

## **Alborea: A New Cultigen Developed from Hybrids of Alfalfa X Medicago arborea**

Edwin T. Bingham<sup>1</sup>, John A.G. Irwin<sup>2</sup>

(1. Emeritus Professor Agronomy, Univ. Wisconsin-Madison, WI, USA;

2. Emeritus Professor, School of Agriculture and Food Science, University of Queensland, St. Lucia, Australia)

### **Abstract**

Alfalfa, *Medicago sativa*, and *M. arborea* are autotetraploids ( $2n=4x=32$ ) assumed reproductively isolated until 2003. Then, an alfalfa genotype was identified in Wisconsin that produced a few seeds and progeny with hybrid traits after a large number of pollinations by *M. arborea*. At the same time in Australia, a derivative of this alfalfa genotype also produced a low frequency of progeny with hybrid traits. Thus, the hybridization barrier was weakened by selection of parents. Hybrids from both events expressed traits from *M. arborea*, and *M. arborea*-specific DNA bands. More of the *M. sativa* genome was retained, based on the DNA results; nonetheless, there was considerable genetic transfer from *M. arborea*. To date, more than 35 hybrids with an array of *M. arborea* traits have been obtained. Traits have been genetically transmitted in crosses with alfalfa, and hybrids have been crossed with each other to pyramid selected *M. arborea* traits. The latter material needed an identifying name for ease of recording and reporting. Alfalfa and *M. arborea* were abbreviated and combined for the name Alborea. The characteristics of Alborea, and its anticipated uses will be described.

### **Introduction**

*Medicago sativa* (L.) alfalfa or lucerne, is an important forage grown around the world. *M. arborea* is a woody shrub native to the islands and areas around the Mediterranean Sea that has been used as a browse plant since Greek and Roman times. *M. arborea* has many traits of potential use in alfalfa breeding. It can grow to a height of 4 meters, it is remarkably drought resistant, and it is the longest-lived *Medicago* species. *M. arborea* also has large seeds, disease resistance, and morphological traits that may be useful in restructuring alfalfa. Also, frost resistance has been observed in Wisconsin, with plants remaining green down to minus 8-10 C, before they need to be moved to greenhouse. This was unexpected, but has been observed each year for 15 years.

Wisconsin has had an interest in the potential usefulness of *M. arborea* traits for over 40 years (2). Crosses of alfalfa and *M. arborea* were made in 1970 and 1985, but, no hybrids were obtained. Then, in 1998, a new program began screening for alfalfa genotypes that supported degrees of embryo development after hand crossing with *M. arborea* pollen. Eventually, an alfalfa genotype that produced progeny with hybrid

traits was identified in 2003 (2). Seed from this alfalfa was sent to Australia where the hybridization was repeated (1). Recently, anthracnose resistance was transferred from *M. arborea* to alfalfa (1), and alfalfa X *M. arborea* derivatives have been used in alfalfa breeding (4,5,6). Also, traits such as large seed, robust growth, and traits of special interest are being backcrossed into alfalfa (2).

Alborea lines have been developed by intercrossing hybrids to pyramid *M. arborea* traits in specialized lines identified by a suffix. Thus, Alborea-YS stands for yellow-flowered synthetic; Alborea-P/V indicates purple/variegated flower color. Other specialized lines include large seed, arborea leaf, winter active, and various morphological traits.

### Materials and Methods

**Plant genotypes used to obtain hybrids:** In both Wisconsin and Queensland, male sterile alfalfa genotypes were used as seed parents to maximize the pollen parent contribution, and to minimize the amount of self seed and number of progeny to be screened. Between 1998 and 2003 no alfalfa male sterile was found that supported hybrid seed production (3). Then, in 2003, alfalfa clone MBms produced the first hybrids with *M. arborea*. Clone MBms is a selection from a cross of a male sterile plant from cultivar ‘Magnum III’ and a normal plant from ‘Blaser XL’. It behaves as a cytoplasmic male sterile (3).

In 2004, a second male sterile clone M8 was identified in Wisconsin that supported hybrid seed production. This clone has a complex pedigree involving subspecies of *M. sativa*, and is described elsewhere (3).

In Queensland, two cytoplasmic male sterile genotypes with MBms cytoplasm, were used. WA2071 is a selection from the cross of MBms x (Blaser XL, clone P), generated in Madison, and sent as seed to Brisbane (1). Clone WA2625 was generated in Wisconsin from the cross (MBms x Peruvian) x Sequel. Both the Peruvian and Sequel clones used as males are non-dormant. *M. arborea* plants used in Wisconsin and Queensland, were similar in pollen production, and are described elsewhere (3). Crossing procedures and plant growth conditions were similar to those commonly used in alfalfa breeding, and are also described elsewhere (3).

**Hybrids used to develop Alborea lines:** About half of the hybrids produce sufficient pollen to make hybrid X hybrid crosses. The first ten hybrids produced in Wisconsin were used in partial diallel crosses with the best pollen producers as pollen parents. These single cross products, named Alborea lines, were selected for flower color and other traits, and advanced by crossing with each other, or backcrossing to a particular hybrid to concentrate a trait. As new hybrids were produced annually, they were added to the Alborea program. The hybrids produced in Wisconsin have not been intercrossed with Australian hybrids, but likely will be as breeding progresses. Thus, development of Alborea lines is an ongoing program, with new hybrids still to be included.

### Description of Alborea and Discussion

Alborea YS will be described because it has yellow flowers, and other *M. arborea* traits. And, it is

available to collaborators. Other Alborea lines are similar except for their specialized traits.

**Fertility:** Selection for pollen and seed production has been very effective and still ongoing. At this time, Alborea YS seed production ranges from 60-80% of alfalfa in hand crosses in the greenhouse. Continued increase in fertility is expected with selection. In the field with bee pollination (honey bees, leafcutter bees, bumble bees, etc.) seed production is about 60% of alfalfa, when Alborea is isolated, and there is no competition with flowering alfalfa. But, if blooming alfalfa is in the vicinity, seed production is low because all bees seem to prefer blue/purple alfalfa over yellow flowers. The preference problem is reduced in a mixed planting of Alborea and alfalfa, where seed production on Alborea is better. This permits crossing Alborea with alfalfa in the field, and recovering the larger Alborea seed by size.

**Seeds:** Seeds of Alborea are larger than seeds of alfalfa (ca 500 seeds per gram), and Alborea plants range from 250 s/g to ca 400 s/g, with average ca 300 s/g. Pods: Most plants have pods resembling the large flat pods of *M. arborea* with one to three coils.

**Leaves:** Interestingly, most Alborea plants have broad *M. arborea*-type leaflets near the base of the plant, and gradually change to the narrower obovate leaflet shape of alfalfa toward the apex. Also, *M. arborea* leaflets have minimal serrations, and Alborea is segregating for the degree of serrations.

**Stems:** Stem morphology is variable. Most plants have hollow stems in the first growth in the spring similar to alfalfa. However, a low percentage of plants have solid stems like *M. arborea*. Also, stems vary from stiff and upright like *M. arborea*, to succulent like alfalfa, although Alborea tends to be more upright than alfalfa. No selection has been done for stem type, fiber content, or digestibility; hence, these traits remain variable.

**Roots:** Alfalfa tends to have a tap root, and *M. arborea* roots tend to branch. No selection has been done on root type, and it remains variable.

**Crown:** Alfalfa has a well developed crown, although it is narrow in non-dormant types. *M. arborea* lacks a crown, although there is some bud development after cutting. Alborea varies from narrow alfalfa-type crowns to very little crown. Once again, no selection has been done.

**Dormancy:** On average, Alborea YS and other Alborea lines are intermediate between the dormancy of the alfalfa parents and the non-dormant *M. arborea* parents. There has probably been some natural selection for dormancy, but no direct selection, hence, lines are variable for dormancy. We have observed in Wisconsin that relatively fall active, non-dormant Alborea lines are more winter hardy than expected. This is a potentially valuable trait.

**Frost Tolerance:** *M. arborea* plants have remarkable frost tolerance, much to our surprise. Frost tolerance is segregating in all Alborea lines, and no selection has been done. Hence, frost tolerance is an interesting trait for future breeding programs. It could be useful for stockpiling feed for fall grazing.

### Discussion

Alborea is a unique interspecific hybrid form of *Medicago* with several different uses.

1. Alborea as a cultivated crop per se, where specialized Alborea lines could be selected for environmental niches, or special uses.

2. As a gene donor in alfalfa breeding because it can be crossed with alfalfa, and backcrossed as necessary.

3. Production of hybrids of Alborea X alfalfa, grown together in seed production. Large hybrid seed from Alborea plants can be recovered by grading during seed cleaning. Both the large hybrid seed, and the alfalfa seed (also containing some hybrids) are commercial products.

4. A novel source of new variation for Alborea cultigens per se, and for use in alfalfa breeding. Alborea contains more variation than we have seen in alfalfa, and even more will be discovered when grown in new environments, and subjected to new screening techniques. Finally, new variation appears to be arising by recombination each generation, as expected for interspecific hybrids. The material is still at a “young stage”, so there is much to be discovered in the future.

#### References follow

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