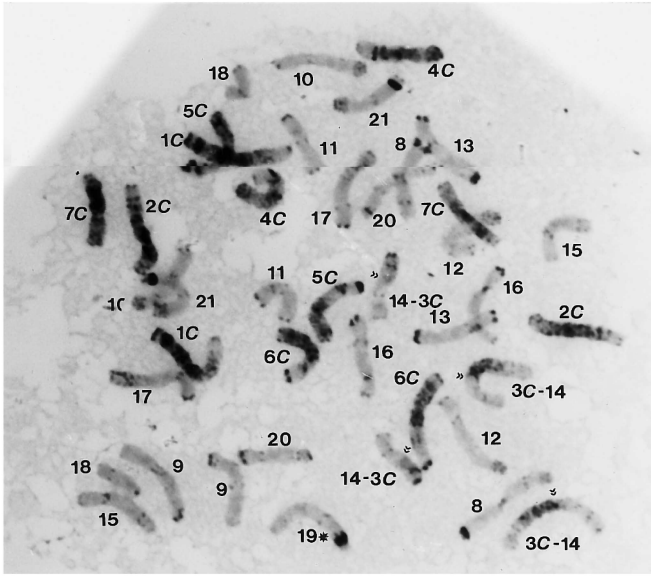

Fig. 2 C-banded root-tip cell from line AVA 91 (Mono XII), monosomic for chromosome 19 (*starred*). An intergenomic translocation involving chromosomes 3C and 14 is indicated with *arrowheads*. This translocation is missing in Sun II lines IV, V, and XVIII, as well as all Sun II aneuploid lines derived from haploids from oat × maize crosses. Magnification is 630

Kanota and has more distinct telomeric and intercalary long-arm C+ bands in Kanota.

Between the Kanota and Sun II monosomic series, we now for the first time have monosomics for all of the chromosomes of hexaploid oat (Table 1). We propose that each line be designated according to its missing chromosome and genetic background for example, "K-mono1C" for a Kanota line missing 1C, or "S-nulli7C" for a Sun II line nullisomic for 7C. As discussed above, it is possible that small chromosomal regions may not be represented in the combined Sun II and Kanota sets of monosomics due to C-banding polymorphisms in the two cultivars for monosomes included in only one of the two genetic backgrounds. Chromosomes 13, 14, and 17 are not represented by monosomics in the Sun II series. In the Kanota genetic background, chromosomes 3C, 4C, 6C, 7C, 9, 10, 12, 20, and 21 are not represented by monosomics. Nevertheless, the existence of a complete monosomic series in oat, albeit in two genetic backgrounds, should greatly facilitate the RFLP mapping effort in this species.

The presence of translocations exacerbates the identification of complete homoeologous groups in oat (Rooney et al. 1994); however, these rearrangements are advantageous in that they can be used to assign molecular markers to physically defined sub-chromosomal segments (Phillips et al. 1995; Fox et al., unpublished results). Wilson and McMullen (1997) have demonstrated that duplicate-deficient lines can be derived from crosses between two oat parents differing for an interchange. Such lines could be used to assign RFLP markers directly to deficient translocation segments, in much the same way as the *Aegilops cylindrica* Host gametocidal chromosome-generated Chinese Spring homozygous deletion lines are used in mapping wheat (Endo 1988; Werner et al. 1992; Endo and Gill 1996). Efforts to identify homoeologous relationships among the Sun II and Kanota oat monosomics using molecular markers (Rooney et al. 1994; Kianian et al. 1997) are ongoing.

The oat × maize crossing technique has greatly accelerated the generation of monosomics, and other aneuploids, in *A. sativa*. In the future, this technique should be useful to derive monosomics from haploids in oat cultivars that are more adapted to the primary growing regions of North America. In addition, this technique might prove useful in the isolation of aneuploids in *Avena* tetraploid genetic backgrounds.

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References

Anderson JA, Ogihara Y, Sorrells ME, Tanksley SD (1992) Development of a chromosomal arm map for wheat based on RFLP markers. Theor Appl Genet 83:1035–1043

Chen Q, Armstrong K (1994) Genomic in situ hybridization in *Avena sativa*. Genome 37:607–612

Chen Q, Armstrong K (1995) Characterization of a library from a single microdissected oat (*Avena sativa* L.) chromosome. Genome 38:706–714

Endo TR (1988) Induction of chromosomal structural changes by a chromosome of *Aegilops cylindrica* L. in common wheat. J Hered 79:366–370

Endo TR, Gill BS (1996) The deletion stocks of common wheat. J Hered 87:295–307

Fominaya A, Hueros G, Loarce Y, Ferrer E (1995) Chromosomal distribution of a repeated DNA sequence from C-genome heterochromatin and the identification of a new ribosomal DNA locus in the genus *Avena*. Genome 38:548–557

Hacker JB, Riley R (1963) Aneuploids in oat varietal populations. Nature 197:924–925

Hacker JB, Riley R (1965) Morphological and cytological effects of chromosome deficiency in *Avena sativa*. Can J Genet Cytol 7:304–315

Hart GE (1979) Genetical and chromosomal relationships among the wheats and their relatives. In: Redei GP (ed) 11th Stadler Genet Symp, Univ Missouri Agric Exp Stn, Columbia, Missouri

Jellen EN, Phillips RL, Rines HW (1993a) C-banded karyotypes and polymorphisms in hexaploid oat accessions (*Avena* spp.) using Wright's stain. Genome 36:1129–1137

Jellen EN, Rooney WL, Phillips RL, Rines HW (1993b) Characterization of the hexaploid oat *Avena byzantina* cv Kanota monosomic series using C-banding and RFLPs. Genome 36:962–970

Jellen EN, Gill BS, Cox TS (1994) Genomic in situ hybridization differentiates between A/D- and C-genome chromatin and detects intergenomic translocations in polyploid oat species (genus *Avena*). Genome 37:613–618

Kianian SF, Wu B-C, Fox SL, Rines HW, Phillips RL (1997) Aneuploid marker assignment in hexaploid oat with the C genome as a reference for determining homoeology. Genome 40 : 386–396

Leggett JM, Markhand GS (1995) The genomic identification of some monosomics of *Avena sativa* L. cv Sun II using genomic in situ hybridization. Genome 38 : 747–751

Linares C, Vega C, Ferrer E, Fominaya A (1992) Identification of C-banded chromosomes in meiosis and the analysis of nucleolar activity in *Avena byzantina* C. Koch cv 'Kanota'. Theor Appl Genet 83 : 650–654

Marshall HG, Shaner GE (1992) Genetics and inheritance in oat. In: Marshall HG, Sorrells ME (eds) Oat science and technology. ASA Monograph No. 33. Am Soc Agron, Madison, Wisconsin, pp 509–571

Mendu N, Rines HW, Silflow CD (1993) Mapping of beta-tubulin genomic sequences in hexaploid oat (*Avena sativa* L.). Theor Appl Genet 86 : 135–140

Morikawa T (1985) Identification of the 21 monosomic lines in *Avena byzantina* C. Koch cv 'Kanota'. Theor Appl Genet 70 : 271–278

O'Donoughue LS, Kianian SF, Rayapati PJ, Penner GA, Sorrells ME, Tanksley SD, Phillips RL, Rines HW, Lee M, Fedak G, Molnar SJ, Hoffman D, Salas CA, Wu B, Autrique E, Van Deynze A (1995) A molecular linkage map of cultivated oat. Genome 38 : 368–380

Phillips RL, Rines HW, Jellen EN, Rooney WL, Wu BX, Kianian S, Riera-Lizerazu O (1995) Oat genome analysis via molecular markers and oat × corn crosses. In: Raupp WJ, Gill BS (eds) Classical and molecular cytogenetic analysis: Proc U.S.-Japan Symp, 21–23 March 1994, Manhattan, Kansas, Kansas Ag Exp Sta Rep 95-352-D, pp 49–57

Price S, Kahler AL (1983) Oats (*Avena* spp.). In: Tanksley SD, Orton TJ (eds) Isozymes in plant breeding and genetics, part B. Elsevier Sci Pub BV, Amsterdam, The Netherlands, pp 103–127

Rajhathy T, Thomas H (1974) Cytogenetics of oats (*Avena* L.). Misc Pub Genet Soc Canada No. 2. Ottawa, Ontario, Canada

Rines HW, Dahleen LS (1990) Haploid oat plants produced by application of maize pollen to emasculated oat florets. Crop Sci 30 : 1073–1078

Rines HW, Riera-Lizarazu O, Nunez VM, Davis DW, Phillips RL (1997) Oat haploids from anther culture and from wide hybridizations. In: Jain SM, Sopory SK, Veilleux RE (eds) In vitro haploid production in higher plants, vol. 4. Kluwer Academic Press, Dordrecht, The Netherlands, pp 205–221

Rooney WL, Jellen EN, Phillips RL, Rines HW, Kianian SF (1994) Identification of homoeologous chromosomes in hexaploid oat (*A. byzantina* cv Kanota) using monosomics and RFLP analysis. Theor Appl Genet 89 : 329–335

Sharp PJ, Chao S, Desai S, Gale MD (1989) The isolation, characterization and application in the Triticeae of a set of wheat RFLP probes identifying each homoeologous chromosome arm. Theor Appl Genet 78 : 342–348

Werner JE, Endo TR, Gill BS (1992) Toward a cytogenetically based physical map of the wheat genome. Proc Natl Acad Sci USA 89 : 11307–11311

Wilson WA, McMullen MS (1997) Recombination between a crown rust resistance locus and an interchange breakpoint in hexaploid oat. Crop Sci (in press)