Oats with Maize Chromosome and Chromosome Segment Additions

Matthew D. Walch[1], Howard W. Rines[1,2], Ronald L. Phillips[1], Richard V. Kowles[3], Adrian O. Stec[1], Ralf G. Kynast[1], Ron J. Okagaki[1], Morrison S. Jacobs[1], Mark W. Galatowisch[1], Christina M. Schmidt[1], Paul A. Huettl[1]

1 - Department of Agronomy and Plant Genetics, Microbial and Plant Genomics Institute, University of Minnesota, 1991 Upper Buford Circle, St. Paul, MN 55108
2- USDA-ARS and Department of Agronomy and Plant Genetics, University of Minnesota, 1991 Upper Buford Circle, St. Paul, MN 55108
3- St. Mary's University of Minnesota, 700 Terrace Heights, Winona, MN 55987

Abstract

Oat (*Avena sativa* L., 2n = 6x = 42) plants crossed with maize (*Zea mays* L., 2n = 2x = 20) yields both haploid oat plants and plants with one or more maize chromosomes added to a haploid oat genome. Recovery of plants requires embryo rescue following partial or full elimination of the maize chromosomes during embryo development. Doubled haploid plants may be produced either as a result of unreduced gamete formation (Rines and Dahleen, 1990) or through colchicine doubling (Davies et al., 2006). Self-fertile single maize chromosome additions to oat (2n = 42 +2) have been recovered for each of the ten maize chromosomes (Kynast et al., 2004). Many of these maize chromosome addition lines exhibit novel phenotypes. Enzymes associated with C_4 photosynthesis have been shown to be present in addition lines with maize chromosomes carrying genes for these enzymes, phosphoenolpyruvate carboxylase (PEPC) in a maize chromosome 9 addition and pyruvate orthophosphate kinase (PPDK) in a maize chromosome 6 addition (Kowles et al., 2005). Plants with both maize chromosomes 6 and 9 are being produced to test possible effects on CO_2 compensation points. No instances of enhanced resistance to oat crown rust were found in seedling tests of the set of addition lines. Expression and possible effects of maize systemic resistance genes are currently being studied in these materials. Treatment of these addition lines with gamma radiation has allowed recovery of "radiation hybrids" with only segments of individual maize chromosomes present. The maize chromosome and chromosome segment additions to oat have been distributed to more than 40 labs as valuable tools for physical mapping of maize genes and for other maize genomic studies, as well as being possible new sources of genes for oat improvement.

Summary of oat-maize addition (OMA) lines

OMA lines are created by emasculation through clipping of oat florets just above the stigma of the primary floret at 1-3 days prior to anthesis. Fertilization with maize pollen occurs at 1-2 days after emasculation, followed by application of an auxin + GA_3 mixture at 1-2 days after pollination. All caryopses without significant endosperm development at 14-16 days after pollination are removed, and embryos are rescued on media. The presence of one or more maize chromosomes is determined by PCR with primers for the maize-specific Grande-1 retrotransposon sequence and the maize-specific CentA centromeric sequence. Individual maize chromosomes are identified using chromosome specific PCR markers. OMAs have been recovered for each chromosome of maize hybrid Seneca 60. When combined, a complete set of OMAs derived from maize inbreds B73 and Mo17 are now available. A complete set of OMAs for each of these two inbreds is currently being pursued. The number of independently derived addition lines in various backgrounds is shown below.

	Added maize chromosome										
Maize chromosome donor	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**B**
Seneca 60	1*	11	2	6	3	3	5	2	9		
B73	1	(2)		1(1)	3(1)			1	1		
Mo17		3(6)	(1)		3(4)	2(2)	(1)			1(1)	
A188				1							
line with bz1-mum9		1						1			
Black Mexican Sweet with B's											2

() Indicate addition lines currently at the F1 generation, DNA is available for all additions
* Numbers indicate independant OMA lines recovered for specified chromosome

Summary of Radiation Hybrid (RH) lines

RH lines are produced through the gamma irradiation of monosomic OMA seeds. Plants recovered from irradiated seed may carry deletions and intergenomic translocations of the maize chromosome. These breaks in the maize chromosome are characterized by markers from the 2004 IBM2 neighbors genetic map of maize. RH mapping panels anchored to existing genetic maps provide a high-throughput mapping tool via presence/absence PCR assays. Low-resolution mapping panels, sufficient to subdivide chromosomes into 10-20 segments, have been produced for all chromosomes, except for chromosome 8. Higher resolution panels exist for chromosomes 2, 4, 6 and 9. The status of the RH panels is summarized below.

	Maize chromosome									
Status of RH Panels	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10[a]**
Total number of RH lines recovered	68	81	39	55	51	121	23	0	202	55
Mapped markers defining lines	63	50	58	42	40	41	40	0	70	36
Core panel lines	10	21	10	22	11	26	10	0	32	11
ESTs mapped on core panel[b]	75	0	45	0	0	40	0	0	0	0

[a] Chromosome 10 RH-map was constructed using a Mo17 addition line
[b] Many additional ESTs have been mapped using a broader array of OMA lines

Schematic Overview

F1 Hybrids
Oat florets are emasculated and fertilized with maize pollen. Embryos are rescued 14 days post-pollination, yielding haploid oat plants that retain from 0 to 10 maize chromosomes. Maize chromatin is detected by PCR assay for maize specific repetitive sequences. Maize chromosomes are identified with previously mapped chromosome-specific markers. Plants positive for maize chromatin are allowed to self-pollinate.

OMA Lines
Transmission of maize chromosomes is monitored by PCR assay. Lines with stable, disomic maize chromosome additions are propagated.

Monosomic Seed
Seed monosomic for the maize chromosome addition is produced by backcrossing OMA lines to the oat-specific parent.

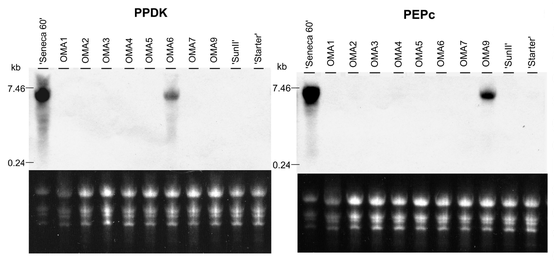
Irradiate and Grow
Seeds monosomic for the maize chromosome addition are irradiated to induced chromosome breaks. The plants grown from these seed contain translocations and deletions of the maize chromosome.

Screen Seedlings
The presence of maize chromatin following irradiation is determined by presence/absence PCR assay for Grande-1 and CentA in the seedlings. Plants containing maize chromatin are propagated as tissue, seed, and DNA.

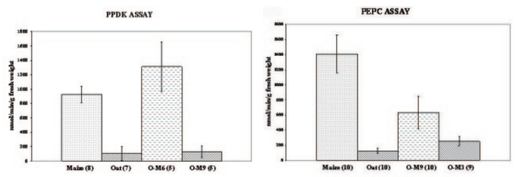
Characterize Breaks
Radiation hybrid lines are characterized with presence/absence PCR assay with markers based on the IBM2 2004 neighbors map. Mapping panels are then constructed by assembling radiation hybrid lines with overlapping segments. These lines can be used to map maize sequences to chromosome segments with presence/absence PCR assays.

Expression of Maize C_4 Genes in Oat

The OMA lines and derivative RH lines may be useful in determining the extent to which individual maize chromosomes and chromosome segments contribute to C_4 photosynthesis. C_3 plants such as oat show decreased efficiency of CO_2 assimilation when compared to C_4 plants such as maize. Current investigations have focused on the expression of two maize C_4 enzymes in the OMA lines. Pyruvate orthophosphate dikinase (PPDK) has been confirmed to be on chromosome 6, and phosphoenolpyruvate carboxylase (PEPC) has been confirmed to be on chromosome 9 by expression analysis in OMA lines.



Total RNA from leaf of maize Seneca 60, oat cultivar SunII, and OMA lines containing maize chromosomes 1-7 and chromosome 9 was run on a denaturing gel. PPDK expression was detected in maize and an OMA line containing maize chromosome 6 with an 800 bp PPDK cDNA clone. PEPC expression was detected in maize and an OMA line containing chromosome 9 with a 601 bp fragment coding for exon 8 of *pepcZm1*

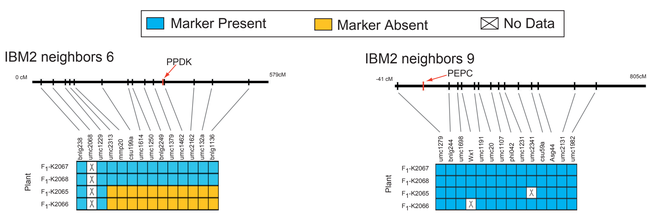


Enzyme assays were conducted with protein extracts from leaf tissue of Seneca 60 maize, SunII oat, and OMA lines. PPDK activity was shown to be significantly greater in the chromosome 6 addition line than in oat or other OMA lines. PEPC activity was shown to be significantly greater in the chromosome 9 addition line than in oat or other OMA lines.

OMAs with maize chromosomes 6 and 9

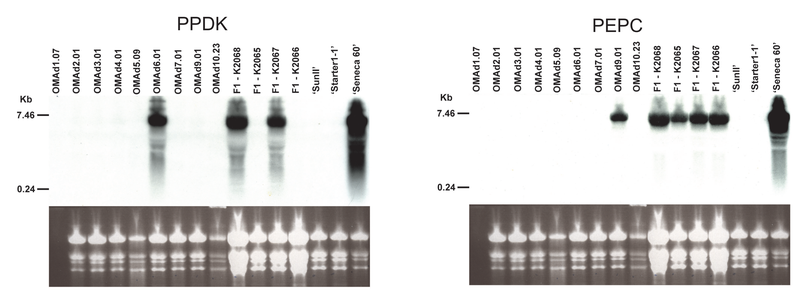
Expression of PPDK and PEPC in plants with both maize chromosome 6 and 9 has been investigated. Crosses between OMA 6 and OMA 9 lines have yielded plants positive for maize chromosome 6 and maize chromosome 9, indicated by marker analysis. RNA gel blot analysis has shown the expression of both PPDK and PEPC in these plants. Additional crosses between OMA lines with maize chromosome 6 and 9 have been made to test possible effects on CO_2 compensation points.

Marker analysis of plants positive for maize chromosomes 6 and 9



Plants shown to be positive for maize chromosome 6 and 9 were analyzed with 14 markers from the 2004 IBM2 neighbors maize genetic map. Two plants, F_1-K2067 and F_1-K2068, were positive for all informative chromosome 6 markers, while two plants, F_1-K2065 and F_1-K2066, were negative for markers on the long arm of chromosome 6. All four plants were positive for markers on chromosome 9.

Expression of PPDK and PEPC



Total RNA from leaf of 4 plants positive for maize chromosomes 6 and 9, as well as maize, oat, and OMA lines 1-7 and 9-10 was run on a denaturing gel. PEPC expression was detected with an 601 bp fragment coding for exon 8 of *pepcZm1*. The blot was then stripped and probed with an 800 bp PPDK cDNA clone. Expression of PEPC was shown in maize and in plants carrying maize chromosome 9. PPDK expression was detected in plants carrying the long arm of maize chromosome 6.

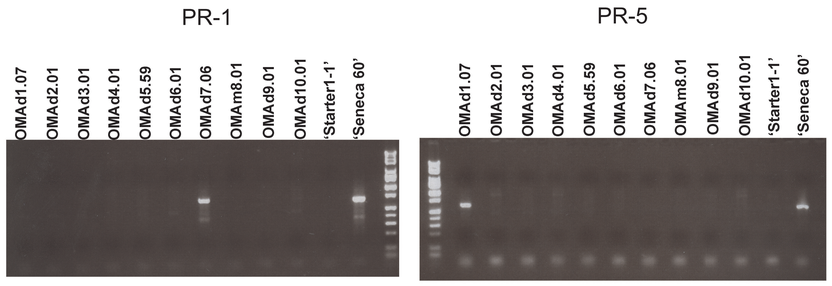
Disease Resistance in OMA lines

Screen for Seedling Rust Resistance

Zea mays is a nonhost for *Puccinia coronata*, the damaging crown rust pathogen of oat. A set of OMA lines have been screened with the crown rust isolates 92MNB254 and 93MNB350 from the USDA Cereal Disease Laboratory. No instances of seedling resistance segregating with the maize chromosome in OMA lines have been observed.

Maize SAR genes in oat

SAR, or systemic acquired resistance, is characterized by the initiation of systemic, broad spectrum resistance. Initiation of SAR has been described following tissue necrosis, caused either by hypersensitive response or pathogen colonization, and is marked by the systemic accumulation of pathogenesis-related (PR) proteins. Morris et al. showed expression of PR-1 and PR-5 genes following pathogen challenge, chemical treatment, and disease lesion mimic (1998).



PCR primers were designed for PR-1 and PR-5 sequences reported by Morris et al. (1998). Primers were shown to be polymorphic between oat and maize. Amplification of PR-1 occurred in maize Seneca 60 and in an OMA with chromosome 7. Amplification of PR-5 occured in maize Seneca 60 and in an OMA with maize chromosome 1. Expression of maize PR-1 and PR-5 in OMAs with maize chromosome 7 and 1 in response to pathogen challenge and chemical treatment will be evaluated.

References

Kowles, R.V., J.M. Minnerath, R.L. Phillips, and H.W. Rines. 2005. Expression of C4 photosynthetic characteristics in oat-maize addition lines. 47th Maize Genetics Conf. Abstr. p.21.

Kynast, R.G., R.J. Okagaki, M.W. Galatowitsch, M.S. Jacobs, A.O. Stec, H.W. Rines, and R.L. Phillips. 2004. Dissecting the maize genome by using chromosome addition and radiation hybrid lines. Proc. Natl. Acad. Sci. USA 101:9921-9926.

Morris S.W, B. Vernooij, S. Titatarn, S. Thomas, C.C. Wiltse, R.A. Frederiksen, A. Bhandhufalk, S. Hulbert. and S. Uknes. 1998. Induced resistance responses in maize. Mol. Plant Microbe Interact. 11: 643-658.

Rines, H.W., and L.S. Dahleen. 1990. Haploid oat plants produced by application of maize pollen to emasculated oat florets. Crop Sci. 30:1073-1078.

Sidhu, P.K., N.K. Howes, T. Aung, P.K. Zwer, and P.A. Davies. 2006. Factors affecting oat haploid production following oat x maize hybridization. Plant Breed. 125: 243-247.

Acknowledgements

We thank Gerald Ochocki of the USDA CDL for assistance with resistance screening. Also, we thank the many undergraduate students supported by the project over the last four years, and most recently, Michelle Nilson and Jenn Gillen (University of Minnesota).

This material is based upon work supported by the National Science Foundation under Award No. 0110134.