Alfalfa Says Hello To The Genome Of *Medicago arborea*

E.Bingham[1], T.Haas[1], J.Irwin[2], J.Mackie[2], J.Musial[2], D.Armour[2], C.Scotti[3], S.Arcioni[4], C.Jimenez[5], and I.Mauriera[6]

ADDRESSES

[1]Dept. Agronomy, Univ. Wisconsin, Madison, WI 53706 USA

[2]Cooperative Research Centre for Tropical Plant Protection,
and The School of Integrative Biology, Univ. of Queensland 4072, Australia

[3]Istituto Sperimentale Colture Foraggere- C.R.A., V.le Piacenza 29,
26900 Lodi, Italy

[4]Consiglio Nazionale delle Ricerche, Istituto di Genetica Vegetale, Perugia, Italy

[5]Avda. General Aviles, 32, 29. 46015 Valencia, Spain

[6]Dept. of Plant Biology, Cornell Univ. Ithaca NY 14853 USA

Abstract

Alfalfa (2n=4x=32) was first hybridized with *M.arborea* (2n=4x=32) with electrofusion of somatic cells by Nenz et al. 1996 (cited in Reference). The near 8x somatic hybrids were sterile. Now, we report sexual hybrids that are near 4x and have sufficient fertility for gene transfer. Alfalfa pollinated with *M.arborea* in general does not produce hybrids, but four alfalfa male sterile sources produced hybrids the last three years. Hybrid frequency is low, about one per 100 flowers pollinated. AFLP analysis has shown that bands unique to the *M.arborea* parent are present in the hybrids, but that not all the *M.arborea* genome is transferred to the hybrids, with bands unique to the *M.sativa* parent predominating. The hybrids differ greatly in morphology and fertility, possibly due to different degrees of chromosome elimination involving both genomes. Hybrids tend to produce a small amount of pollen. Female fertility of hybrids is about half that of alfalfa when crossed with alfalfa, and less when crossed with each other. Nonetheless, this is ample fertility for research including gene transfer to alfalfa. Between 1986 and 2003 a dozen different male sterile alfalfa clones were hand pollinated with *M.arborea* in the winter greenhouse at Madison, WI. Some aborted seeds were produced, but no hybrids. Then in 2003, alfalfa clone MBms produced twelve seeds after several hundred crosses. Clone MBms is from a cross of a Magnum III male sterile plant X a Blazer XL maintainer. The twelve seeds produced one self, one maternal haploid, and ten plants with various hybrid characteristics (see Reference). *M.arborea* is winter active, as are *M.sativa-arborea* derivatives. The general biology and cultivated potential of these materials are being evaluated in Australia, Italy, and North America. Hybrid derivatives survived the recent mild winter in Wisconsin, and biomass of spring growth was impressive. Regrowth after cutting will be examined for the first time in 2006, as will quality. *M.arborea* is a very long-lived perennial, and the impact of this in hybrid derivatives will be interesting. Seedlings and clones of the hybrid materials are very strong and easy to manage. In the F2 and Syn-1 generations, segregations for flower color, leaf shape, and pod and seed characteristics could be due to aneuploidy as well as genetic segregations due to intergenomic chromosome pairing. Additional information is reported in the reference, but many issues need to be studied in future research.

Reference

Reports by Bingham and by Haas in: Medicago Genetic Reports, Vol. 5, 2005

www.medicago-reports.org

INTRODUCTION

The force behind the project to hybridize alfalfa and M. arborea is eighty years of research on the role of the endosperm in seed development that is reviewed in Camadro et al. 2004, and Jansky 2006. The take home lesson being that endosperm development is necessary for embryo development, and cross combinations that produce seed can often be found by screening. Research by Fridriksson and Bolton 1963 showed that fertilization and embryo development occurred after crosses of alfalfa with all Medicago species except M. lupulina. The take home lesson from this study and several others reviewed by McCoy and Bingham 1988 is that alfalfa can be hybridized with almost any other Medicago species. Thus, we began screening for alfalfa parents that would produce interspecific hybrids.

MATERIALS

The alfalfa male sterile clone 6-4ms, of Saranac origin, has been maintained on the Wisconsin project for more than 30 years, and was crossed with M. arborea in eight of those years. Clone 6-4ms kept us optimistic about producing hybrids with M. arborea because it often would complete pod development and produce small dark aborted seeds. However, 6-4ms has never produced a hybrid with M. arborea. Nonetheless, new male steriles were challenged to produce seed almost every year, and in 2004, a male sterile designated MBms produced seed and hybrids, details of which can be found in the abstract and in Medicago Genetic Reports (see refs).

In 2005, three hybrids were produced in Queensland AU using a different alfalfa male sterile and different M. arborea parents. Also in 2005, two other male steriles produced hybrids at Madison WI, and one of them was a genetic male sterile unrelated to the others. Hence, we are optimistic that a relatively broad sample of alfalfa can be hybridized with M. arborea.

Concerning the M. arborea materials, at least three different M. arborea genotypes have been involved in hybrids in Queensland and Wisconsin, indicating that perhaps most M. arborea genotypes can be used for hybridization. The problem with M. arborea in Wisconsin is that although we have gotten it to flower every winter in the greenhouse, we have not yet learned how to control when it will flower, or how profusely it will flower. We welcome any advice you can give us.

M. arborea flowers in the winter greenhouse at Madison, WI.

Flowers left to right; M. sativa MBms (blue); hybrid and sac-9 (variegated); M. arborea (yellow). sac stands for sativa-arborea cross.

Note the flower sizes of MBms (left) and sac-9 (right)

Note the co-expression of purple and yellow pigments in young flowers (upper right); changing to predominantly yellow in older flowers (lower right). MBms is on the left.

Dark purple velvet flowers of sac-10 produced by MBms.

Variegated hybrid of MBP X M. arborea produced at Queensland, 2005.

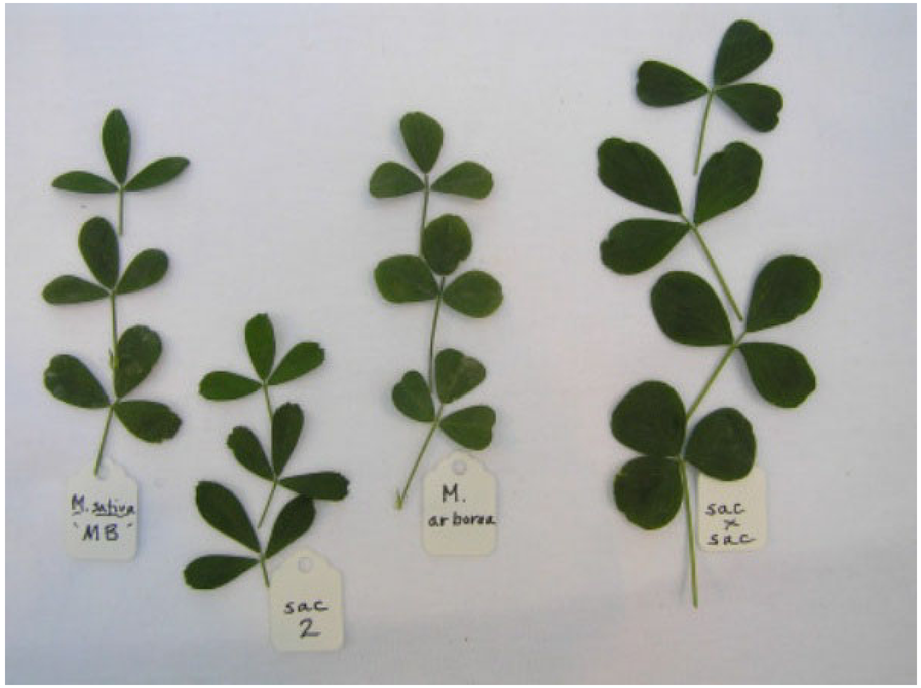
Profuse flowering of a variegated hybrid at Queensland.

Variegated hybrid with pronounced yellow keel produced at Queensland.

Hybrid of MBC X M. arborea (ARC) identified at Queensland.
It has many aborted flowers, slight variegation, and a yellow keel.
It contains AFLP bands unique to M. arborea.

Variegated derivative of sac-9 growing at Lodi, Italy.

Yellow segregate of sac-9 growing at Lodi, Italy.

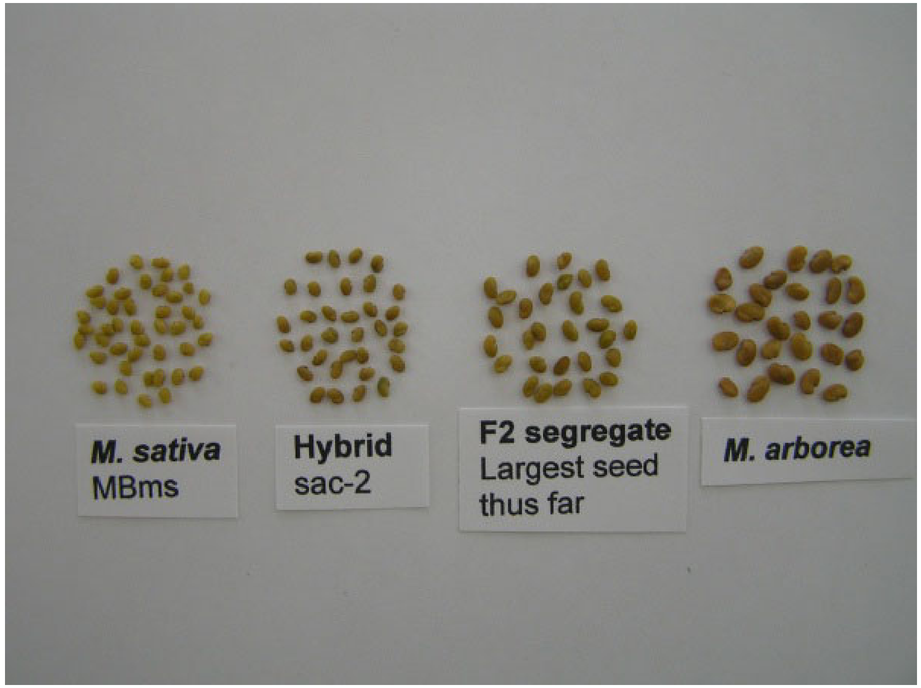


Leaves of hybrids like sac-2 shown here tend to resemble M. sativa. However, some derivatives of sac plants have leaves that resemble M. arborea, especially the lower leaves.

Pods of a sac derivative (left) and M. arborea (right) at Madison, WI. Pods of some hybrids and segregates resemble M. arborea more than do leaves.

Large flat pods of a hybrid in Queensland.

Large flat pods of a hybrid at Queensland.



Seeds of hybrids studied at Madison thus far are only slightly larger than the M. sativa parent. However, segregation in hybrid derivatives has yielded some plants with larger seeds intermediate to the parents.

Crown of an alfalfa plant about 10 months old.

Crown area of a M. arborea plant.

Close-up of the crown area of M. arborea with several crown buds that will develop quickly if the plant is cut.

Hybrid derivative about six months old
with a weakly developed crown.

Rare case (the only one thus far) of a near 8x plant (right)
that occurred as a self progeny of sac-4, a near 4x plant (center).
MBms (4X) is on the left side.

Flower color sectors are rare (circa 1/1000 flowers)
but occur on most hybrids and on some hybrid derivatives.
(continued in next figure)

Sectors are due to loss of the P gene (probably the chromosome with the P gene) during cell division.
(continued in next figure)

In most cases there was loss of one chromosome carrying P during hybrid embryogenesis, and later loss of the remaining chromosome, as shown here. This is a segregate not expressing yellow.

M. arborea exhibits inbreeding depression similar or greater than alfalfa. Plants in the top row are M. arborea X M. arborea S0 plants. Bottom row shows S1 progeny of the female parent in the cross.

Long growth tubes used by Carla Scotti
and her research group at Lodi, Italy.

CONCLUSIONS

Hybrids have been obtained using alfalfa male steriles from commercial cultivars, and M. arborea from the P.I. system. Hence, the materials are available.

Hybrids have ample fertility for gene transfer to alfalfa, and this is underway. Alfalfa has said HELLO to the genome of M. arborea!

Hybrids are easier to produce than haploids! Evidence for this is that only one haploid was obtained along with ten hybrids from MBms.

Application of the germ plasm from the genome of M. arborea is ahead of the basic research. This is often the case in plant breeding, where we exploited heterosis for 100 years while researching the basics.

Issues begging for research include:

-DNA marker study of hybrid genetic transmission or lack of it (segregation distortion). Ideally, use the same male sterile alfalfa to make hybrids with M. sativa, M. coerulea, M. falcata, and M. arborea. This will define aneuploidy, potential uses, and taxonomy.

-Extent of disomic versus tetrasomic segregation in above study. We have seen both types of segregations for the P locus controlling purple flowers. But, the whole hybrid genome needs examination.

-Gene expression; hybrids appear to express more of the M. arborea genome in the winter greenhouse. Is this because of the winter-active nature of M. arborea?

-Longevity; -Adaptation; -Disease resistance; -Quality; -Drought tolerance; -Cold tolerance (M. arborea and some M. falcata materials stay green at -8/9 C, and could extend the grazing season).

-Backcrossing individual M. arborea chromosome blocks into a standard stock(s) would be useful. Concept: Inbred-Backcross of Wehrhan and Allard 1965. See also: www.medicago-reports.org

REFERENCES:

Camadro, E. L., D. Carputo, and S. J. Peloquin. 2004. Substitutes for genome differentiation in tuber-bearing Solanum: interspecific pollen-pistil incompatibility, nuclear-cytoplasmic male sterility, and endosperm. Theor Appl Genet 109:1369-1376.

Fridriksson, S., and J. L. Bolton. 1963. Development of the embryo of Medicago sativa L. after normal fertilization and after pollination by other species of Medicago. Canadian J. of Botany 41:23-33.

Jansky, S., 2006. Overcoming hybridization barriers in potato. Plant Breeding 125:1-12.

McCoy, T. J., and E. T. Bingham. 1988. Cytology and Cytogenetics of Alfalfa. Chapter 24 in Alfalfa and Alfalfa Improvement. Eds.: Hanson, Barnes, and Hill. No. 29 in the Agronomy series, Am. Soc. Of Agronomy, Madison, WI USA

Medicago Genetic Reports. Vol. 5, Dedicated to M. arborea project. 2005. www.medicago-reports.org

Nenz, E., F. Pupilli, F. Damiani, and S. Arcioni. 1996. Somatic hybrid plants between the forage legumes Medicago sativa L. and M. arborea L. Theor Appl Genet 93:183-189.