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ALFALFA

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Abstract

Cultivated alfalfa or lucerne (*Medicago sativa* spp. *sativa*) is the most important forage legume in the world with approximately 32 million hectares cultivated mainly in the temperate regions of both the northern and southern hemispheres. Intensive research has been conducted on the management and physiology of the crop and a major seed industry has developed world wide. Since insect and disease pests are numerous in alfalfa, development of cultivars with the proper fall dormancy and a broad genetic base for pest resistance has been pursued in order to provide increased adaptation, persistence, and yield. There has been a movement lately to introduce more complex genetic traits into these multiple pest, dormancy specific cultivars. Cultivars with grazing tolerance, potato leafhopper resistance (achieved via introgression of genes for glandular hairs from related *Medicago* subspecies), and low bloat incidence are now marketed. Continued alfalfa breeding will be necessary to develop resistance to intransigent pests such as *Lygus*, expand adaption into hostile environments such as saline or acid, aluminum toxic soils, incorporate true bloat resistance, increase N₂ fixation, and maximize economic yield. The use of biotechnology methods to improve the crop has already begun. Molecular markers are now being assessed for their practical use in alfalfa breeding. The introduction of novel genes via transformation is also underway in alfalfa. In addition to its role as the major legume hay, silage, and pasture crop, alfalfa is currently being investigated as a fuel for use in generating electricity, a bioremediation system for removal of harmful nitrates, a source of pulp for paper manufacturing, and a "factory" for production of industrial enzymes. Finally, alfalfa's future use as a component in low management mixed pastures, in sustainable conservation agriculture, or as a viable crop in the tropics is beginning to be examined.

Keywords: Alfalfa, lucerne, *Medicago sativa*, breeding, genetics, management, molecular markers, transformation

Introduction

The primary center of origin for the genus *Medicago* is found in the Caucasus, northwestern Iran, and northeastern Turkey. *Medicago sativa* consists of a complex of several perennial subspecies, both diploids and tetraploids, which are interfertile and have the same karyotype (Quiros and Bauchan, 1988). There are four recognized subspecies of *M. sativa*. *M. sativa* ssp. *sativa*, *M. sativa* ssp. *falcata*, and *M. sativa* ssp. *glutinosa* are tetraploids. *M. sativa* ssp. *coerulea* (progenitor to cultivated alfalfa) and some *M. sativa* ssp. *falcata* are diploids. Cultivated alfalfa (ssp. *sativa*) is an autotetraploid with $2n=4x=32$ (Stanford, 1951). This current paper will concentrate on the cultivated, autotetraploid species known worldwide as either alfalfa or lucerne.

Some important characteristics of cultivated alfalfa which enhance its position as the most widely used forage crop are (Barnes et al. 1988; Bingham, 2000): 1) high nutritional quality for livestock (protein, energy, vitamins, and minerals), 2) high biomass production (the record

yield for one hectare of alfalfa is over 18,000 kg ha⁻¹ of forage) 3) ability to fix atmospheric nitrogen (N₂), 4) wide adaptation to various environments, 5) improvement of soil tilth when growing the crop (excellent basis for sustainable agricultural systems), 6) utility as a model system for genetic studies of autotetraploid species, and 7) its ease of use with the new biotechnologies.

Past Trends

Alfalfa has been spreading world-wide as an important forage crop since the pre-Christian era and was estimated during the 1980s to be cultivated on approximately 32 million hectares mostly within the temperate regions of both the northern and southern hemispheres (Michaud et al. 1988). Nearly 70% of this land area was found in the USA, Argentina, and the southern portion of the former USSR (central Asia, Transcaucasus, and Ukraine) with an additional 17% cultivated in France, Italy, Canada, and China. Although the current world acreage levels have not been estimated recently, decreases in traditional growing areas such as the USA, Argentina, and Italy have probably been off-set with increases in places like China and Australia leaving the 32 million hectare figure still the best estimate for world production (Table 1). However, acreage increases in Australia and China could mark the beginning of a significant shift in the main areas where the crop will be grown in the future.

An important development during the past century was the use of local varieties and land races as base populations for cultivar improvement. Even in areas of the world where alfalfa was only recently introduced, such as North and South America, this early introduction led to the adaptation of the land races needed for future breeding. The approach of using local varieties and land races capitalized on natural selection for adaptation and demonstrates the important role adaptation plays in the success of alfalfa cultivars today throughout the world.

Since insect and disease pests are numerous in alfalfa (Table 2), development during the late 1900s of modern cultivars with the proper fall dormancy, winter hardiness, and a broad genetic base for pest resistance was pursued in many countries within these adapted germplasm pools in order to provide increased persistence and higher yield. Of great value during this time, was the development and use by breeders worldwide of the standard tests developed by the North American Alfalfa Improvement Conference for screening and selection of various diseases, insects, and nematode pests (North American Alfalfa Improvement Conference, 2000; Table 2). In this same on-line publication, agronomic tests for fall dormancy, salt tolerance, winter hardiness, relative feed value, and grazing tolerance can also be found. These standard tests are put to good use in current cultivar development programs.

Nearly all modern alfalfa cultivars are synthetic cultivars. The approach taken is to identify the individual parental genotypes which contain the traits of interest using the various screening procedures. Most synthetics are broad-based usually containing more than 100 parents. This trend of using large numbers of parents began in the early 1970s and was based on the theoretical considerations of alfalfa's autotetraploid genetics indicating inbreeding was reduced by large parental numbers via achievement of maximum heterozygosity for intra-allelic interactions (Busbice and Wilsie, 1966; Hill, 1987). Bingham et al. (1994) recently proposed that a reduction of complementary gene interactions rather than intra-allelic interactions is probably responsible for inbreeding depression in alfalfa.

Intensive research was also conducted throughout the world on establishment methods, soil liming and fertility, *Rhizobium* inoculation, cutting schedules, and methods of grazing management as well as alfalfa's basic physiology (Hanson et al., 1988; Langer, 1967). Finally,

the emergence of a seed industry capable of providing high quality seed of these land races and new cultivars is felt to be greatly responsible for the emergence of alfalfa as the world's major forage legume (Barnes et al., 1988).

Recent Advances

Alfalfa has therefore established itself as a major crop, and the major forage crop, in the world as far as land area in cultivation, use, and sophistication of its management and production systems. Most alfalfa use in its main growing areas is for stored feed; high quality hay, silage, and dehydrated pellets and cubes. Even when used for direct grazing, these pasture systems require high levels of management to establish and maintain the crop as well as grazing plans which maximize yield and animal production yet reduce problems such as bloat. Alfalfa will continue to be used anywhere there is a need for high quality feed for livestock, especially dairy, in the temperate regions. For example, with shortages of feed cereals in China, there has been a substantial increase in alfalfa acreage which is likely to continue until 2010 (Zhang, 1998).

In the high management systems required for stored feed or controlled grazing, the goal is to enhance yield per hectare and feeding value of the crop either through the crop's genetics or the technology required to actually store or manage the crop. Much research and farmer demonstrations have been conducted to supply information on the best methods for establishing and managing alfalfa as a hay, silage, and grazing crop. Management, marketing, and utilization plans are now easily available and accessible in all parts of the world via traditional extension publications for each country or on the internet (Hannaway et al., 1998).

To judge the success of recent cultivar development, one has only to examine performance trials in their respective region to observe that most new cultivars are outperforming the older checks (sometimes as much a 30 to 40% in yield). Another example supporting this view can be seen in the overall USA production statistics (National Agricultural Statistics Service, 2000). Although the total USA acreage dropped during the period 1969-1999 from 10,787,000 to 9,713,000 hectares, the annual yield per hectare increased from 2,545 to 3,125 kg ha⁻¹ thus allowing total production to actually increase from 68,826,000 to 76,119,000 Mg. This increase in overall production is felt to be due to the use of improved cultivars along with new and better management systems.

There has been a movement lately to introduce more complex (from a genetic point of view) traits into the current multiple pest, dormancy specific cultivars. Cultivars with grazing tolerance (Bouton et al. 1993), low bloat potential (Goplen et al. 1993), and potato leafhopper resistance achieved by introgression of glandular trichomes from *M. sativa* spp. *glandulosa* and spp. *glutinosa* (McCaslin, 1994) are now being marketed in the main alfalfa growing regions. Continued alfalfa breeding and management research will be necessary to develop resistance to intransigent insect pests such as *Lygus*, expand adaptation into hostile environments such as acid, Al toxic soil, overcome bloat, increase N₂ fixation, and maximize economic yield.

Future Trends

Biotechnology

The future for alfalfa improvement for high management systems will surely include using molecular markers and transformation technologies to develop the modern cultivars. Molecular markers from genetic maps are being used in crop improvement programs to increase

the efficiency of selection for intransigent genes. These genetic maps also allow identification and selection of chromosome fragments associated with the gene of interest, even traits governed by multiple genes or quantitative trait loci (QTL). Molecular markers are therefore an important tool for plant breeders during selection. Transformation involves movement of specific and useful DNA segments even between two unrelated organisms. It has great promise and has already shown success in making crop plants resistant to insects, viruses, and herbicides and fruits resistant to spoilage. However, insertion of biotech traits with transformation has spawned an entire set of public relations problems for the major grain crops, and its future in alfalfa research, especially in Europe, is still unknown (Veronesi and Rosellini, 2000). A recent survey of researchers showed that many value-added traits are being pursued in alfalfa via these biotechnology methods (Samac et al. 1998; Table 3).

Cultivated alfalfa's autotetraploid, polysomic inheritance complicates the genetic analysis required for genetic mapping. There are diploid subspecies available and diploid genotypes have been developed from cultivated alfalfa. These are all cross fertile with cultivated alfalfa and have greatly simplified the analyses required for developing diploid maps. Five genetic maps of alfalfa were initially published, four of which utilize diploids (Brummer et al., 1993; Kiss et al., 1993; Echt et al., 1994; and Tavoletti et al., 1996) and one which utilizes tetraploid germplasm (Osborn et al. 1998). The recent reports of using single dose restriction fragments (SDRF) (Osborn et al. 1998) or simple sequence repeats (SSR) with single dose allele (SDA) analysis (Diwan et al. 1996) could simplify mapping in tetraploids. One problem with the alfalfa maps constructed with diploid F₂ inbred populations is the high degree of segregation distortion favoring heterozygotes. In some cases, upwards of 50% of the markers show a skewed distortion. One explanation is inbreeding uncovered deleterious recessive alleles (Osborn et al. 1998). The heterozygous loci tended to cluster together in linkage groups, and it is possible that not all linkages are valid. The non-inbred map constructed by Tavoletti et al. (1996) in an F₁ population showed only 8.8% segregation distortion and indicated the use of non-inbred populations may overcome this problem.

The genetic maps developed in both diploid and tetraploid alfalfa have potential for utilization in alfalfa improvement programs for the following practical approaches (Osborn et al., 1997; Bouton and Sledge, 1999): 1) interspecific hybridization analyses, 2) estimating genetic variation among and within the different germplasm sources, 3) marker assisted selection especially introgression of genes from wild and related subspecies, and 4) estimating genetic dissimilarity among parental genotypes for production of high yielding populations or synthetics. For practical cultivar development, marker assisted selection and selection of parents for synthetics have the most potential at this stage.

The DNA delivery systems which are routinely used to introduce genes into alfalfa are the Ti plasmid from *Agrobacterium tumefaciens* and particle bombardment or biolistics (Bingham, 2000). The introduction of novel genes via transformation is now underway in alfalfa with cultivars containing biotech traits speculated to be available in the next few years. The recent announcement of commercialization of herbicide resistance via the Roundup Ready gene by 2004 is an example (McCaslin and Fitzpatrick, 2000). The use of this particular gene also shows the value of alfalfa as a major crop in the USA and as the forage crop of choice for delivery of biotech traits. Another very important potential development for alfalfa would be the insertion of genes for condensed tannins for relief of bloat in animals (Morris and Robbins, 1997). This trait would have immense value to the livestock industry especially for direct grazing.

Other Uses and the Tropics

Alfalfa's traditional role as hay, silage, pasture and dehy crop has been, and will continue to be, refined and expanded as a result of cultivar improvement and agronomic research efforts throughout the world. At the same time, nontraditional uses such as sprouts for salads and nutritional supplements for human diets have already increased for alfalfa (Bouton 1996). The crop is currently being investigated as a fuel for use in generating electricity, a bioremediation system for removal of harmful nitrates, a source of pulp for paper manufacturing, and a "factory" for production of industrial enzymes (Bouton 1996).

Alfalfa's future use as a component in low management mixed pastures or in sustainable conservation management is starting to be examined. Although in Argentina alfalfa has always been an important component of mixed pastures (Basigalup, 2000), using it in these conditions in North America is a new, but growing enterprise (Smith and Singh, 2000). These pasture systems are noteworthy in that they must be conducted in poorer soils, in competition with other species such as grasses, and with less use of resources to establish and manage the crop. In Australia, alfalfa acreage has also increased as the crop is being used to assist with many land degradation issues (Bellotti et al., 1998; Latta et al., 1998). These include relief of dryland salinity by replacing annual plants which contribute to this problem, reduction of soil acidity by recovery of leached nitrate, and as a weed management strategy to control herbicide resistant weeds which have developed with previous cropping systems.

Another important question is can alfalfa become a viable crop in the tropics? An ability to be used in the context of high disease and insect pest pressure (Lloyd and Lowe, 2000) with tolerance to problem soils such as those with acidity and aluminum toxicity (Dall'Agnol et al., 2000) will be a must for the tropics and subtropics. There are now four factors, which if capitalized upon, could make alfalfa a viable crop in the tropics: 1) the availability of very non-dormant, winter active germplasm which allows forage production on a year round basis, 2) the wide availability of germplasm and the use of screening and selection methods for the many disease, insect, and nematode pests (Table 2; North American Alfalfa Improvement Conference, 2000), 3) the increasing interest and need in dairy production and high quality grazing in the subtropics and tropics, and 4) the advances in research which may allow the crop to be grown in problem saline and acid, aluminum toxic soils (Bouton and Parrott, 1997). An ability to provide a nondormant, acid soil tolerant, high pest resistant cultivar would be a big step toward establishment of "tropical alfalfa" systems.

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Table 1 - Alfalfa acreage in selected countries.

<u>Country</u>	<u>1981-83 Census</u> [†]	<u>Current Estimates</u>
	-----ha-----	
USA	10,559,000	9,713,000 (NAS, 2000)
Argentina	7,500,000	5,994,000 (Basigalup, 2000)
Italy	1,300,000	800,000 (Pecetti and Piano, 2000)
China	900,000	1,330,000 (Zhang, 1998)
Australia	115,000	772,000 (Bellotti et al. 1998)
Total World	32,267,000	~32,000,000

[†]From Michaud et al. 1988.

Table 2 - List of major alfalfa pests which have screening procedures listed in the “Standard Tests to Characterize Alfalfa Cultivars” manual (North American Alfalfa Improvement Conference, 2000).

Diseases

Anthracnose - *Colletotrichum trifolii* Bain & Essary
Aphanomyces Root Rot Resistance (Races 1 and 2) - *Aphanomyces euteiches* Drechs.
Bacterial Wilt - *Clavibacter michiganense subsp. insidiosum* (McCull) Davis et al (syns. *Corynebacterium insidiosum* (McCull.) H.L. Jones).
Common Leaf Spot Resistance - *Pseudopeziza medicaginis* (Lib.) Sacc.
Downy Mildew - *Peronospora trifoliorum* de Bary
Fusarium Wilt - *Fusarium oxysporum Schlecht f. sp. medicaginis* (Weimer) Snyder & Hans.
Lepto Leaf Spot - *Leptosphaerulina briosiana* (Poll.) Graham and Luttrell
Phytophthora Root Rot - *Phytophthora megasperma* Drechs. f. sp. *medicaginis*
Pythium Seed Rot and Damping-off - *Pythium spp.*
Rust - *Uromyces striatus* Schroet.
Sclerotinia Crown and Stem Rot - *Sclerotinia trifoliorum* Eriks.
Spring Blackstem and Leafspot - *Phoma medicaginis* (Malbr. & Roum.) var. *medicaginis* Boerema
Stagonospora Leaf Spot and Crown Rot - *Stagonospora meliloti* (Lasch) Petr.
Stemphylium Leaf Spot (Warm Temp. - Eastern Biotype) - *Stemphylium botryosum* Wallr.
Verticillium Wilt - *Verticillium albo-atrum* Reinke & Berth.
Yellow Leaf Blotch - *Leptotrochila medicaginis* (Fckl.) Schuepp.

Insects

Alfalfa Weevil - *Hypera postica* (Gyllenhal)
Blue Alfalfa Aphid - *Acyrtosiphon kondoi* Shinji
Clover Root Curculio - *Sitona hispidulus* (F.)
Pea Aphid - *Acyrtosiphon pisum* (Harris)
Potato Leafhopper - *Empoasca fabae* (Harris)
Spotted Alfalfa Aphid - *Therioaphis maeulata* (Buckton)

Nematodes

Alfalfa Stem Nematode - *Ditylenchus dipsaci* (Kuhn) Filipjev
Root-Knot Nematode - *Meloidogyne hapla* Chitwood, *M. incogita* Chitwood, *M. javonica* (Treub) Chitwood, and *M. chitwoodi* (race 2)
Root-lesion Nematode - *Pratylenchus penetrans* Cobb, Filipjev and Schur-Stekhoven

Table 3 - Traits of alfalfa needed for the future and the biotechnological methods being investigated to achieve them (from Samac et al. 1998).

Trait	Method
Acid Soil, Aluminum Tolerance	Transformation; Molecular Markers
Apomixis	Molecular Markers
Bloat Control via Condensed Tannins	Transformation
Cold Tolerance, Winter Hardiness	Transformation, Molecular Markers
Disease Resistance	Molecular Markers, Transformation
Genetic Distances; Synteny	Molecular Markers
Herbicide Resistance	Transformation
Heterotic Blocks; Yield Enhancement	Molecular Markers
Salt Tolerance	Somatic Hybridization, Transformation