Report on Progress Hybridizing Herbaceous *Medicago sativa and* Woody *M. arborea*

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Medicago sativa (L.), alfalfa or lucerne is a widely grown forage crop. *M. arborea* is a woody shrub native to the islands and areas around the Mediterranean Sea. It can grow to a height of 4 meters, and is remarkably drought resistant. Moreover *M. arborea* is the longest lived *Medicago* species. Whereas the old growth is woody, the young growth is relished by livestock. History records cultivation for livestock feed before Roman times, and use of the dark heartwood of old growth for knife handles and beads. *M. arborea* is still cultivated on some Greek Islands. Our interest is in genome relationships of perennial *Medicago* species, and transfer of traits from *M. arborea* to *M. sativa*. In particular, we are interested in large seeds, longevity, disease resistance, and morphological traits that may be useful in restructuring alfalfa. The hybridization barrier between *M. sativa* and *M. arborea* (both tetraploids) is post-zygotic failure of endosperm/embryo development. In 1998 we began screening for *M. sativa* genotypes that supported degrees of embryo development , and in 2003, a genotype (MBms) was discovered that produced 12 seeds/progeny after ca 2000 flowers pollinated. One plant was haploid, one from selfing, and ten showed hybrid characteristics. The ten varied widely in flower color, morphology and fertility. To test the repeatability of sexual hybridization, seed of MBms X 'P' (both parents from commercial alfalfa cultivars) was sent to Australia [2] and five plants were obtained in 2006 exhibiting asymmetrical hybrid characteristics. In 2007, genotype (M8) was identified that produced eight hybrid progeny in only about 500 flowers pollinated. M8 is derived from crossing *M. sativa* subspecies *sativa-coerulea-falcata*. The subspecies derivatives including M8 have fertility issues that apparently have weakened the hybridization barrier between *M. sativa* sensu lato and *M. arborea*. *M. arborea*-specific SSR markers have been verified in 2003, 2006, and 2007 hybridization events.

Home of *Medicago Arborea*

M. arborea flowers

Medicago sativa	***Medicago arborea***
Alfalfa Lucerne	Moon Medic; Tree Medic Tree alfalfa; Tree Lucerne Cytisus
Tetraploid: 2n = 4x = 32	Tetraploid: 2n = 4x = 32
Herbaceous	Woody Shrub
Blue/Purple Flowers	Yellow Flowers
Height: circa 1 meter	Height: circa 3+ meters
Perennial, 3-5 years with 3 or more harvests	Perennial, 25 – 50 years, if browsed lightly
Native: Asia Minor, now cultivated almost world-wide	Native: Mediterranean region and adjacent parts of Asia and Africa. History of sporadic cultivation and currently on some Greek Islands.
A sample of alfalfa male sterile genotypes crossed by hand with *M. arborea* pollen in GH in late winter. Alfalfa genotypes screened for degrees of pod development and seed set.	Pollen from a sample of regional genotypes used in crosses, in GH in late winter.

***M. sativa* (left) and *M. arborea* (right).**

M. sativa grows faster in summer.

M. arborea grows faster in winter.

M. sativa MBms (left) selected for the genetic tendency to produce a low frequency of hybrids with *Medicago arborea* (ca 1/1000).

Hybrid (right) with variegated flowers that express mainly anthocyanin pigment early (top) and yellow pigment later (bottom).

Hybrids have unbalanced chromosome sets from Parents probably due to chromosome elimination during embryogenesis, and in growing points.

HISTORY OF HYBRIDIZATION

1996	Somatic hybrids produced by electrofusion of *M. sativa* and *M. arborea* (Nenz, et al.1996). No hybrids prior to this.
1998-2002	3 to 4 different alfalfa male sterile genotypes pollinated each year with *M. arborea* pollen. Aborted seeds, but no hybrids.
2003	Alfalfa clone MBms produced 12 seeds from ca 1000 flowers pollinated by *M. arborea*.
2004 2005	The 12 seeds yielded one self progeny, one haploid, and ten hybrids.

2006	Australian group uses an MBms derivative and obtains five "asymmetric" hybrids involving *M. arborea* (Armour, et al. 2008)
2007	Subspecies-Bridge-cross derivative **M8** produced **10 hybrids** in only **500 flowers pollinated** by *M. arborea*. **M8** Pedigree = Six diverse subspecies parents in a 3-way hybrid X 3-way hybrid. [(*M. falcata*-a X *M. sativa*-ND) X *M. falcata*-b] X [(*M. coerulea*-2 X *M. falcata*-c) X *M. sativa*-D]
2008	confirmation of Hybrids by DNA markers.
2009	Production of two new hybrids of **M8** X *M. arborea* from a small number of crosses in Spring of 2009

Traits of Potential Use in Alfalfa Breeding

Large flowers (right) are associated with large seed, with some exceptions.

Plants with large flowers also are robust.

Large Seeds

Backcrossing large seed into alfalfa is progressing with no negative linkages at this time.

SOLID STEMS for:

- Lodging Resistance
- Reduce Alfalfa Weevil egg deposition

Hollow stems of spring growth on *M. sativa* cultivars (left), and solid stems of selected F_2 segregates (right).

Variation in leaf size and growth habit that may be of value

↓

Backcrossing required

Traits That are Neutral or Undesirable

M. arborea pod shape.

Appears independent of seed size.

Large stems and no regrowth.

Interesting new variation.
Probably not for livestock feed,
but may have new uses.

Variation in foliage color

Small, odd segregates,
some sterile,
some never flower,
some near sterile

Origin of Bridge-Cross Parent M8

Genotype **M8** was discovered while screening resources from our "Genetic Toolbox". **M8** is one of 28 progeny that were evaluated for hybrid breakdown (aka outbreeding depression), of a 3-way X 3-way hybrid.

Each of the six parents involved in the complex hybrid was normal in fertility and morphology. Hybrid breakdown in fertility was obvious; not one of the 28 progeny studied was normal in pollen production or seed set.

Hybrid breakdown in morphology was evidenced by multifoliolate leaves on 5 of the 28 plants, one of which was almost entirely multifoliolate.

Also, there was a range in vigor among the plants studied, but no data were collected.

M. sativa Bridge-cross Seed Parent **M8** (left) has cream-colored flowers.

M. arborea Pollen Parent (right) has dark yellow flowers.

Hybrid plant (bottom middle) has light yellow flowers.

Leaves of *M. sativa* seed parent **M8** (top left), *M. arborea* pollen parent (bottom row), and hybrids 2, 3, 4, 7 and 8, from transplants in GH fall of 2008.

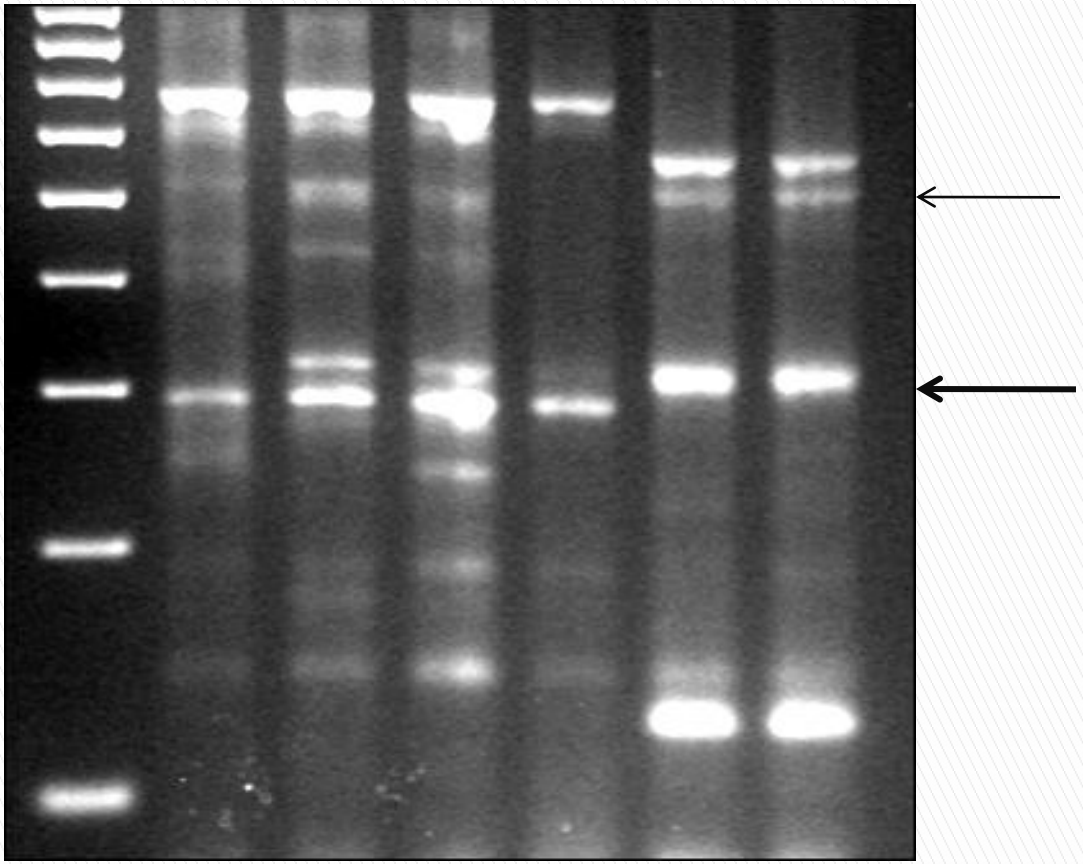
The shape of lower leaves of hybrids resembles *M. arborea (shown above)*, whereas upper leaves (not shown) tend to resemble *M. sativa*.

Chromosome Loss

Yellow sectors in flower petals are due to loss of *M. sativa* chromosome with gene for anthocyanin production.

Sectors occur on hybrids; 1/100- 1/1000 flowers.

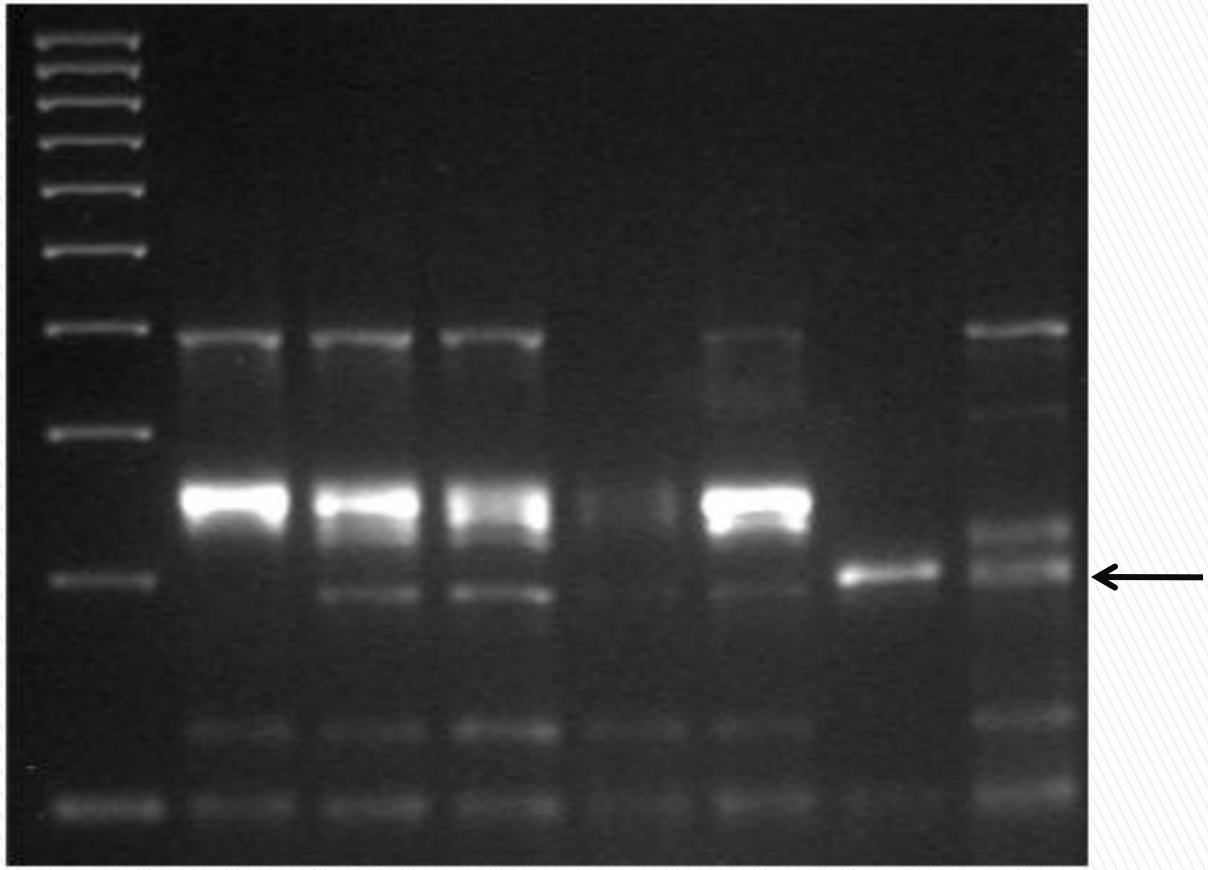
Vegetative sectors in leaves and side branches indicate all chromosomes are involved.



M8 2 3 4 A D

M. sativa s.l. Hybrids *M. arborea*

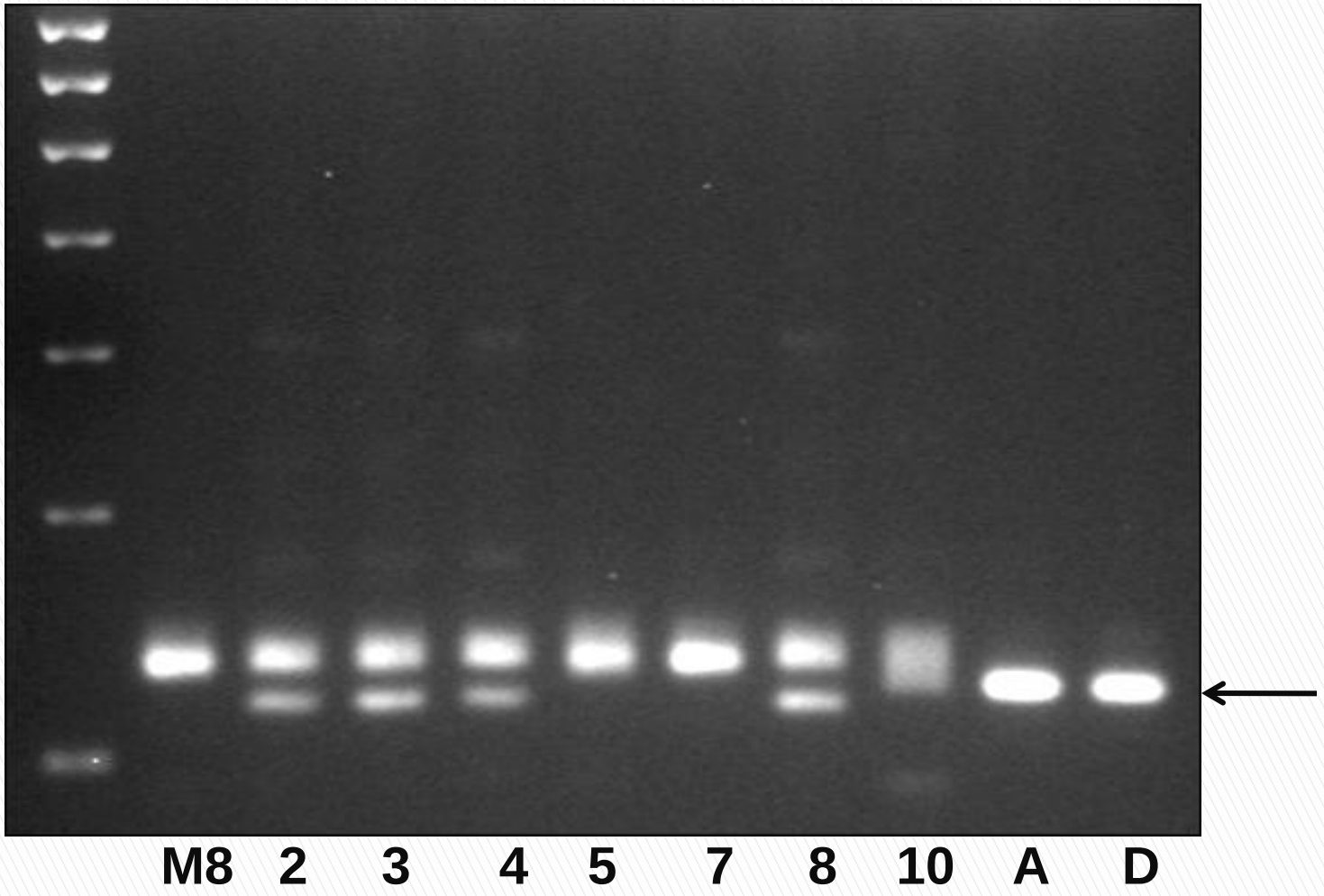
M. truncatula SSR primer: 71m126 Chromosome 8



M8 2 3 4 7 A D

M. sativa s.l. | Hybrids | *M. arborea*

M. truncatula SSR primer: MTIC354 Chromosomes 2



M. truncatula SSR primer: MTIC051 Chromosome 3

CONCLUSIONS

Hybrids have near the tetraploid number of chromosomes, 2n = 4x = 32.

Hybrids have low fertility, but normal fertility can be restored by backcrossing.

- Hybrids range from 5% - 50% of normal.
- F_2 segregates range from sterile to 60% of normal.
- Following two backcrosses to alfalfa with selection for large seed and fertility, seed number is 80% of normal, and seed weight is greater than 100% of normal.

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