In this Wisconsin selection nursery, some individual plants thrived, while others succumbed over time.

Bringing alfalfa to market takes many years

by Robin Newell

THE perennial forage plant commonly known as alfalfa in the Americas, alfa-alfa in the Middle East, and lucerne in many other parts of the world, is Medicago sativa. The species is thought to have originated in Iran, and there are records of its cultivation for grazing and hay in ancient Greek and Roman civilizations. No doubt there is an equally long history of selection and breeding in the crop.

In Europe, there were Flemish types described as having earlier harvest. When brought to the New World, settlers found their European ecotypes lacked the winter survival needed for much of North America where alfalfa is grown today. An early effort to improve winter survival came from a European emigrant to Minnesota. He selected his European seed for winter survival over a period of decades during the latter 1800s, developing a Minnesota cultivar known as "Grimm."

Alfalfa began to be known as the "Queen of Forages." Breeding and selection efforts in the last century led to improved varieties like Ranger (Nebraska 1942, improved yield) and Vernal (bacterial wilt resistant). By the 1950s several breeders were working in alfalfa, and by the 1970s most who were not at universities were employed by companies that brought larger breeding expertise and resources to bear. In North America, there were more than 20 alfalfa breeding programs of some note by the 1980s, followed by consolidation that led to just a handful of ongoing alfalfa breeding and variety development programs today in North America.

Alfalfa is different

From a modern breeding and variety development standpoint, alfalfa is unique and different from most other crop plants. Individual alfalfa plants have a high degree of self-incompatibility and exhibit significant inbreeding depression. This severely affects viable seed production after just one to two cycles of self-pollination. Several generations of inbreeding, as required for true homozygous inbred development as we think of it in corn hybrids, is generally unsuccessful.

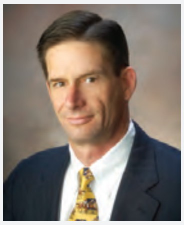
Commercial alfalfa cultivars do not achieve the uniformity among plants made possible by several generations of inbreeding in the product development process of other major crops. Therefore, all commercial alfalfa cultivars, whether marketed as varieties or semi-hybrids, are segregating populations of related plants. Like a human family, an alfalfa cultivar is comprised of related individuals having both similarities and differences.

How then do alfalfa breeders improve the crop given its genetic peculiarities?

They typically maintain several germplasm lines and employ selection techniques that are suitable to the crop. You can think of a germplasm line as

ROBIN NEWELL

The author is the vice president of North American sales for S&W Seed Company.



an extended family that has a shared set of characteristics. For some examples, a breeder might maintain several lines based on fall dormancy, plus a leafhopper resistant line, perhaps a salt tolerant line, or even transgenic lines. Alfalfa breeders strive to improve their germplasm lines over generations, mainly using 1) phenotypic recurrent selection for simply inherited traits and 2) genotypic selection to improve more complex traits.

Picking and proving

Phenotypic recurrent selection is used for traits you can observe directly, such as robust plant type, or reaction to a disease or pest. Plants that exhibit desired characteristics are selected from the line (population) and bred (crossed) onto others in hopes of improving the frequency of desirable types in successive generations of the germplasm line. Effort is underway to develop genetic markers for more rapid phenotypic selection, but this is not yet far advanced in alfalfa.

Genotypic selection takes the added step of testing progeny performance in order to prove the genetic contribution of parent plants. This progeny testing can be likened to testing the daughters of dairy bulls, proving the breeding value of the bull. Genotypic evaluation through progeny testing is not always used for new alfalfa cultivar development. But it is more effective than phenotypic recurrent selection for making progress in multigenic traits such as yield, persistence, and forage quality.

The typical path for alfalfa germplasm improvement starts with a polycross among a varying number of plants selected by the alfalfa breeder. Seedlings and plants grown from the resultant seed are evaluated for phenotype and selected for improved characteristics. A savvy breeder will expose seedlings to seedling diseases to remove susceptible plants. Seedlings that thrive can be further inoculated with disease organisms, transplanted into selection nurseries outdoors, and monitored over multiple years for disease resistance, insect resistance, persistence, winter survival, and yield appearance.

A breeder can siphon off plants to use as parents in a new potential cultivar at this point. This can be a fast development path for single gene dominant traits based on phenotypic selection, but parental yield is not yet tested at this stage, and breeding value is unknown.

After multiple years in the selection nursery, and exposure to the growing environment, the most promising plants are selected for clonal propagation in the greenhouse. When fully developed, the clonal plants are randomly crossed by bees in the greenhouse, then seeds are harvested separately from each clonal plant. Seeds from each plant are planted in a single short row. The resultant plants in each half-sib row are from a single mother plant, while each plant within a half-sib row has an unknown father.

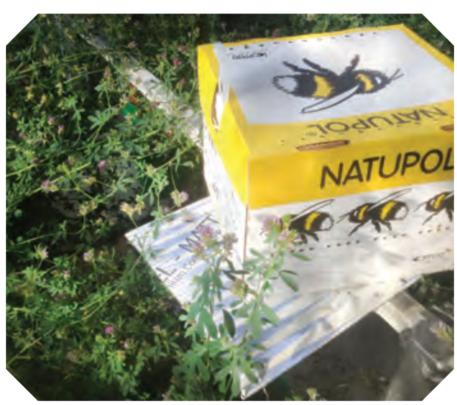
Several hundred potential parents can be evaluated in a half-sib selection nursery, where each row represents the offspring from a single potential parent plant. This is another point where the breeder can siphon off potential parent plants based on phenotypic selection, selecting the best plants from the best half-sib families to use as parents for a new cultivar.

Finding the best mothers

In a further step, yield of the half-sib rows can be compared across multiple locations and years. This extra step can require additional growing seasons but enables breeders to prove the mother plants' breeding value, through progeny evaluation for yield potential, forage quality, and general combining ability of each potential parent. Mother plants remain in a holding nursery while this multiyear evaluation unfolds.

Those mother plants that are represented by the best half-sib rows can be selected as parents and intercrossed among each other to create new breeding lines and potential cultivars. Here again the breeder can select parent plants, this time selecting mothers of the best yielding half-sib rows. These selected plants can be used in a poly- ▸▸▸

Cuttings are taken from the most promising plants in the selection nursery for clonal propagation in the greenhouse. This provides additional "copies" of the mother plant. Here, small cuttings are misted several times hourly until rooted (about two weeks), then transplanted into pots for winter seed production in the greenhouse.



Growing in pots over winter in the greenhouse, plants are randomly intercrossed by bees. Seed from each plant can be harvested separately, thus having one plant as a mother, with all other plants in the greenhouse as pollen sources (many fathers) for the resultant seed.

In the half-sib selection nursery, each short length of row is a family of plants derived from the same mother plant, but multiple unknown fathers as a result of random crossing in the greenhouse. Performance of each row predicts the breeding value of the original plant, a concept akin to selecting sires based on bull proofs

cross to synthesize a new cultivar, or a new breeding line in the ongoing cycle of population improvement.

Regardless of selection method, the vast majority of commercial alfalfa cultivars are synthetic cultivars, synthesized by intercrossing from 10 to 200 parent plants selected for individual robustness and desired characteristics. Plants chosen as parents are typically intercrossed using bees in isolation such as a greenhouse or screened cage to prevent the introduction of pollen from outside sources. The small amount of breeder seed thus produced represents the initial generation of seed for an experimental cultivar.

A portion of this breeder seed will be used for small plot forage trials and characterization that includes testing for disease and pest resistance. Another portion can be used to grow a small field of foundation seed in isolation from other alfalfa types. The remainder can be archived in controlled storage for future breeding and selection, and/or reconstitution of foundation seed if needed in future years.

Applying standard tests

A critical step on the path to commercializing a new cultivar is the evaluation for overall forage yield, persistence, and characterization of pest and disease resistance ratings. Remember, individual plants within a cultivar are different from each other. For this reason, commercial cultivars receive ratings for levels of disease and pest resistance based on percent resistant plants within the cultivar (see table). This characterization, along with forage yield and quality trials, takes from two to four years of additional testing for an experimental cultivar.

Characterization for disease and pest resistance is performed according to standard tests approved by the National Alfalfa Variety Review Board, and subject to yearly review by a panel of industry and university experts. An experimental cultivar must be adequately described in accordance with review board standard tests before being sold as certified seed in the United States. To learn more, see www.naaic.org. To see a current list of many commercially available cultivars with their pest and disease resistance ratings, visit www.alfalfa.org.

A well-known development in plant breeding is the use of genetic modification techniques that usually add small amounts of genetic material to the plant's DNA, thus expressing a novel protein or enzyme change not normally found in the crop. The Roundup Ready and HarvXtra traits are the two examples available commercially.

The transformation in genetic material is performed in a lab environment on undifferentiated callus cells, which are then selected for the desired change in protein or enzyme expression. When adequately regenerated into a viable plant, these can be cloned as donor lines, transferring the transgenic trait into more desirable breeding lines, and eventually commercial cultivars. ●

Alfalfa resistance ratings

% Resistant plants	Resistance class	Class abbreviations
0-5%	Susceptible	S
6-14%	Low resistance	LR
15-30%	Moderate resistance	MR
31-50%	Resistance	R
>50%	High resistance	HR

Next issue:
Field seed production