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ORIGINAL PAPER

Addition of individual chromosomes of maize inbreds B73 and Mo17 to oat cultivars Starter and Sun II: maize chromosome retention, transmission, and plant phenotype

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Abstract Oat-maize addition (OMA) lines with one, or occasionally more, chromosomes of maize (*Zea mays* L., $2n = 2x = 20$) added to an oat (*Avena sativa* L., $2n = 6x = 42$) genomic background can be produced via embryo rescue from sexual crosses of oat × maize. Self-fertile disomic addition lines of different oat genotypes, mainly cultivar Starter, as recipient for maize chromosomes 1, 2, 3, 4, 5, 6, 7, 9, and the short arm of 10 and a monosomic addition line for chromosome 8, have been reported previously in which the sweet corn hybrid Seneca 60 served as the maize chromosome donor. Here we report the production and characterization of a series of new OMA lines with inbreds B73 and Mo17 as maize chromosome donors and with oat cultivars Starter and Sun II as maize chromosome recipients. Fertile disomic OMA lines were recovered for B73 chromosomes 1, 2, 4, 5, 6, 8, 9, and 10 and Mo17 chromosomes 2, 4, 5, 6, 8, and 10. These lines together with non-fertile (oat × maize) F_1 plants with chromosome 3 and chromosome 7 of Mo17 individually added to Starter oat provide DNA of additions to oat of all ten individual maize chromosomes between the two maize inbreds. The Mo17 chromosome 10 OMA line was the first fertile disomic OMA line obtained carrying a complete chromosome 10. The B73 OMA line for chromosome 1 and the B73 and Mo17 OMA lines for chromosome 8 represent disomic OMA lines with improved fertility and transmission of the addition chromosome compared to earlier Seneca 60 versions. Comparisons among the four oat–maize parental genotype combinations revealed varying parental effects and interactions on frequencies of embryo recovery, embryo germination, F_1 plantlets with maize chromosomes, the specific maize chromosomes retained and transmitted to F_2 progeny, and phenotypes of self-fertile disomic addition plants. As opposed to the previous use of a hybrid Seneca 60 maize stock as donor of the added maize chromosomes, the recovered B73 and Mo17 OMA lines provide predictable genotypes for use as tools in physical mapping of maize DNA sequences, including inter-genic sequences, by simple presence/absence assays. The recovered OMA lines represent unique materials for maize genome analysis, genetic, physiological, and morphological studies, and a possible means to transfer maize traits to oat. Descriptions of these materials can be found at http://agronomy.cfans.umn.edu/Maize_Genomics.html.

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Introduction

Cytogenetic stocks of plants have been widely available and used in studies of chromosome pairing, recombination, gene mapping, genome structure, evolution, and for many other purposes. The application of molecular biological techniques using such stocks led to the field of molecular cytogenetics allowing specific DNA sequences to be mapped to the physical chromosome. The production of a set of novel oat–maize addition (OMA) lines with each of the ten chromosomes of the hybrid sweet corn 'Seneca 60' (*Zea mays* L. $2n = 2x = 20$) added individually to an oat (*Avena sativa* L. $2n = 6x = 42$) genomic background has been described (Kynast et al. 2001, 2004). Here we report the generation of sets of OMA lines with the widely studied maize inbreds B73 and Mo17 as chromosome donors. These inbreds as maize chromosome sources allow greater specificity in the use of the derived OMA lines in physical mapping to chromosome of maize non-genic as well as genic DNA sequences. Use of maize inbred lines as chromosome donors and two oat cultivars as recipients also enable insights into the effects of parental genotypes in the oat × maize sexual crosses on specific maize chromosome retention, transmission, and plant phenotype.

Recovered OMA lines are valuable maize genomic tools due to individualized maize chromosomes isolated in an oat genomic background, which may serve to simplify analysis of the maize genome about tenfold. A set or panel of DNA extracts from the various OMA lines allows mapping of maize sequences to chromosome by a simple presence/absence hybridization or PCR assay. This simple procedure has been used to physically locate to chromosome expressed sequence tagged sites (ESTs), sequence tagged sites (STSs), duplicated genes, members of gene families, and BAC-end sequences (Okagaki et al. 2001; Zhang et al. 2006; Mica et al. 2006; Phillips and Rines 2009). Other types of studies employing these OMA lines have included analyses of maize knob and centromere structure (Ananiev et al. 1998a, b, c; Jin et al. 2004), meiotic chromosome behavior (Bass et al. 2000), physical mapping of single-copy sequences on maize chromosomes by fluorescence in situ hybridization (FISH) (Koumbaris and Bass 2003; Amarillo and Bass 2007), flow-cytometry sorting of an individual maize chromosome (Li et al. 2001), and expression of maize genes in the genetic background of oat. The latter has been done both to study gene regulation aspects (Muehlbauer et al. 2000; Cabral et al. 2007) and to possibly introduce new traits such as C4 photosynthesis (Kowles et al. 2008) and disease resistance (Walch 2007) from maize into oat.

The simplification of the maize genome into even smaller units or subchromosomal segments was attained by gamma-irradiating seed of OMAs each containing a single copy of a maize chromosome. The derived progeny included both lines with a partial maize chromosome and ones with a maize segment(s) translocated onto an oat chromosome (Riera-Lizarazu et al. 2000). These materials, termed radiation hybrids, could be delineated using mapped molecular markers to allow construction of a set or array with overlapping segments of a chromosome that enables mapping of a maize sequence to a sub-chromosomal segment, again by a series of simple presence/absence assays. Radiation hybrids and panels are available and described for all the maize chromosomes except chromosome 8 (Okagaki et al. 2002, 2004a, b; Kynast et al. 2002, 2004; Phillips and Rines 2009).

In the generation of OMA lines, the sexual cross of oat (sub-familia *Pooideae*) and maize (sub-familia *Panicoidae*) produces hybrid zygotes ($2n = 21 + 10$) which subsequently undergo uniparental loss of maize chromosomes during embryonic cell divisions, as had been described earlier in wheat (*Triticum aestivum* L.) × maize crosses (Laurie and Bennett 1986). Unlike in wheat × maize crosses where maize chromosome elimination appears rapid and complete resulting in only haploid wheat plants recovered by embryo rescue (Laurie and Bennett 1989), one-third to one-half of plants from oat × maize rescued embryos retain one or more maize chromosomes added to a complete haploid oat genome (Riera-Lizarazu et al. 1996). Furthermore, partial self-fertility as a result of unreduced gamete formation, as shown in normal haploid oat plants (Rines and Dahleen 1990; Davis 1992), also occurs in haploid plants carrying maize chromosomes and allows transmission of an added maize chromosome to form disomic addition ($2n = 42 + 2$) progeny (Riera-Lizarazu et al. 1996).

In the report of the initial complete set of OMA lines described by Kynast et al. (2001) where the sweet corn hybrid Seneca 60 served as the maize chromosome donor, various oat genotypes were used as recipients, but mainly cultivar Starter. Self-fertile disomic additions that could be maintained over generations were obtained for Seneca 60 maize chromosomes 1, 2, 3, 4, 5, 6, 7, and 9, but only a monosomic addition was available for chromosome 8 and a disomic addition for a partial chromosome 10 (Kynast et al. 2002, 2004). In spite of the widespread use of the Seneca 60-derived OMA lines, these materials have limitations, such as for mapping genomic sequences from inter-genic regions of other maize genotypes. These regions tend to be quite highly variable in sequence among maize genotypes (Wang and Dooner 2006). Thus, there was a need for OMA lines produced with the maize inbreds B73 and Mo17, two maize genotypes that are the most widely used in maize genetic and genomic studies including B73 as the selected genotype of the maize genome sequencing project (http://www.maizesequence.org). Also, there was a desire to have these maize chromosome additions in as uniform oat background as possible to limit gel band interpretation

problems from possible oat background sequence homologies and polymorphisms.

Here we describe the recovery of OMA lines with B73 and Mo17 as the maize chromosome donors and oat cultivars Starter and Sun II as the alien chromosome recipients. A series of steps are involved in the process leading to the final recovery of fertile disomic additions of specific maize chromosomes with the various maize and oat genotypic combinations. These steps include recovery of an embryo from maize pollination of an oat floret, in vitro germination of the embryo into a vigorous plant, evidence of maize chromatin in the plant, identification of the specific maize chromosome(s) in the plant, and the capture of the added chromosome in a self-fertile disomic addition line. The frequencies of success in these steps are described for the various genotype combinations and how the parental genotypes do or do not relate to the recovery of a particular chromosome addition, its transmission, and the phenotype of the derived plants. Although the OMA lines recovered from B73 and Mo17 are not quite complete sets, they do include the first fertile disomic additions of an intact chromosome 10, disomic additions for chromosome 8, and a disomic addition for chromosome 1 with much improved seed production compared to the Seneca 60 OMA line set.

Materials and methods

Plant materials

Seed of the popular maize inbreds B73 and Mo17 were obtained from the USDA North Central Regional Plant Introduction Station, Ames, Iowa. Seed of the two oat cultivars used in this study, Starter and Sun II, traced to earlier single plant reselections in the respective cultivars. The two oat cultivars were selected as recipients because Starter was the primary recipient in the earlier Seneca 60 OMA series and Sun II in other studies has shown tolerance to chromosomal aneuploidy (Jellen et al. 1997). The initial hybridization scheme included the four combinations of crosses: Starter × B73, Starter × Mo17, Sun II × B73, and Sun II × Mo17. B73 and Mo17 pollen for the crosses to oat all came from field-grown plants because of difficulties in obtaining consistently vigorous pollen production of these inbreds in off-season greenhouse plantings. Transplanting of young plants started in the greenhouse plus periodic field plantings provided a source of fresh pollen from mid-July to early September. Oat plants for the maize crosses were produced by periodic plantings of seed in 15-cm diameter pots of two parts soil, one part potting mix beginning mid-April. After an initial 6–8 weeks in growth chambers under short-day conditions (11 h light at 20°C, 13 h dark at 16°C) to promote vegetative growth, the oat plants were shifted to long days (16 h light at 20°C, 8 h dark at 16°C) in growth chambers or greenhouses for reproductive development, maize pollinations, and F_1 seed development.

For crosses and embryo rescue, oat plants 1–3 days prior to anthesis were emasculated by cutting off the end of the florets and the anthers therein, 24–48 h later freshly collected maize pollen was applied, and 1–2 days post-pollination the florets were treated with a solution of 100 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and 50 mg/L gibberellic acid (GA) to promote embryo development and to delay endosperm collapse. At about 15 days post-pollination, embryos were rescued onto culture medium, as described in detail earlier for oat × Seneca 60 maize crosses (Rines et al. 1997; Kynast et al. 2001). Plantlets from germinating embryos, once they were vigorous enough for transplanting to soil, were tested for the presence of the maize retrotransposon *Grande-1* as indicative of retained maize chromatin (Kynast et al. 2001). *Grande-1*-positive F_1 plants attaining vigorous vegetative growth with multiple tillers were tested with a series of maize chromosome-specific SSR markers, two per chromosome arm, to determine the identity of the maize chromosome(s) present. The plants were then transferred to long-day conditions (16 h light, 8 h dark) to promote flower induction. Seed produced on these F_1 partial hybrid plants, as a result of unreduced gamete formation and self-fertilization, were planted and resulting F_2 plants PCR assayed to confirm the transmission and identity of the added maize chromosome(s).

DNA extraction and PCR analyses

DNA extractions and PCR analyses were accomplished as described by Kynast et al. 2004. Briefly, DNA was extracted from small leaf segments, using the RED Extract-N-Amp Plant PCT kit (Sigma, St. Louis, MO) according to the vendor's recommendations. For initial tests for the presence of maize chromosomes in F_1 plantlets and F_2 seedlings, maize-specific primers for *Grande-1* and for the maize centromere-specific repeat *Cent A* were utilized. To identify specific maize chromosomes present, larger amounts of tissue were extracted with the DNA Easy Plant Mini kit (Qiagen, Valencia, CA) and PCR conducted using simple sequence repeat (SSR) markers that were selected from the maize genetics and genomics database (http://www.maizegdb.org). Two markers, including one near the distal end, were selected for each chromosome arm (Table 1). Transmission of an added maize chromosome from an F_2 plant to >80% of its progeny was taken as an indication that the F_2 plant was disomic for the added chromosome, whereas monosomic alien chromosomes are expected to transmit exclusively maternally at only about 10% frequency (Kynast et al. 2004; Phillips and Rines 2009). Ideally, each plant or representative plants from each selected F_2-derived line would be visually

Table 1 Selected set of maize chromosome arm-specific SSR markers used to identify retained and transmitted maize chromosomes in the genetic background of oats and their corresponding chromosome arm identities

Chromosome arm	SSR markers	Chromosome arm	SSR markers
1S	*umc1354*, *umc2217*	6S	*umc2310*, *umc2313*
1L	*umc1689*, *umc1744*	6L	*umc1656*, *bnlg1136*
2S	*umc1165*, *umc1259*	7S	*umc1159*, *umc1401*
2L	*bnlg1138*, *bnlg1893*	7L	*umc1393*, *umc1407*
3S	*umc1746*, *umc2002*	8S	*umc1139*, *umc1974*
3L	*umc2267*, *umc1641*	8L	*umc1415*, *umc1069*
4S	*umc1164*, *umc1088*	9S	*umc1279*, *umc1698*
4L	*umc1775*, *umc1180*	9L	*umc1191*, *bmc1129*
5S	*umc1445*, *umc1692*	10S	*umc1380*, *umc2067*
5L	*umc1524*, *umc1153*	10L	*umc1272*, *umc2021*

checked cytologically by genomic in situ hybridization (GISH), as was done in earlier work with the Seneca 60 OMA lines (e.g. Riera-Lizarazu et al. 1996; Kynast et al. 2004). Such analysis would help ensure that a plant was a disomic addition for an intact single maize chromosome pair with neither it nor any additional maize chromosome segments present as translocation segments on oat chromosomes. The general consistency we have found with PCR assays showing a maize centromere and two markers per chromosome arm in the high transmitting lines indicative of a disomic addition line provides confidence in the efficient PCR scoring technique. There remains, however, a risk in sequence assignment to chromosome due to undetected maize chromosome segments being present or to homologies in gene families.

Data analysis

The effect of oat–maize genotype combinations upon frequencies of rescued embryos from maize-pollinated oat florets, germination of embryos, and *Grande-1*-positive plantlets was analyzed with the help of Dr. Sanford Weisberg of the Statistical Consulting service of the University of Minnesota using the data presented in Table 2. The differences between oat and maize combinations were modeled using standard factorial main effects and interactions. The year-to-year effect was modeled using a random effect for years. Different models were compared using the AIC criterion (Bates 2007).

Results

Embryo recovery, germination, and maize chromatin retention

Because of the rare and sporadic retention of particular maize chromosomes in the F_1 (oat × maize) plants and the low frequency of recovery of fertile F_2 disomic OMA plants due to different transmission frequencies, the efforts to recover a full set of OMA lines with B73 and Mo17 chromosomes involved maize pollinations of almost 60,000 emasculated florets of Starter and Sun II oat extending over 4 years (2003–2006). Frequency data as influenced by maize and oat genotypes are shown for successful embryo rescue, embryo germination, and *Grande-1* retrotransposon presence (Fig. 1; Table 2). While there were often variations between years in the success frequencies, for statistical modeling the year-to-year effect was considered to be the same for all crosses in analyzing for oat/maize effects.

In the first step of the process—the recovery of embryos from oat × maize crosses—three of the four oat × maize combinations had the same probability of success of about 6% embryos (Table 2). The fourth combination, Starter × Mo17, produced significantly more or about 11% embryos. The portion of the embryos that germinated to give small

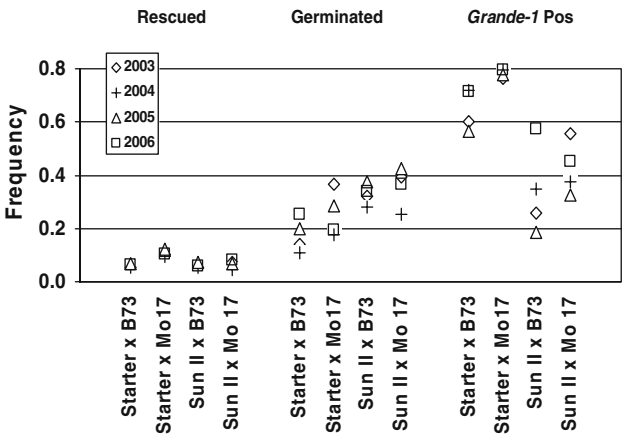

Fig. 1 Frequencies for years 2003–2006 of rescued embryos from oat × maize pollinations, embryos that germinated, and F_1 plants testing positive for retrotransposon *Grande-1* indicative of maize chromosome presence. Data are shown for combinations of oat cultivars Starter and Sun II by maize inbreds B73 and Mo17. Actual numbers and frequencies over years are given in Table 2

Table 2 Frequencies of embryos rescued from maize pollinated oat florets, embryos that germinated, and plantlets testing *Grande-1* positive from crosses of two oat cultivars each by two maize inbreds, 2003–2006

Cross	Year	Oat florets maize pollinated	Embryos rescued	%	Embryos that germinated	%	Plantlets *Grande-1* tested		
							Total	Positive	%
Starter × B73	2003	3,930	239	6.1	33	13.8	30	18	60.0
	2004	4,430	235	5.3	26	11.1	25	18	72.0
	2005	5,256	355	6.8	70	19.7	67	38	56.7
	2006	5,557	359	6.5	91	25.4	49	35	71.4
	Total	19,173	1,188	6.2	220	18.5	171	109	63.7
Sun II × B73	2003	4,129	243	5.9	78	32.1	70	18	25.7
	2004	3,102	164	5.3	46	28.1	43	15	34.9
	2005	3,027	216	7.1	81	37.5	81	15	18.5
	2006	3,567	210	5.9	71	33.8	61	35	57.4
	Total	13,825	833	6.0	276	33.1	255	83	32.6
Starter × Mo17	2003	647	71	11.0	26	36.6	17	13	76.5
	2004	5,041	475	9.4	83	17.5	74	59	79.7
	2005	4,397	535	12.2	153	28.6	129	100	77.5
	2006	4,011	424	10.6	82	19.3	39	31	79.5
	Total	14,096	1,505	10.7	344	22.9	259	203	78.4
Sun II × Mo17	2003	1,004	74	7.4	29	39.2	27	15	55.6
	2004	5,175	242	4.7	61	25.2	59	22	37.3
	2005	1,733	118	6.8	50	42.4	46	15	32.6
	2006	2,110	172	8.2	63	36.6	53	24	45.3
	Total	10,022	606	6.1	203	33.5	185	76	41.1
Grand total		57,116	4,132	7.2	1,043	25.2	870	471	54.1

plantlets was influenced mainly by the oat parent genotype although the maize genotype also had a minor effect. The two oat–maize combinations involving Sun II as the oat parent yielded similar portions over years of about 33% germinating embryos, which was higher than the 19 and 23% for combinations of B73 and Mo17, respectively, with Starter oat. Germinated plantlets with vigorous growth were then tested for the presence of *Grande-1*, a maize-specific, high-copy retrotransposon element distributed throughout the maize genome. The element's presence usually indicates one or more retained maize chromosomes. The oat parent and the maize parent both exhibited main effects on the portion of plantlets testing positive for *Grande-1* with a weak but significant oat/maize interaction effect. Combinations of B73 and Mo17 with Starter oat (64 and 80%, respectively) have about double the frequency of plantlets testing *Grande-1* positive compared to ones involving Sun II (33 and 41%, respectively) (Table 1). Of interest is that while combinations involving Starter oat gave a much lower frequency of embryos germinating than combinations involving Sun II oat, the Starter oat combinations gave a much higher portion of plantlets testing positive for the presence of maize chromatin (i.e. *Grande-1* positive).

All four oat-maize combinations had a much higher frequency of embryos germinating compared to the value of about 11% reported in earlier studies involving Seneca 60 maize (Kynast et al. 2004). The difference may be due not only to maize parent difference as improved embryo rescue techniques were employed in the present study, including the use of a tape with improved gas exchange (3M^R venting tape) to seal the Petri dishes during embryo culture. Embryos recovered from oat × maize crosses tend to be much smaller and less well developed than ones from oat × oat crosses and can require up to 9 weeks for germination to occur (Kynast et al. 2001). The improved rescue techniques likely accounted for, or at least contributed to, the higher proportion of embryos germinating in this study.

Specific chromosome retention and transmission

Recovered F_1 plants that tested positive for maize-specific *Grande-1* and which attained growth adequate for potential reproductive development were then assayed for *Cent A* and a series of maize SSR markers, two specific for each maize chromosome arm (Table 1). The incidence of each maize chromosome being present, either singly or in combination with other maize chromosomes, in F_1 plants is shown in Fig. 2 for each of the four oat–maize combinations used in this study along with values from oat × Seneca 60 maize crosses reported by Kynast et al. (2001). Also shown for each combination are the numbers of F_1 fertile plant lines with a particular maize chromosome

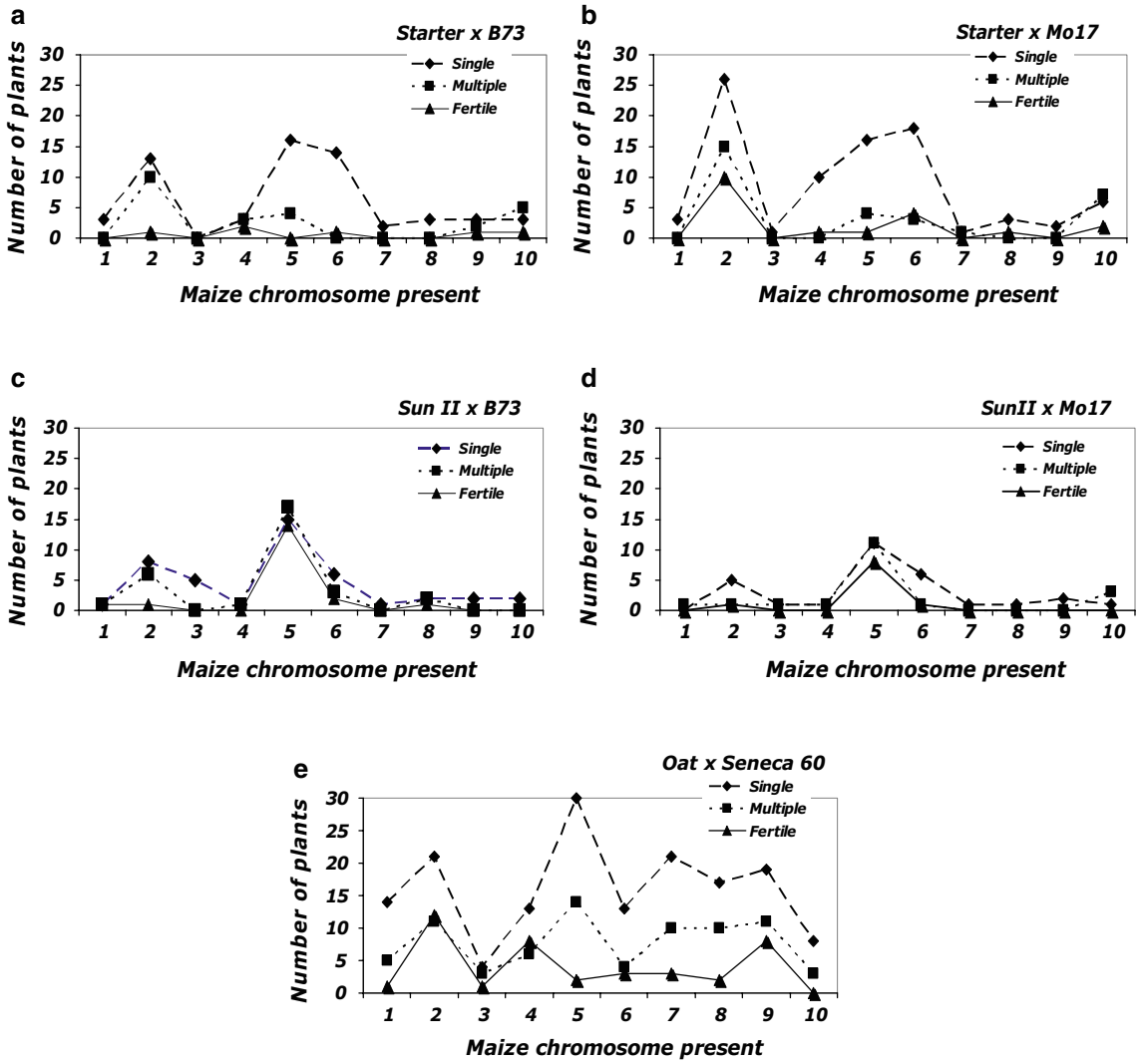

Fig. 2 Numbers of (oat × maize) F_1 multiple maize chromosome plants, F_1 single maize chromosome plants, and F_2 fertile lines for each of the ten maize chromosomes as additions to oat for combinations of oat cultivars and maize inbreds Starter × B73 (**a**), Starter × Mo17 (**b**), Sun II × B73 (**c**), and Sun II × Mo17 (**d**). Also shown for comparison are earlier reported numbers (Kynast et al. 2001, 2004) for various oat lines, mainly Starter, × sweet corn hybrid Seneca 60 (**e**)

transmitted to F_2 progeny. A pattern of occasional loss of maize chromosomes through oat-maize F_1 plant development and transmission into F_2 plants was described by Kynast et al. (2004).

The frequencies with which a particular maize chromosome was observed in F_1 plants and its transmission to F_2 progeny varied greatly among the maize chromosomes and were influenced by the oat and maize parental genotypes. Also, as reported by Kynast et al. (2004), the frequency with which a particular chromosome was present in F_1 plants did not always correlate with its chances of being retained in a F_2 line. Chromosome 5 was the most frequently retained chromosome in F_1 plants either singly or in combination with other maize chromosomes in three of the four oat × maize combinations in this study (Fig. 2), just as it had been in the crosses involving Seneca 60 maize reported previously (Kynast et al. 2001; 2004). In the fourth combination, Starter oat × Mo17 maize, chromosome 5 was still frequently retained, especially in plants with multiple retained chromosomes. A marked effect of the oat parent was observed in the B73 and the Mo17 combinations. Retention of chromosome 5 singly in F_1 plants and its transmission to F_2 fertile lines plants were both much less frequent in a Starter oat background than in a Sun II oat background. In earlier studies with Seneca 60 as maize parent, transmission of chromosome 5 to F_2 fertile lines was infrequent with both Starter and Sun II oat (Kynast et al. 2004). In the one fertile disomic chromosome 5 addition of Seneca 60 maize in a Starter background, transmission of the added maize chromosome was reported as erratic (Kynast et al. 2004). Transmission in the several lines of B73 and Mo17 chromosome 5 additions in Sun II oat was

found here to be quite consistent, with >80% of progeny from self-fertilization of disomic F_2 plants containing the maize chromosome 5 addition. In contrast, the one (Mo17) F_2 chromosome 5 addition line recovered in Starter oat had infrequent maize chromosome transmission to progeny.

Maize chromosome 2 was the next most frequently retained chromosome in the oat–maize F_1 plants being first, second, or third most frequent among all oat × maize combinations (Fig. 2). For chromosome 2, both the oat and the maize parent genotypes appeared to play a role in its retention. The Starter × Mo17 combination was highly favorable, particularly in production of F_2 plants, compared to other B73 and Mo17 combinations. The Seneca 60 combinations also appeared favorable for maize chromosome 2 retention and production of F_2 plants. Although at least one F_2 fertile disomic maize chromosome 2 plant was recovered in all four possible B73 and Mo17 oat–maize combinations, a large differential effect of the oat genotype was seen on the vigor of both the B73 and the Mo17 chromosome 2 addition plants (Fig. 3). The chromosome 2 addition plants in the Starter oat background were similar to normal Starter plants in vigor and seed production, whereas the ones in the Sun II background grew quite slowly and produced only 1–3 panicles and a total of 5–30 seeds.

Fig. 3 Plants with B73 and Mo17 maize chromosome 2 additions to Starter and Sun II oats showing a strong differential phenotypic effect between the two oat genotypes from the added maize chromosome 2. The growth of Starter plants with a chromosome 2 addition from either B73 or from Mo17 is similar to that of normal Starter oat, whereas the growth of Sun II plants is greatly reduced with the presence of chromosome 2 of either maize genotype. Seed for all four plants were sown on the same day

Maize chromosome 6 was the next most frequently retained chromosome after chromosomes 5 and 2 in the oat × maize combinations in this study, although less frequently retained in Sun II than in Starter oat background (Fig. 2). At least one instance of F_2 chromosome 6 addition plants was obtained for each of the B73 and Mo17 combinations, as was the case with chromosome 2 additions. In the chromosome 6 additions, however, there were no strong oat background differences on the phenotypes of the F_2 plants and progenies. All four genotype combinations showed variable older plant disease lesion mimic phenotype as observed earlier in the Seneca 60 chromosome 6 additions (Kynast et al. 2001). Chromosome 9 and chromosome 4 were retained much less frequently with either B73 or Mo17 as maize parent than in the earlier crosses with Seneca 60 as maize parent. Only a single instance of an F_2 addition line was obtained for chromosome 9, and that was from a Starter × B73 cross. Two F_2 chromosome 4 addition lines were obtained from Starter × B73 and one line from Starter × Mo17.

Only one maize chromosome 1 and two maize chromosome 8 F_2 addition lines were recovered from the B73 and Mo17 crosses. However, each of these F_2 addition lines presents an improvement compared to the earlier recovered Seneca 60 versions in our efforts to obtain a highly fertile, stably transmitting disomic addition line for each of the ten maize chromosomes. Plants of the two F_2 chromosome 8 addition lines, one from a Sun II × B73 cross and one from a Starter × Mo17 cross, were highly self-fertile disomic additions that transmitted the added chromosome at high frequencies (>90%). Of the two transmitting chromosome 8 addition lines previously available (Kynast et al. 2004), one was a GAF-Park oat × Seneca 60 maize line monosomic for the chromosome 8 addition. The addition monosomic chromosome, lacking a meiotic pairing partner, transmitted to only about 10% of progenies and always as a monosomic addition (Kynast et al. 2002). This is normal cytogenetic behavior of a univalent chromosome; that is, low frequency transmission through the female and little or no transmission through the male due to gamete (pollen) competition by segregating normal pollen. The other F_2 chromosome 8 addition line was a Starter × Seneca 60 line that, although disomic initially, had very weak plants and poor transmission of the added chromosome. The chromosome 1 stock recovered here from a Sun II × B73 cross is also an improved stock. Although it has the novel plant phenotype of the earlier Starter × Seneca 60 chromosome 1 addition line in having a thick culm and upright leaves as described in Kynast et al. (2001), it is more fertile with higher transmission of the maize chromosome 1 to progeny, making this chromosome 1 addition line much easier to maintain.

The F_2 maize chromosome 10 addition lines from the cross Starter × Mo17 were the first recovered to contain a

transmitted complete maize chromosome 10 (Kynast et al. 2005). In the first complete set of oat–maize addition lines reported (Kynast et al. 2001), the chromosome 10 addition was available only as an F_1 of GAF-Park oat × Seneca 60 maize; this line was maintained vegetatively. Although it did set one seed, no maize chromosome was transmitted. In later Seneca 60 crosses, several plantlets were recovered that retained the chromosome 10 in single or multiple chromosome additions (Kynast et al. 2004). Twenty-eight maize chromatin-positive F_2 plants were produced from one of the Sun II oat background F_1 plants; however, upon testing of seven chromosome 10-specific SSR markers distributed along the length of the maize genetic map, only the three short-arm-specific markers tested (*p-phi041*, *p-phi117*, and *p-umc1293*) were present in each of these plants. This result indicated that only a short-arm telosome was transmitted and raised the possibility that there might be a factor on the Seneca 60 chromosome 10 long arm preventing its transmission. However, transmission to F_2 plants of an apparent complete added maize chromosome 10 was eventually found in two Sun II × Mo17 crosses as evidenced by the presence of the four tested long-arm-specific markers (*umc1272*, *umc1084*, *umc2021*, and *csu48*) as well as the short-arm-specific markers (Kynast et al. 2005). The recovery of two chromosome addition lines of Mo17 plus a subsequent recovery of a F_2 line with a complete chromosome 10 addition of B73 means that a full set of fertile lines of all ten maize chromosome additions to oat are now available.

In all the oat × maize crosses involving B73 and Mo17 as maize parents, no fertile additions were recovered for chromosomes 3 or 7 (Fig. 2). However, an F_1 plant of Sun II × Mo17 with chromosome 3 as a single addition and an F_1 plant of Starter × Mo17 with chromosome 7 as a single addition were recovered. Limited amounts of DNA isolated from leaf tissue of these plants were obtained which can be used in mapping maize sequences to these two chromosomes.

Phenotypes of B73 and Mo17 OMA lines in Starter and/or Sun II backgrounds

Distinctive phenotypic features of B73 and Mo17 OMA lines compared to their respective oat parents are summarized in Table 3. Photos of OMA plants exhibiting these phenotypes are available at http://agronomy.cfans.umn.edu/Maize_Genomics.html. In Table 3 features are listed for a representative line of each oat/maize genotype combination from which fertile disomic lines were recovered; these features were typical for all lines of a genotype combination when more than one line was obtained. The distinctive features or syndrome imparted by each of the B73 and Mo17 chromosomes as additions are similar to those described by Kynast et al. (2001) for the respective Seneca 60 chromosome additions. The differences between the parent oat and maize genotype involved seem to be more on degree of syndrome expression rather than novel effects and somewhat sensitive to environmental conditions, particularly stress conditions. The degree of expression of a particular maize chromosome addition syndrome also in some cases varied among siblings, even ones grown side-by-side in growth chambers or glasshouses. For example, sibling plants with less severe syndrome expression had to be chosen for seed production for line maintenance for B73 and Mo17 maize addition chromosomes 6, 8, and 9 where many progenies were too stunted to produce any seed or they produced only a few seed. In some cases there appeared to be a strong effect of parental genotype on syndrome expression, as illustrated in Fig. 3 by the effect of Sun II versus Starter oat parent on chromosome 2 additions from either B73 or Mo17 as maize donor. The variability in degree of syndrome expression in maize chromosome addition plants, which in some cases was strongly affected by oat or maize genotype or specific genotype combination and in some cases appeared erratic among sibs, may explain the rather sporadic occurrence of various OMA lines as well as the oat–maize genotype influence on OMA line recovery observed in our studies.

Discussion

This study using maize inbreds B73 and Mo17 in crosses to oat allowed comparisons with earlier results with the maize hybrid Seneca 60 as the maize parent, in addition to producing desired new OMA lines. The influence of different maize parents and their interactions with different oat parents was analyzed relative to the recovery of additions of each maize chromosome and on the steps leading to their recovery. Four oat × maize combinations were evaluated—B73 and Mo17 maize crossed with Starter and Sun II oat. One particular oat genotype × maize genotype combination (Starter × Mo17) produced a significantly higher frequency of recovered embryos from maize-pollinated oat florets than did the three other combinations, which showed frequencies similar to one another. In germination frequencies from the embryos, oat parent genotype played a larger role than maize parent genotype in the combinations studied. Specific maize chromosomes differed widely in their frequencies of forming F_1 OMA plants and in transmission to F_2 plants. Maize parent genotype, oat parent genotype, and interactions between them all influenced the frequencies depending on the specific maize chromosome. Some maize chromosomes rarely were recovered as additions to oat in any oat × maize combination. The parental genotype influences and interactions observed indicate that recovery of a complete fertile chromosome addition set for a specific

Table 3 Phenotypes of B73 and Mo17 oat maize addition lines in Starter and/or Sun II oat backgrounds

Added maize chromosome	OMA line no.	Oat × maize cross	Phenotype compared to oat parent[a]
0		Starter oat parent	Earlier heading, shorter stature than Sun II oat
		Sun II oat parent	Later heading, taller stature than Starter oat
1	1.36[b]	Sun II × B73	Thicker stems, upright leaves, compact panicles, later heading
2	2.51[b]	Starter × B73	Shorter plants, later heading
	2.48[b]	Starter × Mo17	Shorter plants, later heading
	2.61	Sun II × B73	Much smaller plants, later heading, few seeds
	2.60	Sun II × Mo17	Much smaller seeds, later heading, few seeds
3	U6855-1 F1[b]	Sun II × Mo17	Non-fertile, DNA only
	3.01	Sun II × Seneca 60	Liguleless, crooked peduncle, compact panicles, aerial axillary buds
4	4.43[b]	Starter × B73	Smaller plant, early leaf senescence
	4.42[b]	Sun II × Mo17	Fewer, mostly smaller tillers
5	5.60[b]	Sun II × B73	Later heading
	5.61[b]	Sun II × Mo17	Later heading
6	6.33	Starter × B73	Heavy leaf necrosis, degree varies among sibs
	6.31[b]	Starter × Mo17	Light leaf necrosis, degree varies among sibs
	6.34[b]	Sun II × B73	Heavy leaf necrosis, degree varies among sibs
	6.36	Sun II × Mo17	Heavy leaf necrosis, degree varies among sibs
7	W6608-2 F1[b]	Starter × Mo17	Non-fertile, DNA only
	7.06	Starter × Seneca 60	Smaller plant, later heading
8	8.05[b]	Sun II × B73	Smaller plants, early leaf senescence
	8.06[b]	Starter × Mo17	Smaller plants, early leaf senescence
9	9.41[b]	Starter × B73	Short plants, compact panicles, erratic premature plant senescence
10	10.26[b]	Starter × B73	Shorter, later plants; some sibs quite small
	10.23[b]	Starter × Mo17	Shorter, later plants

[a] Almost all OMA lines have reduced fertility with less than full transmission of the maize chromosome

[b] Lines used to supply requests for DNA or seed of sets of B73 and/or Mo17 OMA lines

maize inbred may require the using of multiple oat genotypes as recipient.

The primary objective of the work reported here was to produce chromosome addition lines in oat of the maize inbreds B73 and Mo17 to complement the already widely used Seneca 60 addition lines as a maize DNA sequence mapping tool, particularly in the mapping of non-genic sequences. Fertile disomic addition lines were recovered for chromosomes 1, 2, 4, 5, 6, 8, 9, and 10 of B73 and chromosomes 2, 4, 5, 6, 8, and 10 of Mo17. Fertile addition lines thus are available in one or the other inbred for each of the maize chromosomes except 3 and 7, but DNA from Mo17 non-fertile F_1 addition plants are available for those two. Seeds of addition lines for chromosomes 3 and 7 are available among the previously generated Seneca 60 OMA lines. The chromosome 10 OMA lines in B73 and Mo17 are the first available fertile OMA lines for a complete chromosome 10. Also, the B73, and Mo17 OMA lines for chromosomes 1 and 8 are stocks with improved fertility and transmission stability compared to the previously available Seneca 60 OMA lines. In Table 3 representatives of the B73 and Mo17 disomic fertile addition lines are indicated that will be distributed upon request for sets of available B73 and/or Mo17 chromosome addition lines for use in physical mapping of maize sequences to chromosome or other studies. A list of all fertile OMA stocks, including the Seneca 60 and other maize parent lines described previously can be found at http://agronomy.cfans.umn.edu/Maize_Genomics.html. This web site also includes a listing of the radiation hybrids and radiation hybrid map panels for maize chromosomes 1, 2, 3, 4, 5, 6, 7, 9, and 10 developed from OMA lines and described in Riera-Lizarazu et al. 2000; Okagaki et al. 2001; Kynast et al. 2001, 2002, 2004; and Phillips and Rines 2009. OMA materials can be obtained by contacting either co-author H. W. Rines or R. L. Phillips.

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