Legume perspectives

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LEGUME PERSPECTIVES

Alfalfa: Back to the Future

Lucerne: The first letter in the legume alphabet

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Front cover photo
Paolo Annicchiarico

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Legume Perspectives is an international peer-reviewed journal aiming to interest and inform a worldwide multidisciplinary readership on very different aspects of the research and use of all kinds of legume plants and crops, including genetics, agronomy, animal production, human nutrition and health and economics.

Our journal prefers review articles on different legume species and crops and various legume research topics. The structure of review articles is rather loose by not strictly following structure of original research articles. Please write your article so that it will assist in the exchange of information between people working in different expert fields of legume research: write to the length requested; provide a review of the most important information on your topic; try to explain specialist words, unusual jargon and acronyms; emphasise results and conclusions; choose titles carefully and add subheadings that tell readers something. *Legume Perspectives* prefers a clear, simple and comprehensive writing style that would make its articles interesting and useful for both academic and amateur audience.

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EDITORIAL

This monographic issue on alfalfa (alias lucerne) reflects the recent evolution of AEP (Association Européenne de Recherche sur les Protéagineux) into ILS (International Legume Society), i.e., a wider scientific community with a focus on both grain and forage legumes. This change recognizes the large affinities existing between these groups of crops for genetic and genomic characteristics (as confirmed by legume synteny studies and the key role played by Medicago truncatula as a model species) and in other respects, e.g.,the interactions of plants with nitrogen fixing bacteria, or the dynamics of associations of legume and non-legume plants. There is also increasing opportunity for research projects devoted to improving simultaneously annual and perennial legumes, such as the FP7-ArimNet project REFORMA (Resilient, water- and energy-efficient forage and feed crops for Mediterranean agricultural systems), which provided the context for the current editorial work. ILS initiatives, including this journal, will respond to the need for greater cultivation of legumes - of all kind.

Paolo Annicchiarico and
Bernadette Julier
Managing Editors of
Legume Perspectives issue 4

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…Paolo Annicchiarico[1] and

Bernadette Julier[2]

Alfalfa: Back to the Future

Alfalfa (alias lucerne) has played a major agricultural role in ages of the human civilization that featured technological progress and economic stability, such as the most prosperous periods of the Persian Empire in western Asia, the Roman Empire in the Mediterranean region, the Han Dynasty in China, the Arab Empires in the Middle Ages, and the Renaissance in Europe. These conditions favoured the exploitation of this crop for specialized forage productions, as well as for enhancing the soil fertility. The appreciation of this crop by farmers of temperate and subtropical crop-livestock systems has risen until the second half of the 20th century, when the low cost of energy and the ignorance of the impact of a high-carbon economy on climate change favoured the adoption of crop-livestock systems that are manifestly unsustainable.

In West Europe, the maintenance of alfalfa cultivation in vast areas during this period is remarkable, when considering its much lower level of EU subsidization compared with other major crops (with subsidies that were granted only to the industry of dehydration).

We believe that alfalfa is going to play a crucial role in a new, environment-friendly chapter of the human civilization in which agriculture is asked to produce more and better, using far less energy (for nitrogen fertilizers, provision of extra-farm feed proteins, soil tillage, weed and pest control, etc.). The return of this crop to its destiny of a bright future is favoured by its wide range of possible target environments and utilizations (hay, silage, grazing; high-protein feedstuff; dual-purpose for forage and energy). ■

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Perspectives of alfalfa in Europe

by Christian HUYGHE[1]*, Luc DELABY[2], Daniele ROSELLINI[3] and Fabio VERONESI[3]

Abstract: Sustainable agriculture shall reconcile high productivity with preservation of the environment, and at the same time compete in a global market. Alfalfa can help to meet the challenges of climate change and pollution reduction, by favoring crop rotations capable of better preserving the soil and controlling weeds. The utilization of alfalfa through grazing may also reduce risks of pollution and production costs. Alfalfa will keep an important place in European agriculture, because it sustainably supports high quality dairy productions. These unique features can be further improved through technical and varietal innovation.

Key words: crop rotation, forage, grazing, *Medicago sativa*, sustainable agriculture, weed control

Stakes of the European agriculture and worldwide context

After a period when an increasing production was the only objective of agriculture, followed by a decade when the environmental issues were the main concerns, it is now well accepted that a sustainable agriculture shall reconcile high productivity with environment preservation. A highly productive agriculture must meet the food and non-food demand of the European societies and also be competitive on the world market, considering that most protein sources for supplementation of animal feed are imported, and that these products have shown large price variations in recent years.

The environmental issues are most complex to identify, because they have both merchant and non-merchant aspects, such as biodiversity preservation, and because they have different time horizons. In a short term perspective, the reduction of pollution by nitrates and pesticides is essential and prescribed by the European directive on pesticides and quality of the water resources. In a mid-term perspective, water availability and energy resources for agriculture have to be considered. In a long-term perspective, climate change will dramatically modify the conditions for agricultural activity. As a consequence, on the one hand we have to anticipate such changes, on the other hand we should reduce as much as possible the present emission of greenhouse gas (GHG), namely, CO_2 from production of agricultural inputs, CH_4 from ruminants, and N_2O from application of nitrogen fertilisers. Research and innovation are required to meet these objectives.

Potential of alfalfa in this context

Alfalfa acreage in Europe is devoted either to production of dehydrated forage, mainly in Spain, France and northern Italy, or to on-farm use of forages, mainly as hay. While the production of dehydrated forage tends to plateau or to decline due to the cost of energy for dehydration, the on-farm use of alfalfa is slightly increasing after a long period of steady decline.

In Europe, plant and animal productions tend to be separated, with specialized farms and territories. Alfalfa has the peculiarity to be inserted in grain crops rotations, and also used in animal farms. While this was considered as a downside, it appears to offer a high potential for future farming needs. In grain farms with specialized short rotations, farmers have to cope with difficulties in controlling weeds, which become highly specialized and sometimes resistant to herbicides. The insertion of perennial alfalfa in rotations with a high proportion of winter wheat has proved to be able to control well the weed flora. The results reported in Fig. 1 show that the species composition of the weed community depends on the crops, and the presence of alfalfa in rotations reduces dramatically the risk of specialization of the weed flora compared with cereal monocultures (thereby reducing the risk of generating weed flora resistant to herbicides) (2). Moreover, alfalfa in rotation with winter cereals has improved the soil structure and has provided a substantial amount of nitrogen for the two crops following alfalfa (3), thus reducing energy consumption and emission of GHG. In the same process, it prevents nitrate leaching at the rotation scale. In organic agriculture, the usefulness of alfalfa in weed control and as a source of nitrogen fertility at the rotation scale is also highly recognized in many European regions.

Production of feed proteins on the farm or in nearby cereal farms makes it possible to reduce the dependence of animal production on imported soybean feedstuff, whose prices show a high volatility. This meets the demand of farmers to be less exposed to risks. In particular for dairy cow nutrition, alfalfa (which has high protein and calcium contents) is an excellent companion of maize silage. However, even though a high protein production per unit area is a real advantage of alfalfa, the high protein solubility and the difficulty to preserve it when harvested as hay (loss of leaves) or silage (protein degradation) require innovation.

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Which alfalfa crops for the future European agriculture?

Because of its high agronomic potential and its ability to produce large amounts of protein per unit area, alfalfa has a role to play in European agriculture, even if some challenges have to be faced. The achievement of high biomass and protein production remains a key objective and this will be obtained mainly by improving the resistance to biotic (leaf and soil fungal diseases, nematodes) and abiotic stresses. Lodging resistance is essential in Europe to optimize spring harvest, which is the one providing the highest biomass and protein production (Fig. 2).

Persistency is not a critical issue for hay or silage production, as the present duration of most alfalfa crops (> 3 years) fits correctly in most cropping systems. It is a key issue, however, for the grazed crop, which has gained interest in the last two decades for extensive livestock systems especially in southern Europe, in order to reduce the environmental impact of intensive animal husbandry and to decrease production cost. Conventional alfalfa varieties generally do not persist under grazing. Therefore, Spanish, French and Italian research teams have started selection programs for grazing tolerant alfalfa, releasing cultivars with better survival under grazing. In the near future, European varieties specifically adapted to grazing will hopefully enter the seed market (4).

The ability to grow in mixture with a perennial grass is of interest for animal farms as it makes it possible to achieve a high biomass production without extra nitrogen fertilization, reduced weed damages, and facilitates grazing or the harvest and conservation as silage. Indeed, the water soluble carbohydrates of the associated grass will help to get low pH and improve conservation.

A major effort should be devoted to harvesting machinery. For example, mowing machines equipped with roll conditioners would make it possible to speed up the drying process, thus reducing quality losses. This is of course of major importance for hay production, but it is also valuable for ensiling with a pre-drying phase, or for production of wrapped round bales.

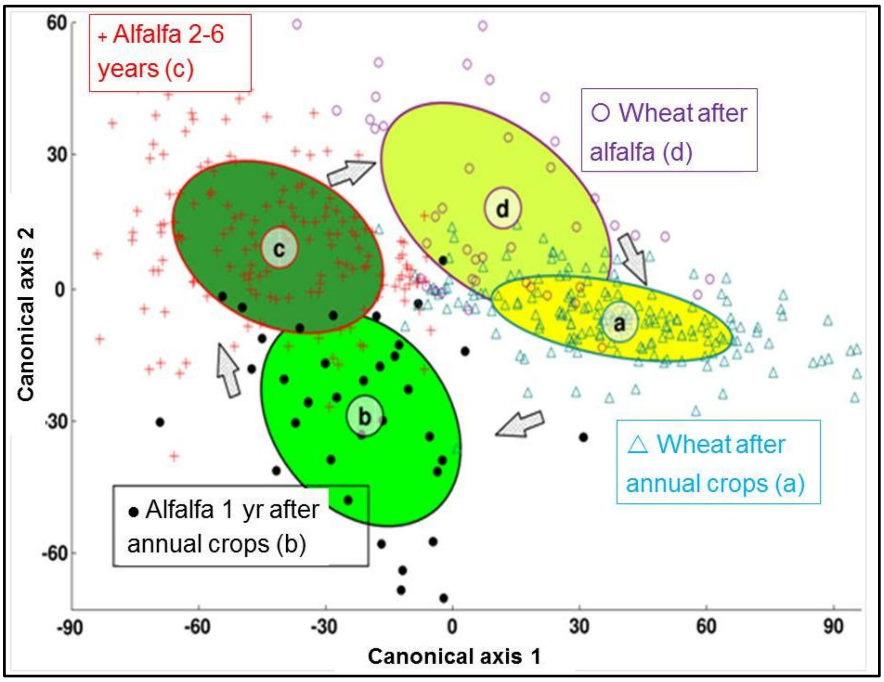

Figure 1. Canonical discriminant analysis of weed communities in 4 groups of plots (a), (b), (c) and (d) representing the four key-steps of rotations including annual cereals and alfalfa. Initial data represented the abundance of the various weed species in the crops (1)

Figure 2. A stand of the alfalfa crop at the stage of full vegetative development

On-farm barn drying is an emerging technique in some European regions, especially where high-quality products, such as protected-designation-of-origin cheeses, are produced. Alfalfa is extremely well adapted for such a production system, as it dries more quickly than other forages in the drying cells under ventilation.

In conclusion, we believe that alfalfa will keep an important place in European agriculture. Its favourable features can be further enhanced through technical and varietal innovation aimed at improved energy efficiency and reduced costs. ■

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Perspectives of alfalfa in North America

by Neal P. MARTIN

Abstract: Harvested alfalfa for hay was 12.3 million ha in 2011 in U.S. and Canada. However, acreage is declining due to competition for land area due to high value cash crops and lower yields than corn (silage and grain), soybean and wheat. Top farmers within US harvest 2 to 3 times national or regional agricultural statistical averages. Reduced-lignin transgenic alfalfa can increase forage fiber digestibility for lactating dairy cows, or allow for 8 to 12 days delayed harvest. The latter has potential for eliminating one or more annual harvests while increasing yield by over 20%.

Key words: fiber digestibility, forage quality, hay, milk production, yield

Introduction

Alfalfa is the most widely harvested forage legume worldwide and the fourth most valued crop in the U.S., contributing more than 10 billion USD annually to the Nation's farm economy. Farmers harvested alfalfa for hay from 7.8 and 4.5 million ha in 2011, from U.S. and Canada, respectively. Alfalfa has primary value as an animal feed, but also diversifies farming production risks, provides a source of biologically fixed nitrogen for subsequent crops in a rotation, reduces soil erosion, captures nutrients from annual cropping fields that can contaminate surface and ground water, disrupts pest cycles of rotation crops, and promotes soil carbon sequestration.

Competition for land and global climate change dictate increasing alfalfa yields on marginal lands. Drought tolerance, salt tolerance and biotic stress resistance will be needed on many of western acres. Livestock producers will need increased yield from fewer cuts to remain economically competitive. New research efforts are needed to develop new harvest, storage and processing methodologies to maximize protein utilization produced by the plant and to develop new markets for alfalfa co-products. In addition there is a need to develop new tools for accelerating and enhancing breeding programs, and demonstrating the importance of alfalfa in environmental sustainability.

Yield

Yield of alfalfa over the last 60 years did not increase substantially in the absence of disease pressure, but new cultivars out yielded older checks by as much as 40% in environments where diseases are prevalent (2). Average reported whole-farm hay yields are 50 to 70 % higher than in 1962 and regularly exceeded corn grain until only 15 years ago (6). In depth analysis of 2007 Census of Agriculture data showed that yields of top tier producers across U.S. in non-irrigated or irrigated production, although small in number, are 2 to 3 times higher than averages, and matching in some regions the yield of cultivar small plot trials (Fig. 1). More on farm programs that estimate field yield and quality such as Wisconsin Alfalfa Yield and Persistence Program (5) are needed to set yield goals closer to the crop genetic potential and to demonstrate to producers, educators, and scientist that alfalfa is an economically competitive perennial legume feed source, with potential to compete for biofuel feedstock production and with efficient water use.

A review of several alfalfa cutting management studies showed as the interval between harvests increased, annual DM yield increased while nutritive value decreased (1). Neutral detergent fiber digestibility (NDFD) of the diet is an indication of potential DM intake and milk yield in dairy cows (4). Summarizing a range of studies, the authors found that a one-unit increase in herbage in vitro or in situ NDFD was associated with a 0.17-kg increase in DM intake and 0.25-kg increase in 4 % fat-corrected milk per day. During spring and early summer, DM is being produced and nutritive value is declining more rapidly (NDF increases and NDFD decreases in early spring) than during late summer (1). Under humid growing conditions early harvest and shorter cutting intervals are needed in spring and early summer, but slower changing nutritive value in late summer (more arid) fit delaying harvest to allow additional yield accumulation. Utilizing nutritive value estimates of fiber and fiber digestibility to decide on cutting management have resulted in quality forage needed to maintain high feed intakes and digestibility, but have also made successful harvest more challenging.

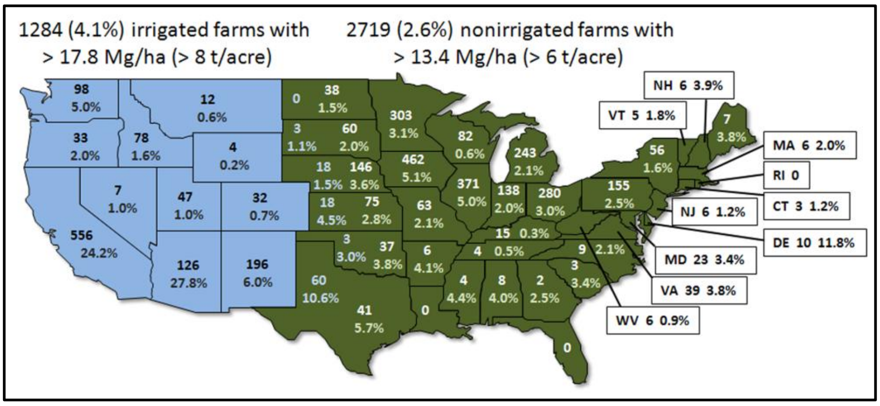

Figure 1. Number of farms (% of total) that reported annual alfalfa hay yield (13 % moisture) greater than 18 Mg ha^{-1} (irrigated) or 13.4 Mg ha^{-1} (nonirrigated), based on a subset of the 2007 Census of Agriculture. For states with more than 50 farms, estimates for irrigated were made by interpolation of best-fit sigmoidal curves

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Role of alfalfa in animal diets

Alfalfa is the most important forage legume for dairy cows. It is relatively low in fiber and high in protein compared to other forages, and typically results in high intakes and levels of milk production. The major disadvantage of alfalfa is low yields when compared to corn silage and the need for multiple harvests. Multiple harvests not only increase the labor and equipment costs for alfalfa, but also expose the forage to multiple maturities and harvesting environments, such as rain damage, that increase the variability in nutritional quality. Intensive cutting schedules may also be the root cause of poor stand survival and reduced yields.

The fiber (cell wall) component of forage represents a major source of energy; however, less than 50% of this fraction is readily digested and utilized by the animal (3). If alfalfa could be engineered to increase the extent of fiber digestion, the amount of energy derived from cattle diets would increase or the potential amount of carbohydrate available for cellulosic ethanol fermentation could increase; each of which has potential to increase the demand for alfalfa. Proof of concept reduced-lignin transgenic alfalfa hay fed in total mixed diets with corn silage measured increased fiber digestibility in both lactating dairy cows and rapidly growing lambs (7). Digestible dry matter of one transgenic increased 3.5 % fat corrected milk over the same plant population without the reduced-lignin gene by 1.3 kg hd^{-1} day^{-1}. Late harvested transgenic lines had the same NDFD as their control populations harvested 8 to 12 days earlier (7). Producers using this trait may be able to delay harvest while maintaining forage quality, potentially eliminating one or more annual harvests while increasing yield by over 20%.

Immature alfalfa silage is high in protein in which plant proteases can degrade 44% to 87% of forage protein into ammonia, amino acids, and small peptides during silage fermentation (3). Decreasing protein degradation during ensiling and in the rumen would decrease the need for supplemental protein and reduce the loss of nitrogen to the environment on the dairy farm (8). Recently, the U.S. Dairy Forage Research Center has successfully tested the hypothesis that PPO and *o*-diphenols inhibit proteolysis in alfalfa leaf plant extracts (3). Although alfalfa has at least one gene encoding PPO, expression has not been detected in any tissues except developing seed pods. A cloned red clover PPO gene (PPO1) was constitutively expressed in transgenic alfalfa (PPO1-alfalfa). Proteolysis was inhibited in leaf extracts of the PPO1-alfalfa when a substrate with similar activity of a o-diphenol in red clover (caffeic acid) was added (3).

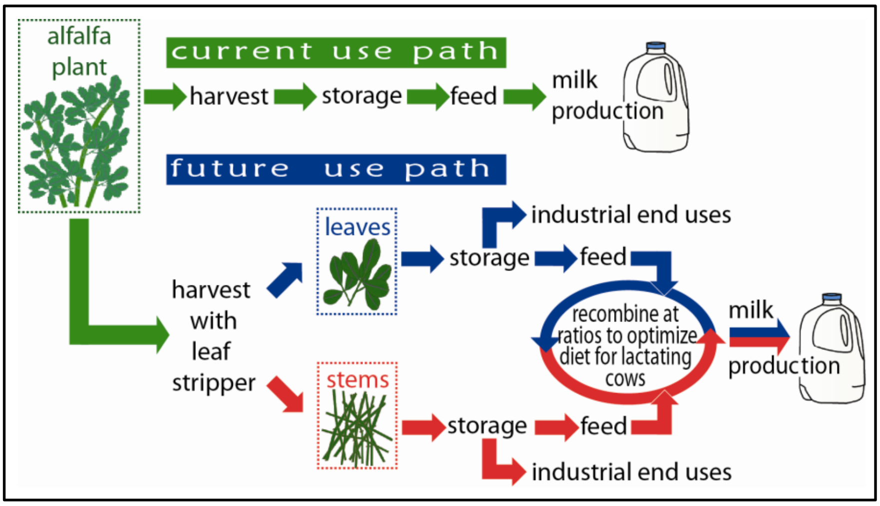

Figure 2. Novel harvest system to alter alfalfa harvest and storage to obtain leaves and stems for new animal or products uses. US Dairy Forage Research Center, USDA-ARS

Tannins are phenolic compounds that generally bind with proteins, decreasing the rate and extent of protein digestion. Forage legumes (e.g. birdsfoot trefoil) that produce tannins in leaves or stems have increased stability of the protein in the rumen, thus more protein escaping degradation in the rumen. An optimum level of tannins supported an increase of 5 kg per day of milk from cows fed normal tannin containing birsdfoot trefoil over alfalfa silage (3). Unfortunately, alfalfa does not produce tannins except in the seed coats. With new knowledge about tannin biosynthesis (Dixon group, Noble Foundation), it may be possible to engineer alfalfa to produce tannins that provide protein protection in the rumen and may also lead to less bloat.

New harvesting system and value-added products

Difficulties in managing alfalfa for high protein and low fiber has inadvertently resulted in the expansion of corn-based feed and silage into feeding rations. Development of novel alfalfa management and field harvesting equipment that separate high protein leaves from high fiber stems allows recombining these fractions in proportions that optimize the diet requirements for the target animal (e.g., high producing dairy cow, dry cow, and heifer) (Fig. 2). It also reduces the impact of adverse weather on crop harvest, increases yields by capturing the approximately 30% loss typically occurring during harvest, and opens the harvest window for the farmer (uncoupling harvest from stage of plant development). Finally, such an approach creates a value-added stream for products. Protein from harvested leaves can also be used to produce high protein supplements in diets for other animals, or serve as a raw material base for proteins destined for more industrial applications, while stems could be used in a biomass to bioenergy scheme. Newly constructed facilities will soon begin production of fuel from crop residues, primarily corn stover. Widespread stover harvest could have decidedly negative environmental impacts, particularly loss of soil organic matter and increased erosion. Systems that separate leaves from stems open the door for wider use in crop rotations to provide greater environmental and economic stability. ■

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Perspectives of alfalfa in Australia, China, Africa and Latin America

by Daniel BASIGALUP[1]*, John IRWIN[2], Fugui MI[3] and Meriem ABDELGUERFI-LAOUAR[4]

Abstract: The importance of alfalfa in Australia, China and some African and Latin American countries is summarized. Present planting areas, average forage yields, main use (hay or grazing) and crop conditions (rainfed or irrigated) are briefly reported. Finally, the crop perspectives for the near future in each region are discussed. Tolerance to abiotic stresses (drought, salinity, acidity, cold and heat) will play a crucial role in expanding alfalfa over the four continents. The importance of landrace genetic resources in traditional breeding and biotechnological approaches (transgenesis and marker-assisted selection) for developing adapted cultivars for those limiting environments are also highlighted.

Key words: Africa, alfalfa, Australia, China, Latin America

Australia

Alfalfa is Australia's most important temperate and sub-tropical perennial forage legume, being grown extensively from north Tasmania to the Tropic of Capricorn (6). Total Australian alfalfa area (grazing and hay) has been estimated at 3.5 M ha, more than half grown in New South Wales (NSW). Hay production has increased by 40% over the period 1990-2005, principally through an increase in cropping area. In 2005, about 250,000 ha were grown solely for hay, with an average yield of 4.96 t DM (dry matter) ha^{-1}, and NSW (40%), Victoria (25%) and Queensland (18%) as the leading states (8). Seed production is mostly concentrated in South Australia (85%), having reached a total of 7913 t in 2007, 85% of which was exported. It is estimated that over 3 M ha of dryland alfalfa is grazed in Australia by either sheep or cattle, mostly in NSW. Using modeling studies it has been calculated that an additional 95 M ha could be sown to dryland alfalfa, especially in NSW and Queensland.

Up to 1977, the Flemish type cultivar Hunter River occupied > 90% of the Australian alfalfa planting area, but now over 50 cultivars are marketed. Pest and disease resistant cultivars have made a major impact on Australia´s alfalfa production, particularly in irrigated hay crops where diseases such as *Phytophthora* root rot and anthracnose can cause serious losses (6). Most of the modern Australian cultivars have multiple pest and disease resistance incorporated. Grazing tolerant cultivars have also been recently deployed (5).

Perspectives. To increase the alfalfa area in Australia, there are several traits which will have to be bred into future cultivars. Tolerance to acid soils is under development in the US and Australia (5). In sub-tropical areas, increased winter activity and the capacity to compete with C4 grasses will be important (6). Other valuable traits include drought and salinity tolerance, increased seed size, and non-bloating to bovines. If these traits are addressed, there are good prospects for overcoming the present forage yield stagnation.

China

Alfalfa is an important forage crop in China, where it was introduced from Persia in 138-119 BC by the emperor Han Wu as a source of forage for his horses. Therefore, China has more than 2,000 years of history on the crop. At present, it is widely distributed throughout the country. Just between 1930 and 2012, about 400 alfalfa accessions were introduced from all over the world. As a centre of diversification, China holds a large set of genetic resources. The country has 13 species of the genera *Medicago*, mainly distributed in the Western plateau and in parts of the Changjiang river basin, roughly between 35-43° N.

Nowadays, China grows nearly 3.6 M ha of alfalfa, 77% of which are harvested as hay. Most fields (80%) produce at least three cuts per year, providing forage with crude protein contents between 14 and 18%. The overall production (about 60 M t of hay DM in 2010) is insufficient for the feeding needs.

A total of 62 alfalfa varieties are presently registered in the Chinese forage catalogue (24 bred cultivars, 20 landraces, 15 introduced cultivars and 3 domesticated species). While having different fall dormancy classes, all of them are adapted to cold environments. Most cultivars were developed by traditional breeding (including crosses between *ssp. sativa* and *ssp. falcata*), but a few recent ones (named Zhongmu No.1, 2 and 3) were bred by tissue culture selection.

Perspectives. Chinese policies are emphatically promoting the crop by articulating activities from government business, production farms and scientific research units. Three research topics have been prioritized, namely: harvesting and processing methodologies, genetic improvement of cold and drought tolerance, and the development of biotechnology approaches contemplating transgenesis or marker-assisted selection.

Africa

Alfalfa is cultivated in just some African countries located in northern and southern regions. Morocco grows nearly 106,000 ha (22.8% of country's total fodder crop land), with an average yield of 5.93 t DM ha^{-1}. In Algeria, alfalfa occupies about 10,000 ha, with a mean production of 3-4 t DM ha^{-1}. In Tunisia, alfalfa is the main forage and extends over about 13,000 ha (77% in oases). The small area planted in Mauritania is concentrated in oases. Alfalfa is the fourth

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Figure 1. Irrigated alfalfa in one oasis of Adrar in Algeria, in rotation with wheat (photo kindly provided by Ahmed Chaabena)

Figure 2. Alfalfa under rotational grazing for dairy cows in Argentina

forage crop in Egypt, where it reached 36,000 ha in 2008 all under irrigation. Locally adapted populations (landraces) are extensively used in Northern Africa oases, where alfalfa production is almost continuous (8-10 cuts year^{-1}) and intervals between cuts are very short (1). South Africa is the main alfalfa growing country in the southern hemisphere, with about 300,000 ha (9) mainly under irrigation, and where dairy cow diets contain up to 40% of alfalfa hay.

Perspectives. Alfalfa cropping has potential for expansion in Africa, particularly under efficient irrigation systems (e.g. pivot irrigation) and with the development of dehydration units at the farm level. Also rainfed cropping of drought-tolerant germplasm in semi-arid regions has greater potential than hitherto believed (3). The evaluation and exploitation of local (2) and exotic (3) genetic resources has crucial importance for producing varieties with better tolerance to major stresses (e.g., drought, salinity, heat) or to frequent cutting. The implementation of variety registration, reliable seed multiplication and commercialization chains are required in most countries for promoting adapted, improved varieties. Similarly in India, warm southern locations favour early flowering, while the opposite is the case in the north (6).

Latin America

The importance of alfalfa in Latin America varies greatly among countries. Brazil grows only 40,000 ha, mostly for hay production in the Southern states. The locally adapted cv. *Crioula*, with an average yield of 14 t DM ha^{-1} year^{-1}, is widely used (7). Uruguay has about 70,000 ha mainly devoted to dairy in the SW, yielding 9-13 t DM ha^{-1} year^{-1}. Soil acidity and low P content limit the crop expansion in these countries. Chile´s planting area is 50,000 ha, concentrated in Central and Southern regions, mostly grown for dairy systems in association with ryegrass. Peru grows nearly 120,000 ha mainly in the coastal areas, with yields of 13-18 t DM ha^{-1} year^{-1}. While intermediate (6-8 cuts year^{-1}) to non-dormant (11 cuts year^{-1}) varieties are used for coastal environments, dormant/very dormant (2-3 cuts year^{-1}) are adopted for the high Andean plateau. Mexico grows about 400,000 ha of alfalfa, almost all under irrigation. About 65% is used as green forage, while the remaining is devoted to hay production (with an average yield of 17.5 t DM ha^{-1} year^{-1}). With a few exceptions, most of the varieties are imported from USA.

Argentina grows 4 M ha of alfalfa, mostly under rainfed conditions for dairy and beef production in the Pampas Region. About 60% is planted as pure stands, and 40% in mixtures with temperate grasses. Direct grazing is still very popular, although hay (and more recently silage) production is gaining importance (4). Other areas (NW, W and Patagonia) are devoted to hay and seed production under irrigation. The mean forage yields are 11 and 20 t DM ha^{-1} year^{-1} for rainfed and irrigated conditions, respectively. About 75% of the marketed seed (9,000 t) is imported, mainly from Australia and USA. Over 80 cultivars (with fall dormancy 6 to 10) are marketed, all having multiple aphid and disease resistance.

Perspectives. No significant changes in alfalfa planting are expected in Mexico, Peru, Uruguay and Chile. Conversely, the expected release of new cultivars adapted to tropical environments could quickly duplicate the cropping area in Brazil. In Argentina, the on-going development of cultivars tolerant to abiotic (drought, salinity, acidity) and new biotic stresses (particularly alfalfa enation virus), will extend the cropping area. Transgenic cultivars are being developed with tolerance to herbicides, drought and salinity and with delayed leaf senescence. ■

Acknowledgments

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Environmental benefits of growing perennial legumes in cropping systems

by Michael P. RUSSELLE

Abstract: Alfalfa offers several environmental benefits to farmers and society. Reduced nitrate leaching due to slower water flow and excellent nitrate removal are valuable in farm fields and at remediation sites. Improved N supply to following crops reduces energy use, economic costs, and greenhouse gas emissions, but reliable indicators are needed to identify which fields need supplemental N after alfalfa. Livestock manure is applied to alfalfa on farms, but too little is known about the benefits and risks. Critical needs are on-farm estimates of yield and N_2 fixation and broadened plant improvement objectives to explicitly include environmental benefits.

Key words: fertilizer replacement, nitrate leaching, nitrogen, soil quality, water use

Introduction

In the First Century, Columella wrote that “all cattle whatsoever grow fat with [alfalfa]” and that it “dungs the land.” Today, alfalfa is known as a premier forage crop, primarily as a protein source, but its positive environmental effects are less well recognized.

Widely acknowledged is the contribution of alfalfa to nitrogen (N) availability. Symbiotic N_2 fixation occurs by rhizobia housed in root nodules and fed by C compounds from the plant in exchange for the N-containing amino acids the bacteria produce. Any factor that affects plant growth or yield likely affects the amount of N fixed – supply of nutrients (P, K, S, and micronutrients), soil pH, inorganic N supply in the soil, the degree of defoliation at harvest or by pests, and inadequate or surplus water availability, to name a few. In pure alfalfa stands, estimates of annual N_2 fixation range from 40 kg N ha^{-1} to 600 kg N ha^{-1} and from 10 kg N ha^{-1} to 320 kg N ha^{-1} in mixed alfalfa-grass stands (6). However, N_2 fixation varies within and among fields and regions, due to the myriad factors that modify it. Because we still can only guess at N_2 fixation rates in farm fields, a key research objective should be to develop a reliable technique to use on farms.

Nitrate removal

Alfalfa’s large N requirement is due to high herbage yield and protein concentration. Alfalfa does not fill this need solely by N_2 fixation, but rather it absorbs nitrate from the soil solution effectively enough to reduce nitrate leaching losses. Nitrate assimilated from the soil reduces the rate of N_2 fixation, thereby reducing N input when plant-available N supply is high.

High yield potential of this C3 forage crop results in high water use, although water use efficiency (WUE, g dry matter g^{-1} water transpired) is excellent in comparison to other C3 crops. Furthermore, water use begins earlier and continues longer than most annual crops, such as maize and soybean. This timing of water use makes alfalfa highly effective in reducing nitrate leaching losses. Managed as a harvested forage crop, alfalfa maintained annual nitrate losses through subsurface tile drains to < 5 kg N ha^{-1}, even when drainage volumes were twice the normal amounts (5). In contrast, loss under continuous maize or a maize-soybean rotation averaged 40 kg N ha^{-1} with normal and 75 kg N ha^{-1} with high drainage volumes (5).

Nitrate removal capacity is affected by stand density (plants m^{-2}). As alfalfa stands decline with age, disease, winterkill, or damage by wheel traffic, nitrate can accumulate in soil and be leached in percolating water (3). Where leaching should be minimized, the stand should be rotated to an annual or perennial crop that can absorb mineralized N. Alfalfa increases the amount of mineralizable N in the soil, which is released earlier, faster, and longer than N mineralized following maize or wheat (1). If the following crop cannot capture this mineralized N, nitrate leaching can be significantly higher after alfalfa than after other crops, even in semiarid environments.

Nitrogen supply

This higher N supply reduces the need for fertilizer N in crops following alfalfa. On medium-textured soils, alfalfa usually provides the entire amount of N required by maize, resulting in substantial economic savings and greatly reducing the energy cost of maize production (8). On fine- and coarse-textured soils it is more common to find a fertilizer N response of first-year maize after alfalfa than on medium-textured soils (Fig. 1), probably because of slower nitrification and enhanced denitrification on fine-textured soils and greater nitrate leaching on sands. Still undiscovered are means of identifying fields where the first crop after alfalfa will require additional N. With alfalfa, decreased fertilizer inputs and overall lower denitrification contribute to greatly reduced net energy requirements (8) and greenhouse gas emissions (4).

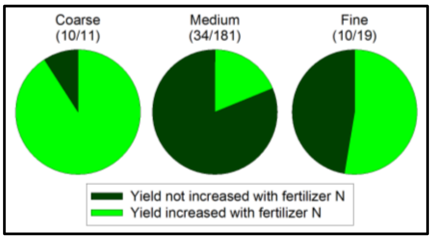

Figure 1. Yield response to fertilizer N of first-year maize grain yield after alfalfa is more likely on fine- and coarse-textured soils than on medium-textured soils, with the number of N-responsive sites and total sites in each soil texture class (8)

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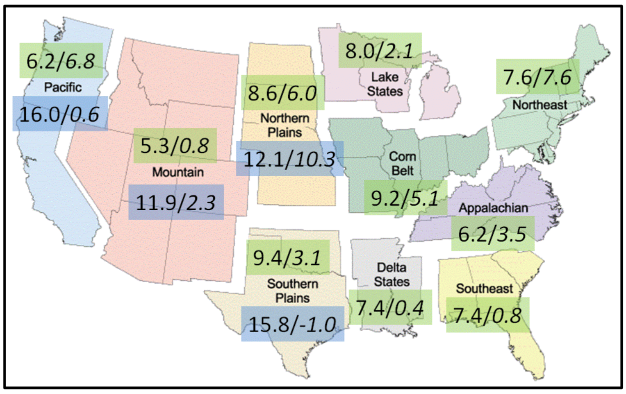

Figure 2. Alfalfa yield (t dry matter ha^{-1}) on the top 10% of US farms and the yield gap (italics) compared to regional cultivar performance trials, with irrigated (blue shading) and without irrigation (green shading) (7)

Deep rooting

Roots of alfalfa extend vertically about 1 to 2 cm day^{-1} at typical soil temperatures, totaling 1.5 to 2 m annually in temperate environments and in a favorable soil matrix (low penetration resistance, adequate aeration, and appropriate chemical conditions). There are anecdotal reports of extremely deep rooting, but measurable alfalfa root activity has been evidenced by water and nitrate depletion to 5 m and by uptake of ^{32}P at 5.5 m. Consequently, alfalfa is used to prevent or reduce nitrate leaching from abandoned feedlots and other sites.

High water use by alfalfa has been used to prevent expansion of salt-degraded soils. Replacement of native perennial vegetation with annual grain crops in arid environments has resulted in intrusion of saline groundwater into the root zone. Remediation of soils degraded by salinity is difficult and expensive. An economic means of prevention is to plant deeply rooted perennials, such as alfalfa, which simultaneously provide income. Although there have been breeding efforts to increase tolerance to salinity, most alfalfa cultivars do not tolerate moderate salinity conditions.

As secondarily thickened roots decay, improved macroporosity in subsoil facilitates deeper root penetration of subsequent crops and enhanced water infiltration when macropore flow occurs. A potential hazard is that macropore flow also can transmit suspended and dissolved materials, such as surface-applied pesticides, fertilizer, or manure. However, this occurs only when the hydraulic conductivity of the soil matrix is exceeded, e.g. intensive rainfall or irrigation, accumulation of runoff, snowmelt and urination by livestock. Serious impacts due to root channels have not been detected.

Reduced soil erosion

Perennial forages often are recommended to reduce nutrient and sediment runoff. Alternating strips of alfalfa and maize that follow the contour of a hillslope help reduce soil erosion by surface runoff. Although sediment movement is reduced, perennial forages damaged by freezing or drying (e.g., during desiccation for hay production) can release soluble, reactive P into runoff water.

Where plant growth is limited by cold temperatures, soil often is left for several months without cover or living vegetation. Cover crops can reduce soil erosion and nitrate losses, but they are not widely adopted without supplemental payments or regulation. A “living mulch” of Kura clover (*Trifolium ambiguum*) in row crops shows promise when competition can be managed. The following year, clover can be grown as a forage, or an annual crop can be interseeded again. This system, first examined by KA Albrecht, reduces nitrate leaching and probably runoff, but requires further development to maintain maize yield. It remains to be determined whether alfalfa can be used in this way.

Manure utilization

Livestock manure is a valuable resource, but is applied mostly for annual crops. The facultative nature of symbiotic N_2 fixation provides the opportunity for alfalfa to utilize manure N effectively, while other manure nutrients also can benefit alfalfa. Manure application methods to alfalfa vary from broadcasting solid or liquid forms, through partial incorporation, to shallow injection, but relatively few experiments have been published. Swine manure applied through irrigation greatly increased alfalfa yield (2). Only part of this increase could be attributed to nutrients other than N, and yield increases of 20 to 40% with manure may be due to lower photosynthate requirement for inorganic N assimilation than for symbiotic N_2 fixation. There is a clear need for more research to document benefits and risks.

Enhancing the benefits

The value of alfalfa in terms of N_2 fixation, nitrate removal, N supply to subsequent crops, water use, and other benefits is directly related to forage yield. In most regions of the USA, alfalfa yield potential is higher than yields reported by even the best farmers (Fig. 2). Our ability to generate reliable assessments of net impacts of alfalfa on ecosystem services requires better knowledge of yields, stand lifetime, and soil N supply. Elimination of yield-limiting factors will enhance both the economic and environmental benefits of this crop.

Nearly all of these benefits have been demonstrated with commercial alfalfa cultivars. Root system architecture, root extension rate, nitrate uptake, stress tolerance, and perhaps other characteristics related to environmental benefits are moderately heritable. If these traits are introduced, plant development companies could produce alfalfa with greater public and private benefits. ■

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Alfalfa domestication history, genetic diversity and genetic resources

by Jean-Marie PROSPERI[1]*, Eric JENCZEWSKI[2], Marie-Hélène MULLER[1], Stéphane FOURTIER[3], Jean-Paul SAMPOUX[3] and Joëlle RONFORT[1]

Abstract: The domestication history of alfalfa is poorly known. Here, we summarize recent results obtained from the investigation of the genetic diversity available in the *Medicago sativa* species complex, using different molecular markers and morphological characterization. We conclude that a large genetic diversity is still available in the wild form of the species, but original populations are restricted to a relatively small geographic area and in some instances submitted to gene flow from the cultivated form. A short description of the European genetic resources is presented.

Key words: diffusion routes, gene flow, genetic resources, molecular markers, morphological characterization, wild populations

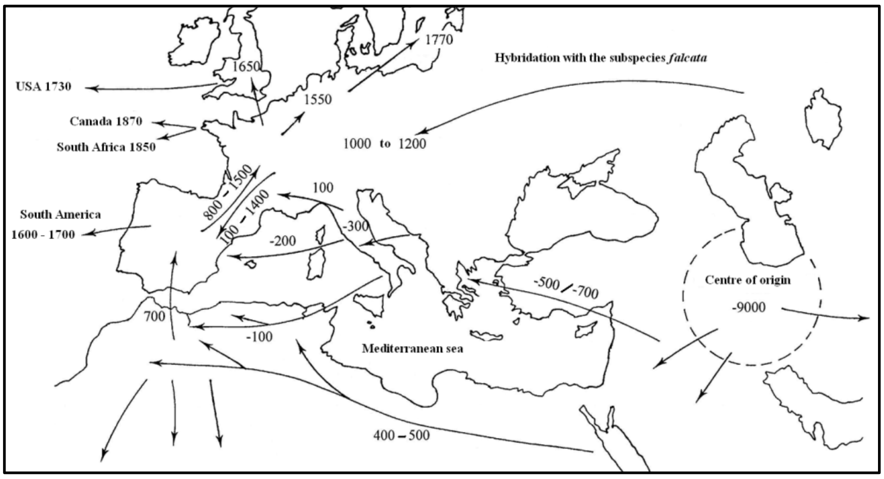

Figure 1. The different routes and approximate dates of diffusion of cultivated alfalfa from its Centre of origin (the symbol (-) correspond to BC dates)

Domestication of alfalfa

It is difficult to identify the first traces of alfalfa (*Medicago sativa* L.) domestication. Different authors suggested that alfalfa was cultivated 9,000 years ago in some rare locations in its centre of origin (Near East to Central Asia). Sinskaya in 1950 suggested that its distribution spread throughout the Middle East by 1,000 BC, and from there, to China and India. But actually, we have no element to validate these assumptions. The first written references mentioned the introduction of alfalfa to Greece by the Medes armies, its spread into Italy and then all over the Roman Empire in Europe. During the Middle Ages, the interest in alfalfa regressed considerably in Europe. Alfalfa was reintroduced into Spain through North Africa with the Moors. It then crossed the Pyrenees around the fifteenth century. Thereafter, it was introduced to South America (Mexico, Peru, Chile) by the Spanish in the sixteenth century, and then to the United States through California at the beginning of the nineteenth century as 'Chilean clover'. These major paths of diffusion are summarised, along with a few minor ones, in Fig. 1 (6).

Feral populations of alfalfa are frequent in a variety of open habitats (roadsides, field borders), but wild forms of *M. sativa* subsp. *sativa* are rare, with the exception of the Iberian Peninsula and its centre of diversity. In other west Mediterranean countries (southern France, Italy, Greece, and North Africa), plants of *M. x varia*, are very common as a result of crosses between subsp. *sativa* and subsp. *falcata* populations. The *M. sativa* subsp. *falcata* appears more frequently as a wild species, from the north of the Mediterranean Sea (Bulgaria, Greece) to the northern limits of Russia. It is particularly well adapted to hard winters, as well as to the hot and dry summers typical of continental climates (6, 7).

Genetic diversity of the cultivated and wild pools of *Medicago sativa*

The evolutionary history of a wild–cultivated complex is the outcome of different factors, among which are the domestication of the cultivated form, the geographical and demographic expansion of the species, and the gene flow between natural and cultivated populations. To clarify alfalfa's history, we sampled several cultivars and landraces, natural populations of *M. sativa* subsp. *sativa* (diploid and tetraploid), as well as populations of the subsp. *falcata* and other perennial species. A special focus was made on the Spanish wild pool. Molecular markers (RAPD, nuclear DNA sequence polymorphism and mitochondrial DNA variation) and phenotypic characterization were used to measure the differentiation between cultivated and wild types and to quantify the importance of gene flow.

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From all these data, we noted:

(i) the marked originality of the wild pools, with specific characteristics such as prostrate habit, rhizomes, tolerance to drought and grazing (3, 7, 8);

(ii) a significant domestication bottleneck measured on nuclear DNA polymorphism, with a loss of diversity from wild to domesticated material of 30% on average (although more data are needed to strengthen this result). We detected no differentiation between diploid and tetraploid forms (3);

(iii) a high level of mtDNA variation, especially in the wild pool. In the wild pool from the presumed area of origin, the diversity was high but the absence of geographical differentiation in our sample hindered a more precise detection of the centre of domestication (4, 5 and Fig. 2);

(iv) in cultivated alfalfa, the geographical structure of mtDNA and nuclear DNA variation strongly suggests at least two independent routes (north and south) of dissemination from its centre of origin (3, 5);

(v) a specific mitochondrial type in the Spanish wild pool. That suggests an old divergence from the centre of origin and the fact that these populations were established in Spain before the introduction of cultivated alfalfa (5 and Fig. 2);

(vi) an occurrence of crop/wild gene flow in many locations in Spain, together with the persistence of wild Spanish populations showing a great agronomic and morphologic originality (1, 2, 7, 8).

Taken together, these results show that *M. sativa* contains a large genetic diversity and that there is probably much to gain from the study, the conservation and the use of the genetic variability available in the wild fraction of this species.

Genetic resources and main gene banks

Numerous gene banks in the world exist that concentrate on *Medicago* (National Plant Germplasm System USA, Australian Medicago Genetic Resource Centre Australia, ICARDA Syria, Vavilov Institute Russia, etc.). For Europe, genetic resources of perennial *Medicago* species are maintained by many countries. Due to past exchanges of genetic materials among research institutes, accessions such as cultivars and landraces are frequently maintained in several gene banks.

Wild perennial species are generally only maintained by the institute that collected them. They are poorly represented or not represented in germplasm collections, especially those from the centre of origin of the genus and from Asia. The diploid forms of the *M. sativa* complex species (i.e. subsp. *glomerata* and subsp. *coerulea*) are also under-represented in gene banks.

In order to promote the use and rationalise the maintenance of plant genetic resources in Europe, the European Cooperative Programme for Plant Genetic Resources (ECPGR: http://www.ecpgr.cgiar.org) was set up in 1980 and is now coordinated by Bioversity International.

Within ECPGR, a working group on forage species brings together 37 countries. This working group has set up Central Crop Databases (CCDB) for several groups of forage grass and legume species. A part of these CCDB is centralized in the EURISCO database, a web-based catalogue which automatically receives the National Inventories of the gene banks from 42 European countries (http://eurisco.ecpgr.org).

The ECPGR Central Crop Database for perennial *Medicago* species references 7874 samples (a third from the Russian Federation) maintained over 25 European national gene banks. Some accessions are present as duplicated samples in several gene banks. A primary holder has been identified for each accession (i.e. a gene bank primarily responsible for the maintenance of the accession). Nineteen species are represented; some of them including several subspecies; but the tetraploid *M. sativa* represents more than 95% of the total. Referenced accessions include cultivars (1920 accessions), landraces (1430 accessions), wild or feral populations (769 accessions) and breeding materials (1260 accessions). All together, these accessions represent the diversity originating from all parts of Europe. Information about the ECPGR Central Crop Database for perennial *Medicago* species can be obtained from the manager of this database (http://www.ecpgr.cgiar.org/germplasm_databases/central_crop_databases/ECCDB_List.html). ■

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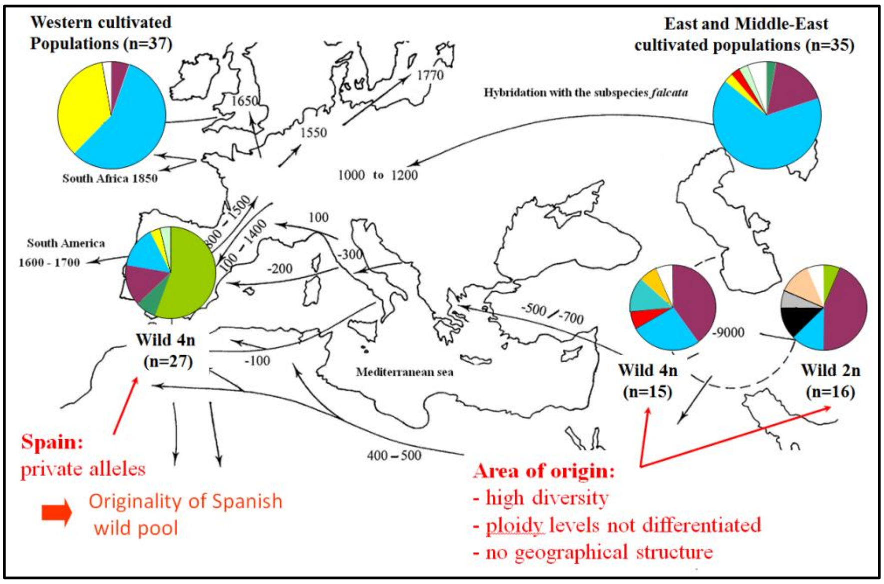

Figure 2. Mitochondrial DNA variation between wild and cultivated pools of *Medicago sativa* in West and East Mediterranean regions. Each mitochondrial haplotype is in a different colour

From the model legume *Medicago truncatula* to alfalfa

by Bernadette JULIER

Abstract: Large efforts have been devoted to *Medicago truncatula* genomics. They offer data and tools to identify *M. truncatula* genes involved in genetic variation for traits related to agronomic value in crop species. This strategy is based on the hypothesis that the same genes are involved in trait variation in both species. These tools are now used to develop alfalfa genomics in order to carry out genomic studies in the target species. Identification of genes or markers in the crop species will contribute to develop tools and theory to implement marker assisted selection in alfalfa.

Key words: genomics, marker, model species, QTL, sequence

Many efforts have been devoted to *Medicago truncatula* Gaertn. genetics and genomics since 1990. A draft sequence has been obtained after sequencing the gene-rich genome portions (euchromatin) (9). Its analysis showed that *M. truncatula* genome is more complex than expected from its short genome size. The genome has been shaped by a whole-genome duplication but also by numerous rearrangements and local duplications. These genome changes allowed the emergence of specific genes involved in rhizobial nodulation. The number of genes is over 62,000, and the genome contains numerous transposons, retrotransposons and transcription factors. Resequencing of a set of 26 accessions has given access to sequence polymorphism (1). A large diversity was found, but linkage disequilibrium was shorter than expected in a selfing species. Transcriptomics data were used to construct a genechip array (https:www.affymetrix.com/products_services/arrays/specific/medicago.affx) that can be used to detect the role of genes in specific functions and organs. In parallel, accessions collected from sites of the Mediterranean Basin were made available to the scientific community in gene banks (http://www1.montpellier.inra.fr/BRC-MTR/). Natural populations have been used to establish mapping populations of recombinant inbred lines, which have been mapped with microsatellite markers developed from EST sequences and used to detect quantitative trait loci (QTL) for traits having agronomic interest in crop species. Fine mapping was used to detect candidate genes explaining the QTL. Collections of tilling or transposon-tagging mutants have been established to detect new alleles of known genes or new genes involved in phenotypic variants.

From *M. truncatula* to alfalfa: strategies

Exploiting genomic knowledge from *M. truncatula* for the genetic improvement of alfalfa is not simple. Detecting genes involved in the variation for breeding traits is often needed to improve breeding programs. Two non-exclusive strategies can be devised. In the first one, candidate genes are detected in *M. truncatula* and are then tested for their effect in crop species (Figure 1). In the second one, the genomics of alfalfa is developed by taking into account the knowledge from *M. truncatula* genomics, and used to detect genes or markers involved in traits. These genes or markers become available to be incorporated into breeding programs (Figure 1).

Identification of genes in *M. truncatula*

In the first strategy, the QTL detection in mapping populations of *M. truncatula*, followed by a fine mapping step, gives access to a small genomic region. Using an *in silico* analysis of the genes annotated in the QTL region and a literature review of the genes known to be involved in the trait under study, a list of candidate genes can be established. This method was successfully used for flowering date. One of the candidate genes, a Constans-like gene, contained sequence polymorphism and showed differential expression between the two parents (7). Analysis of mutants with altered phenotypes (forward genetics), or phenotypic analysis of genotypes showing differential gene expression (reverse genetics) are other ways to detect candidate genes. Association genetics in *M. truncatula* could also help to identify genes or genomic regions involved in traits. The efficiency of association genetics will depend on the linkage disequilibrium (LD) of the population under study, which can be long in this selfing species.

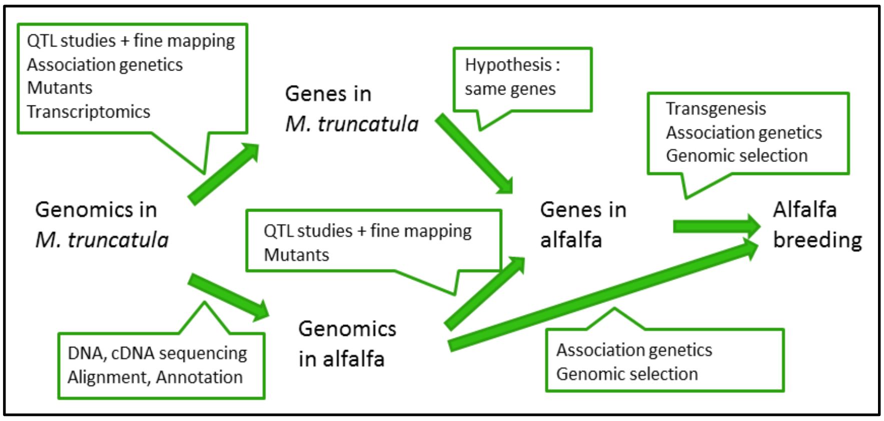

Figure 1. Strategies to transfer genomics information from *M. truncatula* to the breeding program of a legume species; several methodologies (boxes) are proposed to achieve each step

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Once a gene has been identified in the model species, it remains to verify the presence of a homeologue of it in alfalfa which has an effect on the same trait. This demonstration can be achieved and exploited by different methods, such as transgenesis, introgression through backcross, or association genetics (Fig. 1). With transgenesis, the modification of expression of an allele in alfalfa is supposed to confer a new trait variation (8), eventually becoming available in a transgenic variety. Introgression through backcross has the same objective, but the allele originates from alfalfa. With association study, the demonstration results in the identification of alfalfa allele(s) that confer valuable variation, as found for a Constans-like gene (5). These alleles could then be used in a breeding program assisted by molecular markers, to increase their frequency in the synthetic varieties.

Development of alfalfa genomics

The second strategy implies the study of alfalfa genomics by DNA or cDNA sequencing using the Next Generation Sequencing (NGS) technologies (Fig. 1). Read assembly is complex because each individual possibly carries 4 different copies of each gene, in addition to the presence of gene families or repeated elements. The assembly of alfalfa sequences can be considered as a re-sequencing, with *M. truncatula* sequence acting as a reference. However, extensive sequence evolution occurred in the two species, leading to large differences in non-coding sequences between *M. truncatula* and alfalfa. Alfalfa sequencing is currently a scientific challenge that is addressed by a few groups in Europe and the USA. In the meanwhile, genetic mapping and QTL detection are feasible. Theories and software (TetraploidMap, http://www.bioss.ac.uk/knowledge/tetraploidmap/) were developed in autotetraploid species. Microsatellite markers were developed in alfalfa which mostly originated from EST of *M. truncatula* (6). These codominant markers with additional dominant markers such as AFLP or DArT (3) or HRM (4) markers are numerous enough to build maps of any mapping population. The comparison of *M. truncatula* and alfalfa maps proved that both macro-synteny and micro-synteny between the two species are high. Due to autotetraploidy, the precision of QTL position is lower in alfalfa than in a diploid species, so the subsequent use of QTL into breeding is still limited to the genetic background of the mapping population. Fine QTL mapping has not been reported in alfalfa, probably because it would require very large mapping populations and *in silico* analysis of genomics regions is not feasible yet. However, this *in silico* analysis, by using the same anchor markers in alfalfa and *M. truncatula*, can be carried out in the model species. On the whole, gene or marker detection directly performed in alfalfa (through bulk segregant analysis (BSA) or genomic selection) is scarce up to now, but the increasing availability of high throughput markers could change this situation in the future. These markers will now be mainly developed in alfalfa from sequencing and resequencing programs. However, any analysis of their function, position on maps, sequence polymorphism of their genomic regions will benefit from genomic resources in *M. truncatula*.

Perspectives

Current development of alfalfa genomics, now possible because of extensive knowledge in *M. truncatula*, offers new perspective to directly detect genes or markers in the crop species for agronomics traits. All limitations to genetic analysis in alfalfa are not yet overcome and more specifically, polyploidy and heterozygosity will lastingly reduce their efficiency. So *M. truncatula* is still a reference, mainly for all the -omics data, but also for the identification of genes involved in agronomic traits. ■

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Alfalfa marker-assisted selection

by E. Charles BRUMMER* and Maria J. MONTEROS

Abstract: Alfalfa improvement has relied on phenotypic selection for genetic improvement. The availability of single nucleotide polymorphisms (SNP) throughout the genome, as well as methods, such as genotyping-by-sequencing (GBS), to assay SNPs cost-effectively will enable successful integration of markers into breeding programs. GBS will enable breeders to select based on marker breeding values, accelerating genetic gain through repeated cycles of selection. Integrating markers associated with major genes and implementing genomic selection could increase the speed and efficiency of alfalfa improvement. Alfalfa breeding programs may need to modify existing methods to fully realize the power markers can bring to cultivar development.

Key words: alfalfa, genomic selection, genotyping-by-sequencing, marker-assisted selection, SNP

Introduction

In the time since the first alfalfa genetic maps were published 20 years ago this year, few applications of genetic markers to trait selection in alfalfa have been realized. Despite this relative lack of progress, the future indeed looks bright for the potential of markers to make a substantial contribution to genetic gain for major traits in alfalfa. Despite significant investment in a succession of molecular marker systems – RFLP, AFLP, RAPD, SSR – no readily assayed, cost effective markers truly existed for routine breeding programs until SNP methods were developed in the past several years. Currently, large numbers of SNP have been identified in alfalfa (3, 7), and markers polymorphic in different germplasm have been developed, which can be assayed individually using high resolution melting analysis (4) or collectively with an Illumina Infinium array (8). Further, we are well on the way to adapting genotyping-by- sequencing to alfalfa selection programs, thus generating inexpensive, genome-wide markers rapidly. The availability of marker systems that can robustly evaluate anything from single markers to genome-wide markers now opens up a wealth of opportunities to apply markers to breeding programs in a systematic way. In this paper, we suggest three ways markers could constructively be applied to selection: (1) marker diversity assessment, (2) marker-assisted trait integration, and (3) genome-wide selection.

Marker diversity assessment

Perhaps the most extensive use of markers across crop plants has been to characterize genetic variation. Most alfalfa genetic variation resides within populations, so unsurprisingly, most diversity experiments in alfalfa that shown that populations with distinct genetic profiles are also highly phenotypically and/or geographically differentiated. To date, this type of experiment has had limited practical value to breeding programs. Moving beyond these types of experiments to explore specific breeding objectives could help breeders more successfully identify unique sources of germplasm, understand genetic diversity within populations, minimize inbreeding depression, and aid in constructing heterotic groups. The value of markers at identifying heterotic groups has often failed in many crops, but selecting plants within populations to construct *de novo* heterotic groups may be more successful. Part of the problem with using genetically distinct populations as heterotic groups can be that they are otherwise not agronomically desirable or adapted to the target region. We propose a potentially better approach that involves starting with agronomically desirable populations, allocate plants into two pools designated as heterotic groups, and then commence breeding for hybrid performance. Reciprocal recurrent selection has been successfully used to increase yield in maize (2), but has not been applied to any significant extent in alfalfa. We propose that it should be, given the anticipated possibility to improve yield.

Marker-assisted selection

A second use of markers in a broad sense is in marker-assisted selection. The common scenario includes the identification of major genes (or quantitative trait loci, QTL) for key traits and the subsequent introgression of those markers (and genes) into elite breeding populations. Markers can help increase the frequencies of these genes (alleles), moving them toward fixation, so that all or at least most seed of synthetic cultivars will contain these alleles in the certified seed bag that a farmer would purchase. For some traits, such as aluminum tolerance, QTL have been identified in wild diploid germplasm and transferred to tetraploid populations by interploidy hybridization (5). Markers are valuable tools that could be used to track the desirable trait, either from diploid accessions or other tetraploid germplasm, through the breeding process. Other traits undoubtedly exist where commercial germplasm lacks specific desirable alleles, particularly for quantitative traits including abiotic stress responses.

Many of the major diseases and insect pests can be relatively easily assayed using classic phenotypic screens in which thousands or even millions of seedlings are inoculated and survivors selected. However, even in these cases, having markers for the major genes would enable the small subset of elite parents and individual genotypes to be screened for the presence of the resistant/tolerant alleles, and perhaps more importantly, determine the allelic dosage. Because alfalfa is an autotetraploid species, dominant alleles can express a trait despite the presence of three recessive alleles at that locus. This reservoir of deleterious or undesirable alleles will appear during seed increase and the resulting cultivar will include plants that may not have any desirable alleles at that locus. Markers could ensure that any selected parental plant would have multiple copies of that allele, ideally fixed for the correct allele.

Recently, we have used genotyping-by-sequencing (GBS) as a high throughput means to assay SNP variation throughout the genome. With GBS, sequences from the end of restriction fragments are aligned from different individuals and SNP between individuals are identified (1). We evaluated allele frequency shifts in bulked populations selected for and against whitefly resistance. The GBS strategy identified 70,000 SNP loci, and one location on chromosome 8 was identified as a

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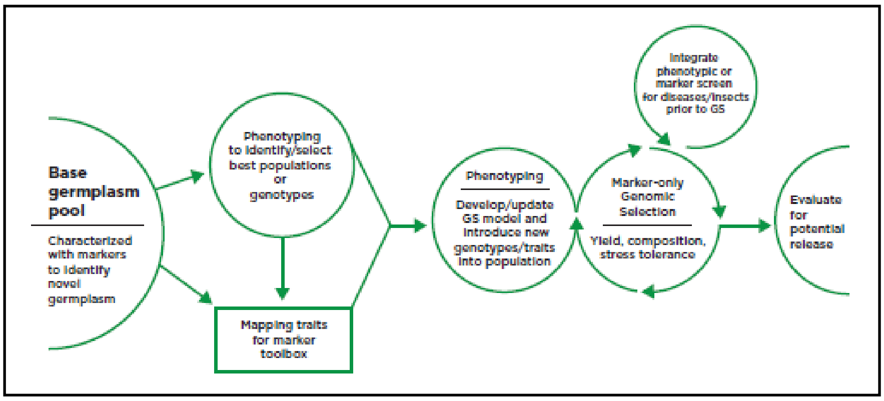

Figure 1. By integrating molecular markers, breeders can identify populations with unique genetic variation, can identify marker-trait associations to complement phenotypic selection, and are able to implement genomic selection (GS; based on a selection index for multiple traits); In the example here, four cycles of genomic selection could be completed for every one cycle of phenotypic evaluation; Phenotyping of progenies from selected plants will continue in a GS program, although mainly for improving the GS model; Phenotypic evaluation of new germplasm could also contribute to an updated model, and genotypes with desirable alleles could enter the next cycle of GS; Prior to any GS cycle, a pre-selection of individuals based on phenotypic or marker screens for disease or insect resistances could ensure that a certain trait threshold is maintained during the marker-only phase of the program; The resulting populations would then be evaluated under field conditions in the target environments

likely candidate region for a major resistance locus, based on large SNP allele frequency changes across cycles. Similar populations selected for resistance to many other diseases and pests are available, and this bulking strategy may facilitate the identification of genomic regions associated with resistance. Mapping relevant loci in segregating populations and identifying functional SNPs are needed to validate the location of a resistance locus, as in our example. Nevertheless, current DNA sequencing technologies greatly expand the potential to identify important loci and alleles and their application to breeding programs.

Genome-wide selection

Marker-assisted selection is best applied to simply inherited traits, or at least, to traits for which one or few QTL of known large effect have been identified. For most complex traits such as yield, the QTL-by-QTL strategy seems less effective. Individual QTL effects are typically inflated in most mapping experiments, and QTL × environment interactions common. Manipulating many QTL simultaneously is difficult, requiring very large population sizes. Identifying all the QTL of importance is time-consuming, and probably population specific as well. Therefore, for these traits, another marker-based approach is needed.

Genome-wide selection, or genomic selection (GS), was proposed over a decade ago for use in livestock breeding (9). In contrast to a typical marker-assisted breeding program, in which certain genomic regions (QTL) are known (or at least hypothesized) to be involved in controlling a trait, GS makes no assumptions about how a trait is structured or which markers are involved but rather estimates genomic breeding values (GEBV) which capture the effects of a large number of genetic markers. The idea is simply to saturate the genome, so that any given gene will be in linkage disequilbrium with at least one marker. In this way, the breeding value of every marker can be estimated. In order for GS to result in genetic gains, an initial model needs to be constructed based on marker genotypes and individual plant (or family) phenotypes, so that marker breeding values can be computed. Once the model has been developed, selection proceeds based on the estimated breeding value of the alleles that each individual in the breeding population possesses. Because GS is marker-only selection, repeated cycles can be recombined in the greenhouse as fast as possible. Because breeding cycles are typically 2-5 years long, the number of recombinations from GS per typical breeding program can be substantial, offering the prospect of significant gains over traditional selection (6).

The limitations of GS are clear. Even if a robust model can be constructed, the multiple - rounds of selection and recombination in the greenhouse still require genotyping, and this can be costly. Currently, GBS markers can be applied to genotypes for about 10 USD each; improved sequencing will undoubtedly bring that number down further. However, a GS model is based on the particular allele frequencies present when selection begins. As cycles are conducted, allele frequencies change, and an updated model needs to be constructed. Therefore, phenotyping really won't be eliminated; in fact, the genotyping cost, however trivial it eventually becomes, will almost certainly be an *additional* cost for the breeding program. Will the added gain pay for the added cost? That's a question commercial programs need to answer, but the answer may be positive if genomic selection results in better cultivars faster. From an academic standpoint, the availability of genome-wide markers seems likely to improve genetic gain for complex traits.

Perspectives

An aspect that requires additional discussion is how breeding programs could be rearranged to further capture the value of markers (Fig. 1). Current methods are probably not the optimum ones given the availability of today's marker resources. One potential change could be a shift from the large population sizes and broad-based synthetics of today's breeding programs to one that is much more focused on fewer, elite clones. The focus would shift from trying to increase allele frequencies for multiple pest resistances in broad populations to using markers to ensure fixation (or nearly so) of the key alleles in all parental genotypes. More effort could then be devoted to extensive progeny testing for yield and other key traits, implementing a genomic selection program to accelerate genetic gain. New germplasm would be extensively tested for combining ability before entering the breeding population. Hybrid cultivars could also be evaluated to capture non-additive effects. Given the new genetic marker realities, current alfalfa breeding methodologies should be revised to most effectively integrate markers, accelerating genetic gain and cultivar improvement. ■

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The challenges of genetically modified alfalfa in Europe

by Daniele ROSELLINI and Fabio VERONESI

Abstract: The application of genetic engineering to alfalfa has large potential, and a genetically modified herbicide tolerant variety is on the market in the US. In Europe, apart from general public opinion issues, the main constraint to the adoption of genetically modified alfalfa varieties is gene flow, because it would be very difficult to completely prevent it. In the future, however, emerging genetic engineering techniques may improve public perception of transgenic crops and allow Europe to take advantage of the potential of genetic biotechnology for alfalfa improvement in Europe.

Key words: coexistence, gene flow, GM crops, *Medicago sativa*, transgenic varieties

The application of genetic engineering to alfalfa has large potential. Many interesting prototypes have been produced in several labs, and there is a number of traits which could improve the productivity, adaptability and product value of the crop (Table 1, 3).

The first, and only, genetically modified (GM) alfalfa on the market to date is Roundup ready alfalfa (Monsanto and Forage Genetics International). This variety was released in 2005, but in 2006 a group of organic farmers and nonprofit organizations sued the US Department of Agriculture for approving the crop without a thorough assessment its environmental and economic impacts. A US federal court stopped its sale, until an environmental impact assessment (5) allowed again its cultivation.

The adoption of GM alfalfa in Europe appears problematic at present. On the positive side, the development of new, publicly available tools could alleviate some intellectual property constraints and some perceived risks; for example, it is now possible to efficiently transform alfalfa without antibiotic resistance marker genes, by using a freely available alfalfa gene (2). This would avoid the environmental release of antibiotic resistance genes when cultivating GM alfalfa varieties.

The European legislation on GM crops poses serious limitations to the cultivation of transgenic alfalfa, especially with regard to gene flow. Alfalfa is pollinated by insects that can fly a long way; consequently, it would be very difficult to completely prevent transgene flow from GM to non-GM crops and to feral alfalfa plants, which are widespread in many European regions. A review study (1) suggests that a distance of 1000 feet (304.8 m) produces an adventitious presence of GM seed below 0.5 % in a non-GM seed crop, which is below the 0.9 % threshold required for the coexistence of GM crops with conventional and organic ones. For seed production, however, the spatial isolation prescribed in US by the Association of Official Seed Certifying Agencies for non-GM seed crops for export is at least 5 miles (8 km) from GM crops.

Organic agriculture makes large use of alfalfa in rotations and is particularly sensitive to the GM crop contamination issue. The same legal threshold for GM contamination is applied to forages for animals producing organic or conventional products in Europe, but the organic market may be affected by any level of contamination above zero due to consumer attitude.

The complete containment of transgenes would be possible if the transgenic crop were completely male-sterile; female sterility would also be desirable, to avoid the establishment of volunteer and feral GM plants; complete suppression of flowering could be obtained by engineering floral development genes (4). Clonal propagation of sterile or non-flowering alfalfa producing high-value molecules, such as vaccines for farm animals, might be economically viable.

Useful traits obtained through genetic engineering that cannot be obtained by classical breeding are already available and others are at hand, but it appears problematic to adopt GM alfalfa in European agriculture under the present regulatory and public opinion scenario. In the future, emerging genetic engineering techniques such as cisgenesis and gene targeting may be applied to alfalfa. Cisgenesis utilizes genes from a species, or from sexually compatible species, for its improvement through genetic engineering. Gene targeting can surgically mutate or replace an allele with another by precise genome editing. These new techniques may contribute to exploit the potential of biotechnology for alfalfa improvement in Europe. ■

Table 1. A selection of traits introduced into alfalfa by genetic engineering

Input traits	Output traits
Herbicide tolerance	Protein quality
Improved phosphorous assimilation	Vaccine expression
Drought tolerance	Improved digestibility
Salt tolerance	Improved fermentable sugar yield
Acid soil tolerance	Reduced protein loss in silage and digestion

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Alfalfa genetic variation for response to tissue culture and *in vitro* regeneration

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Abstract: High *in vitro* regeneration ability of alfalfa is rare and subjected to complex genetic control. Identifying new responsive genotypes that are agronomically valuable is crucial for broadening the genetic base and raising the genetic value of transgenic varieties or *in vitro* selections, and widening the genetic background of material in functional genomics studies. We investigated the response to tissue culture and *in vitro* regeneration of 53 elite, high-yielding genotypes. Good response to tissue culture was fairly common, highly heritable in the broad sense, and with good consistency across experiments. Only one genotype also showed good response to subsequent *in vitro* regeneration, confirming the need for extensive screening.

Key words: genetic transformation, genetic variation, somatic embryogenesis, tissue culture

State of the art

The application of biotechnological techniques that require the use of *in vitro* regeneration systems is limited by the small number of alfalfa genotypes suitable for *in vitro* regeneration and by the modest agronomic value of these genotypes in terms of yield potential and tolerance to biotic stresses (5). Identifying new, agronomically valuable germplasm with good response to *in vitro* regeneration has crucial importance for broadening the genetic base and raising the genetic value of germplasm used for transgenic plant production, as well as for widening the genetic background of material subjected to functional genomics studies (e.g., on gene over-expression, gene silencing, promoter analysis, and T-DNA tagging). Also, the availability of recombinant alfalfa *in vitro* regeneration usually proceeds via somatic embryogenesis (4). The low level of plant regeneration, the genotype dependence, and the lack of reproducibility still represent major shortcomings of somatic embryogenesis protocols for alfalfa *in vitro* regeneration (2). Genetic analyses have indicated that the process of embryo formation from vegetative cells is controlled by at least two independent dominant genes and different gene interactions (3), while the occurrence of maternal effects is controversial. Alfalfa shoot regeneration via direct organogenesis is very rare.

Identification of agronomically-valuable germplasm sources

We have searched for new germplasm sources able to combine high agronomic value with good response to tissue culture and *in vitro* regeneration, by assessing the latter characteristics in 53 elite genotypes that were selected from 4500 genotypes subjected to stratified mass selection for two-year dry matter yield in dense stand (1). The original genetic base of 4500 genotypes was established from a set of landraces and varieties that were highly adapted to the growing conditions of northern Italy. The breeding value of the 53 elite genotypes was further assessed based on two-year forage yield of their half-sib progenies.

For each genotype, leaflets derived from different apical leaves were excised, surface-sterilized, and cultured *in vitro* on a callus induction medium in a growth chamber. After three weeks, calli were transferred to the culture medium lacking growth regulators for regeneration assessment. A genotype was considered as embryogenic competent when it showed at least one callus with one or more somatic embryos. The whole experiment was repeated twice.

Figure 1. Somatic embryogenesis in alfalfa callus derived from leaf explants

All 53 genotypes were able to produce callus, showing high frequency of callus formation. On average, over 78% of the leaflets produced calli, which is a high value compared to various earlier studies. In both experiments, genotype differences in callus fresh weight per explant were highly significant and showed a large broad sense heritability ($h^2 > 0.84$). These differences were largely consistent across experiments (genetic correlation $r_g = 0.67$), while showing no relationship with forage yielding ability as evaluated on the half-sib progenies of the 53 genotypes ($r = -0.01$).

Despite the large number of genotypes that could produce callus, only one genotype out of 53 was able to produce somatic embryos and complete the cycle of *in vitro* regeneration. Only 10% of the calli of this genotype actually produced somatic embryos (Fig. 1), suggesting that not all cells in the growing calli are competent for somatic embryogenesis. The efficiency of embryo conversion into rooted shoots was 40% for this genotype, a value in the range of those in earlier reports for genotypes responsive to *in vitro* regeneration. Interestingly, this genotype was also that ranking first for forage yielding ability.

Identifying agronomically valuable germplasm for *in vitro* regeneration may require the screening of large genotype numbers, but its potential outcome is worth the effort. ■

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Future alfalfa cultivars

by Paolo ANNICCHIARICO[1] and E. Charles BRUMMER[2]*

Abstract: Alfalfa breeding will be influenced by technical advances and various socio-economic factors. Transgenic varieties, where allowed, are likely to justify their investment for just a few traits. While the full potential of hybrid and semi-hybrid varieties still has to be explored, synthetic varieties will become less broadly based. Genomic selection mainly based on a Genotyping-by-Sequencing approach, along with germplasm selection performed in well-defined managed environments or controlled conditions, will jointly contribute to breeding cultivars targeted to definite environmental conditions and utilization modes. Genetic resources and plant physiological characteristics will be fine-tuned to match specific growing conditions.

Key words: GM variety, hybrid variety, selection strategies, semi-hybrid variety

Introduction

A popular aphorism states that "predictions are very difficult, especially about the future". Indeed, the future variety type and selection procedures for alfalfa may be influenced not only by partly unforeseeable technical advances but also by future trends for various socio-economic factors, such as the degree of availability of genetic resources and patented technologies, the structure of global seed systems (e.g. their concentration into a few transnational seed companies), consumers' preferences for crop types (e.g. non-GM) and cropping systems (e.g. organic ones), and regional policies (e.g. supporting the dual-purpose crop for forage and energy). This brief article aims to devise future scenarios without neglecting this complex context.

Transgenic vs. non-transgenic cultivars

Conceptually, transgenic cultivars (commonly referred to as genetically modified or GM crops) could provide numerous new traits to alfalfa produces. However, the time and investment required by current registration and deregulation procedures for GM varieties (where they are established) can be paid off only if the new variety features a remarkable agronomic progress. Thus, practically, only few traits will be commercialized. The transgenic technology is most valuable for traits that can be substantially influenced by a single gene. A polygenic trait such as forage digestibility can be successfully affected by a key gene at a crucial point of the lignin metabolic pathway (5). An upcoming breakthrough which is foreseeable from recent genomic studies in *Medicago truncatula* (10) is the selection of a transgenic alfalfa that is able to express a condensed tannins gene in its foliage (thereby reducing cattle bloat and protein degradation in the rumen). The insertion of transgenes for tolerance to abiotic or biotic stresses may also produce definite genetic progress.

These achievements could increase the public awareness of the potential benefits provided by GM alfalfa varieties. Part of the work generating them may also contribute to the breeding of non-GM varieties, when it could highlight the gene or the genomic region in which a favourable mutation could have a major influence on a relevant metabolic pathway (e.g. one affecting the lignin type or the expression of condensed tannins). This information could be exploited to detect useful allelic variation or to generate it by applying the point mutation technique to elite non-GM germplasm.

The open-pollinated reproduction system of alfalfa makes gene flow to native or feral populations likely, which may hinder the acceptance and use of transgenic alfalfa, especially in regions where organically-grown alfalfa is widespread. European seed companies are likely to keep producing non-GM cultivars for a long time, owing to policy restrictions on cultivation of GM material, difficulties in arranging freedom to operate with components of the GM technology, or the expense of deregulation.

Hybrid vs. semi-hybrid vs. synthetic variety

A transgene is typically introduced to affect a single trait, and breeding to improve other traits – yield, disease resistances, etc. – is still needed to generate a commercial cultivar. Various options exist to develop cultivars, whether transgenic or not. Hybrid varieties of alfalfa offer a simple means of exploiting heterosis between genetically distant, relatively narrow-based parent populations. They are produced through a patented male-sterility technique which is expected to produce about 75% hybrid seed (as male-sterile and male-fertile parents are planted at a seed ratio of 4:1). These varieties have not yet shown a distinct yield advantage over traditional synthetic varieties (8), but their potential is expected to rise.

Heterosis can also be increased by producing semi-hybrids (i.e., free hybrids implying no male-sterile parent) between genetically distant, well complementing populations which previously underwent separate selection (4). *Sativa* × *falcata* semi-hybrids aimed to capitalize on the wide genetic variation between these subspecies are limited by the modest intrinsic yield potential of the latter subspecies, suggesting that developing (or creating *de novo*) heterotic populations from genetically-distant, yet adapted *sativa* germplasm would be a preferable starting point. Thus, the full potential of semi-hybrid varieties still has to be explored. The effective breeding of this variety type will require knowledge and convenient structuring of the potentially useful genetic diversity, which is easier to attain if molecular diversity and heterosis of candidate heterotic populations are substantially related (2). Genomic selection, if concurrently foreseen, should very likely be specific for each heterotic population.

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Synthetic variety breeding probably still has a major role to play in the next several decades. Rather surprisingly, there is little information on the optimal selection scheme among the many possibilities (clonal selection; parent or family selection, based on half-sib progenies, full-sib progenies, or different generations of selfing). Modifying synthetic breeding programs to focus on a few highly selected genotypes may result in better improvement than current broad-based programs.

Selection strategies

Marker-assisted selection (MAS) for alfalfa production traits such as forage yield and persistence is complicated by tetraploidy and is unlikely to exploit candidate genes from *M. truncatula*. However, several quantitative trait loci (QTL) for these traits have already been located, although they have proved largely site-specific (e.g., 9). Genotyping-by-Sequencing has the potential for cutting genotyping costs dramatically, opening the way to genomic selection also for this crop (7). This approach, whose success depends on careful modeling and validation with respect to the targeted parent germplasm and cropping environments, may ultimately and fundamentally change selection procedures. With the decreasing costs of genotyping data, MAS is now limited by the availability of robust, multi-location phenotypic data. More effort will need to be placed on developoing phenotyping platforms suitable for this perennial species and capable of reproducing the genotype responses to different targeted growing conditions (e.g., irrigated and drought-stress environments; monoculture and association with grasses; etc.) (Fig. 1). Increased knowledge on ecophysiological mechanisms that affect alfalfa's adaptation to specific conditions could help define target traits and morphophysiological types to select (2).

Alfalfa landraces and natural populations of the *M. sativa* complex have shown remarkable specific-adaptation effects in relation to the level of drought, salinity, winter cold and utilization regime (mowing or grazing) of the target environment (2). These responses, which are likely to arise from long-standing site-specific selection pressures exerted on these populations in their site of origin, are associated with environment-specific adaptive traits (3). Structuring the genetic diversity and its use in relation to the target environments allows for more efficient selection strategies for wide or specific adaptation (1), and MAS can greatly contribute to that by digging out and assembling QTL associated with the desired adaptation pattern. The thorough exploitation of adaptation to specific regions through local genetic resources and selection environments will help regional breeding programs withstand the competition exerted by large or international programs.

We expect that the development of specific selection methodologies based in highly controlled environments will increase in importance in breeding programs, especially for tolerance to stresses such as soil salinity, aluminium (6; Fig. 2), freezing, drought and some diseases. These methods will mainly be applied to seedlings or young plants to maximize the number of genotypes that can be evaluated, although careful assessment of the relationship between seedling and adult plant responses must be done. Finally, there is still a need for better field-based phenotyping methods and equipment for yield and nutritive value, to make alfalfa breeding more successful and cost-effective. ■

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Figure 1. One of CRA-FLC's phenotyping platforms; it includes four environments formed by the combination of two soil types (sandy-loam or silty-clay) by two possible water regimes (applied via microirrigators under a moving shelter)

Figure 2. *In vitro* selection procedure under development at Noble to select forage legumes for tolerance to pH or aluminium. Plants grown under different pH or Al concentrations are phenotypically selected on the basis of their root length (photo by Maria J. Monteros)

Improving alfalfa tolerance to abiotic stresses

by Paolo ANNICCHIARICO[1]*, Yves CASTONGUAY[2] and Luciano PECETTI[1]

Abstract: Drought, salinity and winter cold are the main abiotic stresses that limit alfalfa yields. Greater drought tolerance can be provided by different adaptive traits, and may be difficult to combine with adaptation to water-favourable environments. Breeding for tolerance to drought or salinity could largely rely on landrace genetic resources and managed selection environments, whereas marker-assisted selection requires thorough investigation by different possible approaches. Controlled selection environments and molecular markers can contribute to selection for cold tolerance. *In vitro* selection is expected to increase in importance, especially for salt tolerance.

Key words: cold tolerance, drought tolerance, frost tolerance, marker-assisted selection, salt tolerance

Introduction

Drought is the main stress constraining alfalfa production at the global scale, while low winter temperatures limit alfalfa production and cultivation in northern latitudes and high elevations. Drought severity is expected to increase in most alfalfa cropping regions, owing to decreasing availability of irrigation water and the combination of higher evapotranspiration and lower and/or less regular rainfall due to climate change (6). The increasing adoption of poor-quality, saline irrigation water will enhance the impact of salinity stress. Frost will hardly lose its importance as a major stress, because alfalfa adaptation to climate change will imply the progressive introduction in northern latitudes of material with lower autumn dormancy, which is intrinsically less cold-tolerant, to take advantage of a longer cropping season. In addition, climate change is expected to increase the frequency of extreme climatic events (6), including early or late frosts, particularly damaging to plants incompletely hardened or partially dehardened as a consequence of unseasonably mild temperatures.

This premise justifies our focus on drought, salt, and low temperature stresses. Actually, drought is frequently associated with heat stress, but little is known on the extent of alfalfa genetic variation for heat tolerance. Other abiotic stresses (e.g., soil aluminum and/or acidic soils; low soil phosphorous available) may well be important for specific regions.

Drought tolerance

Alfalfa is considered as fairly drought-tolerant, mainly because of its deep root system. Investigations on within-species variation for drought tolerance and its underlying physiological mechanisms have been, however, rather limited. In addition, their outcome may be limited by insufficient attention to the specific requirements of the perennial species in terms of evaluation procedures, the choice of physiological traits, and the concept and measure of water use efficiency (which should also take long-term plant survival into account). Physiological studies including a stress treatment should be able to mimic the cultivar adaptive responses across agriculturally relevant stress environments, as in Annicchiarico et al.'s (2) six-month experiment in large containers (Fig. 1) subjected to progressive drought and subsequent recovery. This study highlighted the importance of investigating below-ground organs, suggesting two main tolerance strategies: (i) an osmotic adjustment strategy based on increasing water soluble carbohydrates in storage organs under severe stress; (ii) a water-conservation strategy implying less water used in initial drought-stress phases due to restricted root development that resulted in more water available under severe stress. It also showed the difficulty of introgressing these adaptive traits into widely-adapted germplasm, suggesting instead the selection of varieties specifically adapted either to drought-stress or moisture-favourable conditions (2). This breeding strategy, useful even for irrigated and moderately-stressed, rainfed environments of northern Italy (1), could largely exploit drought-tolerant landrace genetic resources (1, 2).

Other recent work suggests that drought-tolerant and drought-susceptible germplasm may share similar physiological mechanisms of stress response, the main differences being quantitative, e.g., for decrease of stomatal conductance under stress, or production of osmolytes and antioxidants (7). Inexpensive morphological traits, such as lower leaf wilting or delayed leaf senescence under stress (2, 7), can contribute to selection.

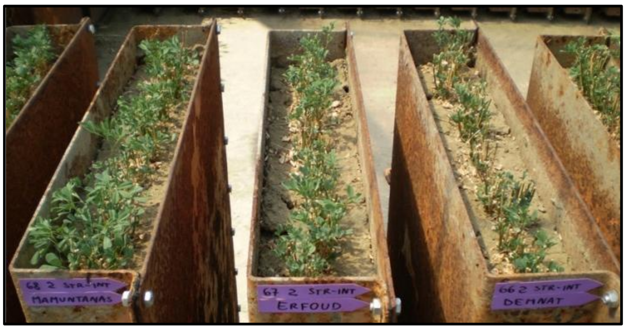

Figure 1. Growth under severe drought stress of Mamuntanas (left; drought tolerant Italian landrace), Erfoud 1 (centre; moderately drought tolerant Moroccan landrace) and Demnat 203 (right; drought susceptible Moroccan landrace) in metal containers (55 cm long × 12 cm wide × 75 cm deep; 21 plants per container) used for evaluating physiological and morphological traits of shoots and roots (2)

Alfalfa marker-assisted selection for drought tolerance still requires proper exploration. Exploiting specific genes involved in tolerance mechanisms is preferable but can be hindered by the complexity of the relevant gene network. Differences in major adaptive traits between annuals and perennials may limit the exploitation of candidate genes from *M.*

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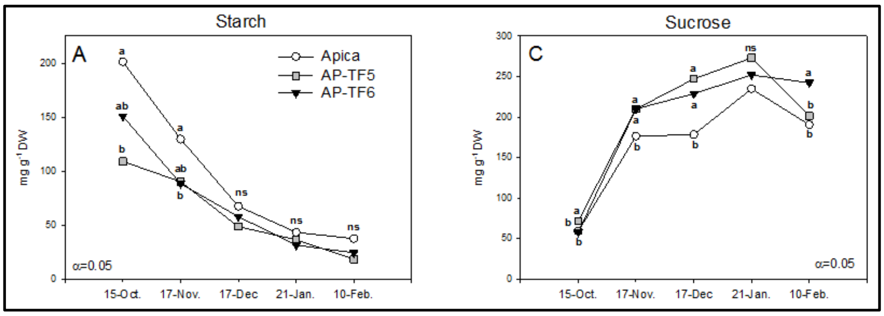

Figure 2. Starch (A) and sucrose (C) concentrations in crowns of alfalfa plants from the cultivar Apica and from two advanced cycles of recurrent selection for improved tolerance to freezing AP-TF5 and AP-TF6 performed within this cultivar; plants were hardened to natural variations in temperature in an unheated greenhouse; reprinted (5) with permission from the Crop Science Society of America

truncatula. In the short term, another useful approach implies the investigation of marker–forage yield association in mapping populations phenotyped in severely drought-prone environments. This approach, if properly devised (e.g. using material derived from genetically distant, drought tolerant populations; avoiding space-plant evaluations; etc.), allows concurrent selection of drought-tolerant varieties. Managed environments (e.g. by rainout shelters and microirrigators) can reduce the negative impact on selection and phenotyping of year-to-year climatic variability (1). *In vitro* selection of alfalfa seedlings is worth being explored for tolerance to drought, salt and low temperatures, exploiting recent work from other species (10).

Salt tolerance

Alfalfa is moderately sensitive to salinity, reducing its forage yield by one-third with a soil electrical conductivity of 8 dS m^{-1} in the saturation extract. However, genetic variation for salt tolerance exists which involves various physiological mechanisms, such as Na^+ exclusion, Na^+ compartmentation into cell vacuoles, and better retention of K^+ in plant tissues (9). While indirect selection criteria based on some of these mechanisms could be devised, breeding strategies for salt tolerance have essentially relied on direct selection under stress conditions or, more recently mainly in China, on the development of transgenic germplasm which exploits stress tolerance genes from other species.

Alfalfa plants may be affected by salinity at germination, seedling growth and mature plant stages. Field selection in naturally saline soils is hindered by the marked spatial heterogeneity of the stress. A seed germination test in saline water recommended by NAAIC has long been adopted as a fast screening criterion for variety evaluation, and contributed largely to the selection of the salt-tolerant variety Ameristand 801S. Another NAAIC test evaluates the forage The greenhouse protocol proposed by Peel et al. (8) also allows for the phenotypic selection of large plant numbers at the adult stage.

Various recent reports point to the possible interest of north-African landraces as valuable salt-tolerant parent germplasm. Knowledge on the mechanisms featuring diverse salt-tolerant germplasm is essential for pyramiding functionally different salt tolerance genes.

Cold tolerance

The capacity of alfalfa to withstand exposure to subfreezing temperature stands out as a core trait that affects productivity in cold climates. The use of cultivars adapted to local conditions and good management practices remain the best options to minimize winter damage to stands of alfalfa. Untimely fall harvest increases the risk of winterkill and can reduce the yield of spring regrowth by impairing the plant capacity to replenish organic reserves in taproots (3). In addition, stubble removal can prevent snow entrapment and allow exposure of perenniating roots and crowns to potentially damaging subfreezing temperatures.

Declining photoperiod and lower temperatures in the fall affects plant development and triggers several molecular changes, including the accumulation of cryoprotective sugars and changes in gene expression, leading to an increase in freezing tolerance. Fall dormancy reflects the sensitivity of cultivars to changes in the photoperiod. However, this trait opposingly affects cold adaptation and seasonal growth. The development of cultivars combining reduced fall dormancy and high level of tolerance to freezing would significantly increase alfalfa productivity in regions with harsh winter conditions. In spite of evidence that the link between the fall dormancy response and freezing tolerance can be dissociated, the development of commercial products remains impeded by insufficient knowledge of the physiological basis of these traits and the lack of accurate phenotypic selection tools. There is a screening methodology entirely performed indoors under environmentally-controlled conditions for the reliable identification of genotypes with superior tolerance to freezing (4). This revealed significant increases in cold tolerance from -25°C to -29°C lethal temperature for 50% of the plants after 6 selection cycles, associated with higher accumulations of cryoprotective sugars (Fig. 2) and superior expression of cold-regulated genes (4, 5).

Perspectives

Molecular technologies provide new opportunities to more efficiently exploit extensive variability in open-pollinated alfalfa. The identification of molecular markers that co-segregate with traits of interest would facilitate the selection of genotypes with desirable alleles and help reduce the number of plants to be phenotypically screened. Next-generation sequencing technologies will accelerate the identification of genomic and transcriptomic sequences that affect tolerance to abiotic stresses. Identification of polymorphisms within candidate genes will pave the way to the development of functional markers to screen a broad range of germplasms. Sustained feedback interactions between breeding and gene discovery programs will be required, to reap the benefits of molecular genetics for the improvement of alfalfa tolerance to abiotic stresses in the 21st century. ■

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Improving alfalfa resistance to biotic stresses

by John IRWIN[1]* and David ARMOUR[1]

Abstract: The biology of alfalfa has up to now necessitated its commercialisation as broadly based synthetics, and the resulting genetic heterogeneity has provided population buffering against genetic shifts in pathogen and pest populations, with new pathotype/biotype development occurring for only a few diseases and pests, e.g. anthracnose. The genetics of resistance is often of complex inheritance, which may also contribute to its durability. Molecular markers, genetic linkage maps, and additional genomic resources from the fully sequenced model legume *Medicago truncatula* are being employed to further understand the genetics of resistance to alfalfa biotic stresses, and improve their management.

Key words: genetic heterogeneity, linkage mapping, *Medicago truncatula* synteny, multiple resistances, stability of resistance

Introduction

Cultivated alfalfa (lucerne) is autotetraploid ($2n = 4x = 32$), and belongs to the *Medicago sativa* complex, which includes subspecies *sativa*, *coerulea* and *falcata*. Because alfalfa is allogamous and individual plants can exhibit high levels of self incompatibility and inbreeding depression, it is chiefly commercialised as synthetic cultivars (5). A consequence of this is that alfalfa cultivars generally include a large number of non-inbred parents (e.g. about 100) or half-sib families, resulting in the cultivar consisting of a heterogeneous population of highly heterozygous plants at the 4th synthetic generation (used for commercialisation). Thus every synthetic alfalfa cultivar varies in its proportion of plants resistant to each individual pest or disease, and if a cultivar possesses > 30% resistant plants to a biotic stress, it is considered resistant to that particular stress. In alfalfa, biotic stresses necessarily have to be managed through breeding for resistance or tolerance due to economic and environmental considerations. This article concentrates on diseases; however the same general principles would apply to nematode and insect pests.

Figure 1. Phytophthora root rot (*P. medicaginis*), an acute (lethal) alfalfa disease. Plant dying in the field due to Phytophthora root rot (left), an actively growing lesion girdling the tap root (right)

Biotic stresses

The biotic stresses which negatively impact upon alfalfa productivity and persistence include diseases, insects, nematodes, invertebrates and weeds. The North American Alfalfa Improvement Conference (NAAIC) lists 29 biotic stresses for which standard screening tests have been developed, including resistant and susceptible checks for each stress, of which 17 are diseases, 8 insect and 4 nematode stresses (3, http://www.naaic.org/resource/stdtests.php). Cultivars exhibiting multiple pest and disease resistances are now widely deployed (3). A review of alfalfa diseases indicates that the development of new races (pathotypes) of pathogens is not a common occurrence for alfalfa. One of the few examples is anthracnose (*Colletotrichum trifolii*) where three pathotypes have been unequivocally established using single differential alfalfa genotypes (6). The reasons for the stability of resistance are the population buffering afforded by the overall genetic heterogeneity and heterozygosity present in synthetic cultivars, and the diversity and complexity of specific genetic resistance mechanisms operating against individual pathogens. *Phytophthora medicaginis* is a good example, where several genetically different systems conditioning resistance have been identified in diverse alfalfa germplasm sources (4).

Breeding for biotic stress resistance

It is likely that in the pursuit of higher productivity, the number of parents used in breeding synthetic varieties will be reduced from around 50-100 or more to 15-20. This will lead to a greater chance of new pathotypes evolving, unless steps are taken to deploy a diversity of resistance mechanisms against individual high risk pathogens. This can only be achieved when genetic mechanisms conferring resistance to a pathogen have been identified including their chromosomal locations, allowing deployment of multiple resistances even in a narrowly based synthetic. The development of molecular markers closely linked to resistance genes would facilitate the breeding of multiple pest resistant synthetics based on a small number of highly productive parents with good combining ability and still deploying a diversity of resistances against individual pathogens and pests. In breeding alfalfa for sub-tropical Queensland, where anthracnose and Phytophthora root rot (Fig. 1) are major lethal constraints to alfalfa productivity, the above strategy is one being pursued (5).

As well as the examples of lethal (acute) diseases mentioned above, alfalfa is host to a wide range of chronic diseases, mostly incited by fungi, in the form of crown rots and leaf spots. For example, *Stagonospora meliloti* causes a chronic leaf spot (Fig. 2), and a chronic to sometimes lethal crown rot. Almost invariably, the highly winter active germplasm shows high susceptibility to pathogens such as Stagonospora, whereas the more dormant material of Flemish origin is relatively less susceptible. The same applies to the lethal diseases, with Turkestan alfalfa generally being the source of resistant breeding material for diseases such as Phytophthora root rot. Within

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the *M. sativa* complex (subsp. *sativa*, *coerulea* and *falcata*), enormous genetic diversity exists, including resistances and tolerances to a wide range of biotic stresses.

Linkage mapping and marker assisted selection

Molecular evidence for the complexity of genetic mechanisms conferring resistance has been uncovered for the alfalfa pathogens *C. trifolii*, *P. medicaginis* and *S. meliloti*. This has largely been achieved through the development of genetic linkage maps using molecular markers of known chromosomal origin, which are anchored to existing genetic or physical maps. For resistance to the above pathogens in alfalfa, the inheritance of resistance could not be explained by the segregation of a single completely dominant tetrasomic gene. However, QTL analysis revealed multiple loci contributing to resistance to each of these pathogens, with both small and large effects (Table 1).

Figure 2. Stagonospora leaf spot (*Stagonospora meliloti*), a chronic (non lethal) alfalfa disease

Table 1. QTLs identified in alfalfa conditioning resistance to three pathogens

Pathogen	Race	% of phenotypic variation explained by largest QTL	Total number of independent QTLs identified	Chromosome location of QTL	Reference
C. trifolii	1	32	2	4, 8	(6)
	2	28	1	2	(6)
	4	40	2	6, 8	(6)
P. medicaginis	-	33	4	2, 5, 6 and 7	Armour and Irwin, unpublished data
S. meliloti	-	17	2	2, 7	(7)

[a] independent QTLs explaining > 6% of the phenotypic variation

Application of *M. truncatula* – derived technology to alfalfa

The extensive genome synteny shared between *M. sativa* and the model legume *M. truncatula* provides a wealth of additional molecular resources which can contribute to the development and understanding of biotic stress resistance in alfalfa. Molecular markers developed in one *Medicago* species will almost invariably map to the corresponding chromosomal location in the other. Importantly, *M. truncatula* is host to many acute alfalfa pathogens and pests, including but not limited to *C. trifolii*, *P. medicaginis*, *Aphanomyces eutiches*, spotted alfalfa aphid (SAA) (*Therioaphis trifolii f. maculata*) and bluegreen aphid (BGA) (*Acyrthosiphon kondoi*). Together with the ability to develop inbred populations and the resources offered by a sequenced and assembled genome, *M. truncatula* is a useful model system to study alfalfa biotic stresses. In *M. truncatula*, single genes have been identified which confer resistance to bluegreen and spotted alfalfa aphids on chromosome 3, *C. trifolii* resistance on chromosome 4, and *A. euticles* resistance on chromosome 3. However, the translation of findings from the model system to alfalfa is likely to uncover additional complexities. A further study into Aphanomyces resistance in *M. truncatula* found that the major gene on chromosome 3 offered broad spectrum resistance, with numerous additional race specific resistance loci on other chromosomes; a situation likely to occur in alfalfa. A further example of this is the finding of a single gene on *M. truncatula* chromosome 4 which confers resistance to all three *C. trifolii* races (8), whereas in alfalfa, molecular and inheritance data indicates a more complex genetic control of resistance operates (6). The increased genetic complexity of autotetraploid alfalfa versus diploid *M. truncatula*, with greater opportunities for inter and intra locus interactions, means that findings in *M. truncatula* may not always be directly applicable to alfalfa.

Perspectives

There are some alfalfa diseases for which no worthwhile resistance has been found, and Rhizoctonia root canker (*Rhizoctonia solanii* AG 6) is one such disease (1). This lethal disease seriously limits alfalfa utilisation in western Queensland, Australia, where an additional area of up to 20 M ha could be grown in dryland pastures. A transgenic approach may be required for such a disease, but only if the exhaustive screening of accessions could exclude the occurrence of resistance within the *M. sativa* complex. Armour et al. (2) were able to transfer resistance to *C. trifolii* from *Medicago arborea* ($2n = 4x = 32$) to alfalfa through the production of partial hybrids, using male sterile alfalfa as the female parent. *M. arborea*, being the longest-lived *Medicago* spp, may be a source of new resistances to biotic and abiotic stresses which can be used to improve alfalfa. However, there is enormous diversity in the alfalfa gene pool, and this should always be exhaustively searched for resistance or tolerance before attempting interspecific crosses as outlined above. ■

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Alfalfa as a grazed crop

by Luciano PECETTI

Abstract: Alfalfa has great potential for grazing, but an acknowledged limit to this exploitation is the poor persistence of common, hay-type germplasm under continuous and intensive grazing. Morpho-physiological traits were identified that may enhance the grazing tolerance. However, direct selection for tolerance under continuous stocking proved more effective than the indirect selection for positive traits such as growth habit and spreading ability, and contributed to the release of tolerant varieties. Successful attempts were made to combine the advantages of favorable morphological traits with those of an effective selection method. Ongoing studies are trying to break a possible relationship between grazing tolerance, high cold-season dormancy, and low vigor.

Key words: crown, dormancy, grazing tolerance, growth habit, persistence, plant morphology

Introduction

Although mainly exploited worldwide for hay-making, alfalfa (*Medicago sativa* L.) can also be a valuable crop for grazing and, as such, it is largely grown in countries such as Argentina, the USA, Canada and Australia. An enhancement of pasture productivity through alfalfa cropping is widely recognized (8). Grazing of alfalfa has traditionally been disregarded as a specific crop exploitation in Europe. It was seen, at most, as a by-product of the hay-making activity in southern countries, where cool-season regrowths were frequently 'cleaned up' by grazing. In recent decades, however, alfalfa grazing is also experiencing an increasing interest in South Europe, in parallel with a re-consideration of the role of grazing. Owing to its acknowledged positive features, such as perenniality, high forage quality, remarkable drought tolerance, improvement of soil fertility and low farming costs, alfalfa can substantially contribute both to increase the environmental sustainability of semi-intensive livestock systems in the more favorable environments, and to maintain the economic profitability in marginal areas where little agronomic alternative to extensive livestock exists.

Possible limits of alfalfa grazing

A major drawback to the use of alfalfa as a grazing crop is the recognized poor persistence of traditional cultivars under intensive exploitation. Although a rational alfalfa grazing should 'mimic' hay cutting, with rest intervals of 30-35 days following short grazing periods of 5-7 days (4), prolonged stocking and overgrazing may frequently be caused by various events, such as, for instance, unfavorable climatic conditions, reduction of pasture areas due to unexpected changes in crop rotations, or excessive animal number following a favorable market conjuncture. There is large evidence that continuous stocking and intensive grazing of hay-type alfalfa cause severe stand thinning. The plants of this kind of cultivars generally have small and shallow crowns, which may easily be damaged by animal trampling and become susceptible to biotic (e.g., fungal infections) or abiotic (e.g., frost) stresses. Moreover, the continuous defoliation determines a progressive depletion of the underground reserves, with further, negative effects on the plant longevity.

Ad-hoc selection of grazing-tolerant alfalfa

The research carried out in the last few decades provided better comprehension of the morphological and physiological mechanisms underlying the tolerance to grazing in alfalfa. Several traits were claimed to contribute to grazing tolerance, including decumbent growth habit, large and deep-set crown, prolific underground budding, large stubble leaf area below the grazing height, and high root carbohydrate reserves. However, indirect selection for these traits generally proved less efficient in selecting tolerant alfalfa than the direct selection for persistence under actual grazing (10). A useful selection method devised in the USA is based on continuous grazing and high stocking rate, which causes overgrazing of the evaluated germplasm and subjects all plants to any possible stress associated with grazing (defoliation, tearing, trampling, excretions), thereby minimizing the chance for non-tolerant genotypes to be selected (9). This method was eventually adopted as a standard test of evaluation by the North American Alfalfa Improvement Conference (2), and resulted in the release of varieties with enhanced persistency that has increased the use of alfalfa as a grazing crop (3). Further work proved that varieties selected by this method could also retain their better stand survival under well-managed grazing systems (1).

Table 1. Growth habit, autumn dormancy, tolerance to continuous sheep grazing, and forage yield under a mowing regime of alfalfa experimental and check cultivars in Lodi, Italy (6)

Cultivar	Growth habit	Autumn dormancy (1)	Survival after grazing (%) (2)	Dry-matter yield (t/ha) (3)
Camporegio	Prostrate	2	90.4 a	25.02 e
La Diana	Semi-prostrate	2	69.2 b	29.38 d
Fontegaia	Semi-prostrate	3	70.4 b	30.39 cd
Mens Sana	Semi-erect	3	60.0 bc	32.29 bcd
Sunto	Semi-erect	4	44.7 c	34.14 bcd
Verbena	Semi-erect	4	68.9 b	34.79 bc
Alfagraze (check)	Semi-erect	2	71.1 b	33.78 bcd
Prosementi (check)	Upright	6	1.9 d	41.70 a

Means followed by different letters differ at $P < 0.05$ according to Duncan's test; (1) Scale from 1 = maximum, to 11 = minimum dormancy, recorded according to NAAIC standard test; (2) Average of two years (average grazing period of ca. 150 days year^{-1}); (3) Sum of three years (total of 12 harvests)

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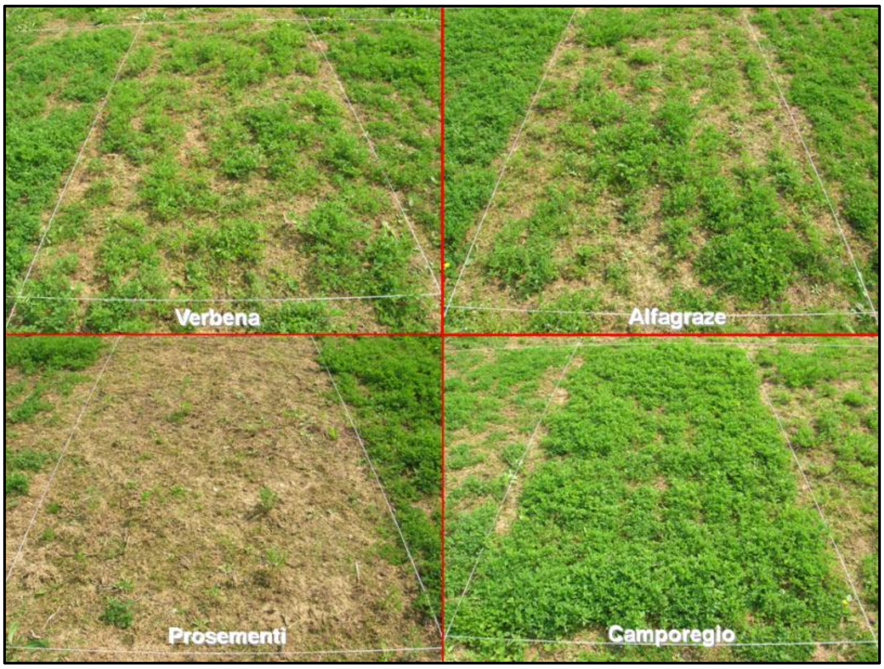

Figure 1. Representative plots of Verbena, Alfagraze (tolerant check), Prosementi (intolerant check) and Camporegio at the spring regrowth following two years of continuous sheep grazing with high stocking rate in Lodi, Italy

In our breeding program at CRA-FLC, we tried to implement the standard test by applying continuous stocking and intensive grazing to germplasm that had preliminarily been selected for the presence of putatively positive traits, such as large and deep crowns, proliferation of crown buds and high plant spreading ability, with the aim of combining the advantages of a suitable morphology with those of an effective selection method. Our activity basically entailed a three-step work, the first one being the screening of large germplasm collections and the identification of plants with the required morphology (7). The identified genotypes were grouped and intercrossed according to 'models', that is, ideotypes defined according to morphology and vigor of the aerial parts and underground organs. These ideotypes ranged from the very rhizomatous, prostrate type with great spreading ability, to the semi-erect model with remarkable shoot proliferation from a deep and robust crown (7). The progenies thus obtained were evaluated under sheep grazing in the second phase with reference to tolerant and intolerant check varieties (5). The most promising germplasm was synthesized into a set of experimental cultivars, which were evaluated under grazing in the last step of the selection process (6). Besides persistence under grazing in two years (with an average grazing period of ca. 150 days year^{-1}), forage yield was also assessed for three years (total of 12 harvests) in a contiguous, mown sub-experiment. The prostrate cultivar 'Camporegio' had outstanding grazing tolerance (Fig. 1; Table 1), although associated with low forage yield in the absence of grazing (Table 1). This confirmed a certain trade-off between plant vigor and trait architecture conferring grazing tolerance, already observed in other studies. As also shown in other work (5), the poor persistence of 'conventional' upright types under grazing is likely to offset any advantage in herbage yield that they may have under a mowing regime. The semi-erect cultivar 'Verbena' possessed a good balance between grazing tolerance and potential yielding ability (Table 1; Fig. 1). Verbena has been registered in the Italian Register of varieties and is currently under registration elsewhere in Europe, while Camporegio is completing its registration process in Italy.

There appeared to be a close, possibly genetic, relationship between grazing tolerance, marked cold-season dormancy, low stature, low vigor and low seed yield (5; 6; Table 1). This may have been determined by the large use of germplasm originating from cold regions (Central Europe or North America) and often introgressed with *M. sativa* subsp. *falcata* (7), which possessed morphological features complying with our breeding targets. While being adapted to temperate, cool areas, the selected germplasm may be less suitable for regions with mild winters, such as those with Mediterranean climate. We have implemented, therefore, a new path of research also involving molecular tools, aiming at investigating the genetic correlations among dormancy, morphology and grazing tolerance, and identifying germplasm tolerant to grazing and characterized by limited dormancy and good seed yield. ■

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Alfalfa intercropping and competitive ability

by Paolo ANNICCHIARICO[1], Bernadette JULIER[2]*, Gaëtan LOUARN[2] and Amel MAAMOURI[2]

Abstract: Alfalfa is subjected to interspecific competition in two situations: (i) intercropping with a forage grass, where the required long-lasting equilibrium between species is frequently threatened by excessive alfalfa competitive ability; (ii) competition against weeds, which can be severe in monoculture and requires high competitive ability to avoid any chemical weed control. In both cases, competition for light interception is the main mechanism that drives the species proportion. Vegetative vigour and plant architectural traits can affect the alfalfa competition dynamics across successive harvests. Once completely unveiled, these traits could be selected for new varieties with improved adaptation to competition.

Key words: interspecific interference, legume-grass mixtures, organic agriculture, plant competition, weed competition

Introduction

Our focus on alfalfa competitive relationships is two-fold here, i.e.: (i) intercropping with a grass companion, to obtain a long-lasting, balanced mixture of the two components of the mixture; (ii) competition against weeds, to avoid any chemical weed control. Legume-grass mixtures are gaining new interest in Europe and elsewhere, owing to the energy and environmental costs associated with the synthesis and use of nitrogen fertilizer required for grass forage production and the quest for greatest self-sufficiency in feed proteins at the farm and the country levels. Legume-grass mixtures are currently grown over 70% of the sown grassland in France, exploited as hay, silage or grazed forage. Alfalfa monoculture is still prevalent in other countries, e.g. Italy, where it is the backbone of organic crop-livestock systems and contributes significantly to conventionally managed systems. Alfalfa competitive ability against weeds is crucial for organic systems and increasingly important for conventional ones, especially for the pure stand.

We briefly review alfalfa competitive relationships mainly with respect to their relevance to breeders. One major issue in this context is whether alfalfa varieties targeted to mixed cropping with grasses, or targeted to cropping under severe weed competition (as in most organic systems), could be successfully selected or recommended from testing trials carried out in the simpler condition of absence of competition. The response, which depends on the degree of genetic correlation between selection and target environments, has far-reaching implications, e.g. for the choice of phenotyping conditions in marker-assisted selection studies, or procedures for assessing the variety value for cultivation and use.

Competition

Alfalfa-grass intercropping and weed-invaded alfalfa crops are situations of interspecific plant competition which imply different targets. The target is maintaining a desirable legume-grass balance in the former case, and excluding the weeds in the latter. Competition for resources is one of the major processes controlling plant growth and explaining the dynamics of plant mixtures. Competition for light is of primary importance, because it determines the energy available for all physiological processes and partially drives the acquisition of other resources. Furthermore, as light availability declines exponentially with the distance from the top of the canopy, minor differences in plant size can have major effects on the relative yield of the intercropped species. The amount of captured light defines the potential growth rate and the nitrogen demand of the crops. Besides energy capture and photosynthesis, competition for water and nutrients is also critical in explaining the dynamics of plant mixtures.

Competitive ability and compatibility with grasses

The companion grass tends to be at competitive disadvantage and less persistent than alfalfa when associated with highly productive alfalfa varieties under favourable cropping conditions, while the reverse may occur when alfalfa is grown in shallow soils or suffers from poor establishment.

In alfalfa, traits related to growth habit, shoot development and branching (leaf area expansion), and internode length (vertical leaf area distribution) showed a prominent role in light interception, and mainly contribute to its advantage compared to grasses (4). For legumes

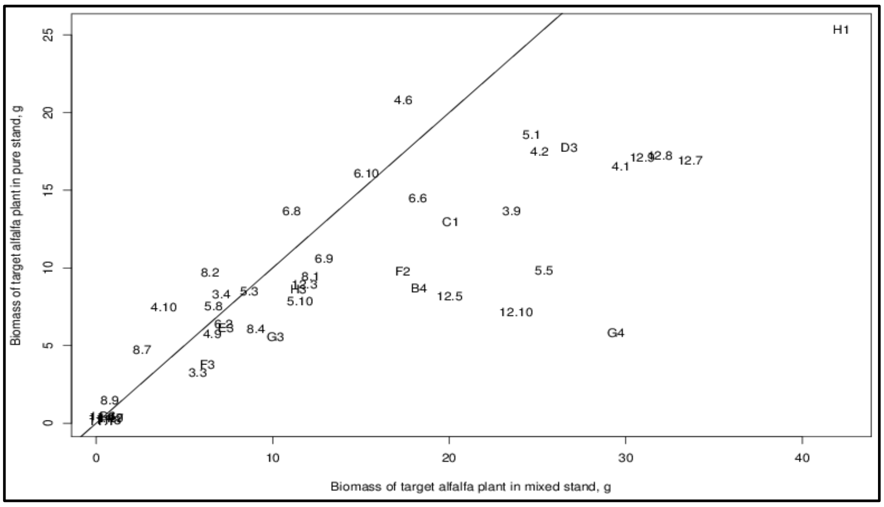

Figure 1. Biomass yield in the first harvest of 2011 of alfalfa genotypes grown in mixtures (one target alfalfa plant surrounded by 2 alfalfa and 4 tall fescue plants) and in pure stands (one target alfalfa plant surrounded by 6 alfalfa plants from the tall, erect cultivar Orca); the line is for equal biomass production in both stands

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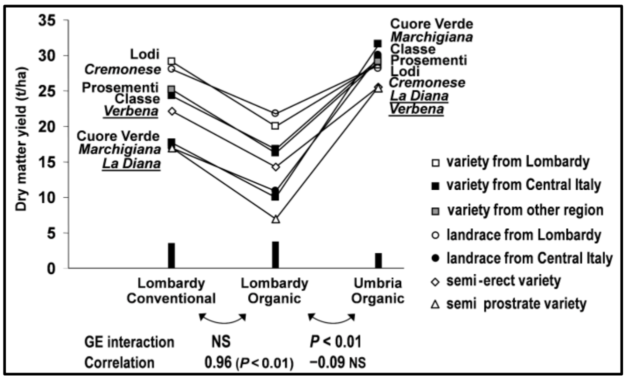

Figure 2. Forage yield of eight alfalfa cultivars across conditions reproducing organic or conventional cropping systems in two geographic areas (Lombardy, northern Italy; Umbria, central Italy), and genotype × environment (GE) interaction and phenotypic correlation for cultivar yield across environments; the graph pools results from the studies (2) and (3)

in mixture, intercepted light amount also determines the maximal amount of nitrogen that can be fixed by nodules (6), which can reach around 180 kg N ha^{-1} year^{-1} in alfalfa pure stands (9).

The root systems of grasses and alfalfa differ strongly in terms of architecture (fasciculate vs. taproot), root length density (high vs. low) and rooting depth (short vs. long) (7). Grasses are better competitors for water and most soil nutrients (mineral N, P and K in particular) than alfalfa, especially within the topsoil layers, but alfalfa explores deeper soil horizons.

However, alfalfa is little affected by soil N competition under conditions favourable to N_2 fixation, owing to its ability to fix atmospheric nitrogen (N_2). The proportion of N derived from fixation is even higher in mixtures than in pure stands as a consequence of the specialization of each species on a particular N resource (atmospheric N for alfalfa; soil N for grasses). N_2 fixation is intensively affected by water limitation, because this physiological process is one of the most sensitive to water stress. A drought event can thus quickly reduce the nitrogen autotrophy of alfalfa, changing drastically its ability to compete with grasses.

The relationships between species in a mixture are not only competitive: they may produce beneficial effects by modifying the environment of the other species. This is frequent is grass-legume mixtures, because a portion of the nitrogen fixed by legumes can be transferred to the associated grasses. Such effects tend to be more limited in alfalfa-grass than in clover-grass mixtures (10).

Maintaining a desirable balance between grass and alfalfa may be achieved by strategic choices at sowing (choice of alfalfa cultivars, grass species, date of seeding; density, seeding pattern) and by tactical management options (fertilisation, cutting). The selection of alfalfa varieties which are specifically adapted to mixed cropping because of greater compatibility with (hence, lower competitiveness against) grasses is also worth being investigated. A set of early studies in Lodi indicated the consistent ranking of alfalfa cultivars for biomass yield across pure-stand and mixed-stand conditions (11). However, preliminary results in Lusignan suggest at least some degree of genotype inconsistency between these conditions (Fig. 1). This experiment, which includes the observation of a number of morphological traits, will be used to test whether other alfalfa traits besides lower vigour may be associated with greater compatibility with associated grasses. We hypothesize that an alfalfa cultivar with lower stem height, more branching and good regrowth dynamics after cutting would have a reasonable high yielding ability in pure stand and would be a less aggressive companion crop for the grass in mixed stand, because of less competition for light interception. Breeding for all other traits that contributes to alfalfa persistency and quality would still be required for these new varieties. Choosing highly competitive grass companions can also be important to ensure more balanced associations.

Competitive ability with weeds

Weed density tends to be higher in alfalfa monocultures than alfalfa-grass mixtures. The false seeding technique, which is frequently adopted in organic agriculture, can be very useful to limit weed density and aggressiveness in the early, crucial stage of crop establishment. Weed competition after the first mowing is limited to species which are able to regrow.

The genetic variation for ability to compete with weeds has been poorly studied in alfalfa. One study whose results are partly summarized in Fig. 2 revealed substantially consistent responses of eight cultivars across conditions of high or almost nil weed competition (reproducing organic and conventional systems, respectively) which were established in one experiment in Lombardy (northern Italy). On average, landrace and variety germplasms showed comparable competitive ability against weeds. Competitive ability of the cultivars was strictly related to their forage yielding ability in the absence of weeds (2), with a semi-prostrate cultivar showing lowest values of both traits (Fig. 2). This relationship agrees with the crucial importance attributed to high relative growth rate for competitive success of genotypes belonging to erect, vigorous forage species grown in relatively favourable environments (5), when considering that high relative growth rate also implies high forage yield in the absence of competition. This relationship may be much looser in less vigorous, subordinate species such as white clover, whose genotype competitive ability mainly relies on the ability to make fine-scale exploitation of light and nutrients from undepleted zones through morphological plasticity (1). The high consistency of cultivar yield responses across high vs. negligible weed competiton conditions contrasts with the low consistency across two Italian cropping regions (northern vs. central Italy) (3; Fig. 2), suggesting that distinct material for each of these regions be bred with no specific selection for organic systems and/or higher competitive ability.

Compatibility with grass companions, and ability to outcompete weeds, likely inquire different optima of alfalfa competitive ability. They are associated with vegetative vigour and plant architectural traits yet to be discovered, which could eventually be selected for to produce varieties with improved adaptation to conditions of competition. ■

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Improving alfalfa forage quality

by Carla SCOTTI[1]* and Bernadette JULIER[2]

Abstract: Morphological and physiological traits contributing to the nutritive value of alfalfa forage are examined at harvesting and during a productive cycle. Leaf-to-stem ratio and the dynamics of development of stem internodes appear the main factors driving the nutritive value at the plant level. Within-population variation for these traits, in addition to between-population variation, can be exploited to improve digestibility and protein content.

Key words: alfalfa, crude protein, fiber fractions, leaf-to-stem ratio

The nutritive value of alfalfa forage is mainly a result of the forage chemical composition and in particular of the content of crude protein and fiber fractions (neutral detergent fiber, NDF, acid detergent fiber, ADF, and acid detergent lignin, ADL) estimating the proportion and composition of cell walls. ADF and ADL contents show strong negative correlation with ruminal digestibility of forage.

An alfalfa stem at harvest time includes a series of internodes separated by nodes carrying a leaf, and a branch (vegetative nodes) and/or a flower or pod (reproductive nodes). Proteins are mainly located in the the green and healthy leaves. Leaf protein content shows a limited variation (25% - 30% of biomass) that is determined by leaf position along the stem, with the highest contents in the upper young leaves exposed to light. Similarly, fiber content of the leaves is rather stable (20% - 25% NDF, 15% - 18% ADF). Stems, on the contrary, show a high variation for fiber content that is related to the developmental stage of the internodes (not yet elongating, elongating or mature). In the basal internodes, the mature stage implies a higher content in secondary lignified vascular tissues (mainly xylem) than in elongating internodes. ADF content of stem portions can vary from 55% in the basal internodes to 40% in the apical ones. In parallel, protein content varies from 9% - 11% to 16% - 20% from the basis to the top of the stem. The dynamics of shoot elongation during a regrowth affects the forage quality through changes in the proportion of leaves and the developmental stage of the internodes (3). At the beginning of regrowth just after harvest, the biomass of leaves is prevailing as internodes have not yet entered the elongation phase. When the stems reach about 30 cm height, the proportion of leaves has decreased but most internodes are still in the elongation stage. Around the phenological stage of 50% green bud stem, when the stems can reach 80 cm to 100 cm height, stem biomass becomes prevalent as a result of internode elongation, and most internodes are in a mature stage with high lignification; leaf biomass remains stable, as newly formed leaves are counterbalanced by the senescence and loss of older leaves triggered by the decline of incidence radiation (4). As a consequence, protein and fiber contents of alfalfa forage are mainly driven by the leaf-to-stem biomass ratio and secondly by the developmental stage of internodes. (Fig. 1).

The main strategies adopted to improve alfalfa protein content and digestibility rely on these morpho-physiological traits. One strategy is improving alfalfa tolerance to an early cutting schedule (e.g. cutting at 5% blooming), which implies plant selection for early restoration of nitrogen root reserves in order to fuel the shoot regrowth and ensure sufficient crop persistence (4). A second strategy is to act on the stem morphology by increasing the node number, decreasing the average internode length and making it more uniform along the stem. That resulted in higher leaf-to-stem ratio (0.80 *vs.* 0.76 in experimental synthetics selected for short and long internodes, respectively) and a final increase of CP production per plant (5). Since the development stage is negatively correlated with plant CP concentration and positively related with fiber fractions content, one further strategy is the uncouplement of growing and development processes by selection for high DM production and delayed triggering to reproductive phase (high vegetative-to-reproductive node ratio).

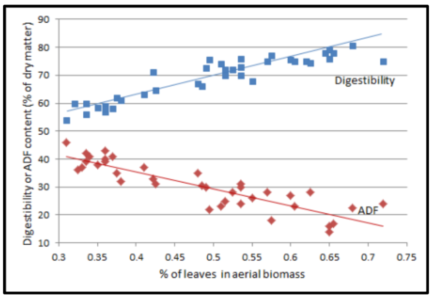

Figure 1. Relationship between forage digestibility or ADF content and leaf proportion in the biomass (from Lemaire and Allirand, Fourrages, 1993)

Within-population variation for plant and stem morphology, multifoliolate trait and rate of secondary wall deposition (1) can account for a major fraction of the genetic variation in digestibility among alfalfa plants with comparable biomass production. High-throughput screening of forage nutritive value by means of near infrared reflectance spectroscopy (NIRS) is currently used to exploit such variation. One last strategy for the genetic improvement of alfalfa stem quality relies on genetic transformation applied to a crucial point of the lignin metabolic pathway (2). Interestingly, alfalfa higher stem digestibility could positively impact not only on ruminant nutrition but also on bio-conversion processing for ethanol production. ■

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Alfalfa private breeding: American and European overview

by Mark McCASLIN[1] and Vincent BÉGUIER[2*]

Abstract: Since the release of the first commercial cultivars like *Vernal* in USA or *Europe* in France, much progress has been achieved in private alfalfa breeding. Private breeding is still very active in North America and in Europe but with some difference in organization (consolidated in USA, diverse in Europe), and in breeding achievements and objectives - particularly with the emergence of molecular breeding and transgenesis.

Key words: alfalfa breeding, alfalfa markets, genetic engineering, molecular breeding

Introduction

Commercial companies have conducted alfalfa breeding programs since the late 1950's. Most of this activity has been in North America, Europe and Australia. Today, 746 varieties are registered on the OECD list (8). Many of these cultivars were developed by industry breeders, demonstrating the significant and successful research investments dedicated to this crop species.

In this article, we will focus on private breeding in North America and Europe.

Private alfalfa breeding in North America

The North American private alfalfa breeding effort has consolidated rapidly in the last two decades. In 2013, only three large U.S.-based alfalfa breeding programs (Dow, DuPont/Pioneer and Forage Genetics International) represent > 95% of the industry-funded alfalfa improvement research in North America (7). This U.S. breeding effort focuses on multiple global markets and spans fall dormancy (FD) groups 2-10, with an emphasis on combining multiple pest resistance and agronomic traits required for adaptation to primary markets. The primary markets for the FD 2-5 varieties are the U.S., Canada and China. The primary markets for the FD 6-10 varieties are the U.S., Argentina, Mexico and the Middle East (3). All the U.S. based breeding programs conduct selection nurseries and/or variety trials in most of the primary markets listed above.

All three of the U.S.-based alfalfa breeding companies conduct both conventional breeding programs and research designed to develop and deploy transgenic traits. The breeding focus has generally been on three traits: forage yield, persistence, and forage quality. All three companies have dedicated resources to develop molecular breeding tools for alfalfa, although marker assisted breeding is in early stages of development in alfalfa compared to corn and soybeans (6). All three companies also have multiple U.S. alfalfa research stations and employ several PhD level scientists to drive their research efforts.

Roundup Ready alfalfa, produced in the U.S. in 2005, was the first transgenic trait to be commercialized in alfalfa (1). Genetically-engineered alfalfa with reduced lignin content is in the early stages of government regulatory review in the U.S. and Canada, and several other potential transgenes are being evaluated by U.S. breeding companies for improved forage quality, increased biomass production, abiotic stress tolerance and/or novel herbicide tolerance (7, 9). It is likely that transgenic varieties first commercialized in the U.S. will also be deregulated and commercialized in Canada, Mexico and Argentina.

Although the scope of industry-funded alfalfa research in North America has broadened greatly to now include molecular breeding, applied genomics, and genetic engineering – the total industry alfalfa research spend has decreased significantly over the last twenty years because of consolidation. This corresponds to a similar loss of alfalfa research funding in the public sector. Fortunately there has been an increase in public/private collaboration, specifically on long term strategic projects with potential high impact (7).

Figure 1. Variety trial in Pennsylvania including numerous experimental varieties resistant to potato leafhopper (green color) and some susceptible commercial varieties (yellow color); resistance to this pest, which is the most damaging one in Eastern US, requires glandular trichomes whose genes were introgressed into alfalfa from wild relatives

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Private alfalfa breeding in Europe

The European private alfalfa breeding is not as consolidated as in North America. Each European country has his own registration list and although a variety registered on a national list can be commercialized in all Europe (defined here as the European Union), we still have strong targeting of local seed markets. Italy (where many varieties derived simply from mass selection of farm landraces) and France are the countries where alfalfa cultivar registration and seed production are most active (Table 1). But in Eastern Europe too, alfalfa breeding has been very active, mainly due to the efforts of universities and state breeding stations.

The European cultivars originate mainly from local landraces developed in varieties in the 1960's and 1970's, from dormancy groups 3-5 in north-western continental Europe, and dormancy groups 6-7 in southern Europe. The Flemish landrace is the main contributor of dormancy group 3-5. For the dormancy 6-7, local landraces from southern European countries have been used, such as the Aragon landrace in Spain and Romagnola in Italy.

The dormancy group 3-5 is characterized by a very good standing ability, and high resistance to Verticillium wilt (caused by *Verticillium albo-atrum*). French breeders have developed varieties with high resistance to stem nematode (caused by *Ditylenchus dipsaci*) (5). For the dormancy group 6-7, resistance to anthracnose (caused by *Colletotrichum trifolii*) has been one of the main objectives.

The latest breeding targets are increased levels of protein content and digestibility (9). These characteristics are now taken in account for the registration of new varieties on the French list of varieties. The latest challenge is developing new alfalfa synthetics better adapted to sustainable agriculture, with a particular emphasis on the adaptation in mixtures with forage grasses.

European breeders are not involved in the development of transgenic varieties but try to use molecular breeding tools (2).

Table 1. Number of alfalfa varieties registered in Europe by country

Country	Number of alfalfa varieties on the list
Italy	153
France	54
Hungary	31
Greece	26
Spain	24
Poland	21
Bulgaria	18
Czech Republic	18
Germany	15
Romania	15
Slovakia	14
Austria	11
Switzerland, Denmark, Lithuania, Luxembourg, Latvia, Netherlands, Norway, Portugal, Sweden, Slovenia	< 10
Belgium, Cyprus, Finland, Ireland, Iceland, Malta, United Kingdom	0

Perspective

With the projected increase in the world population and the increasing need for feed proteins in developing countries, alfalfa should have a promising future. For addressing future needs, commercial breeders have to integrate new tools to be able to make continued progress on improving forage yield and forage quality. Seed production also will be an important feature, as the competition with cereal crops gets globally more and more acute. ■

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Alfalfa management for higher and more sustainable seed yields

by Jacques HACQUET[1]*and Đura KARAGIĆ[2]

Abstract: Seed yield is a major component of alfalfa industry. Alfalfa seed set depends on the activity of insect pollinators. For this reason, the essence of alfalfa seed crop management is providing favourable conditions for flowering and pollination. Key issues for maximizing seed yields are the growing location (soil and climatic conditions), stand density, cutting timing (aimed to insure favourable weather conditions during flowering and presence of pollinators), variety, and control of weeds pests and diseases.

Key words: alfalfa, crop management, ecological conditions, pest control, pollination, seed yield

Introduction

Both crop management and variety determine alfalfa seed yields (1), but climatic factors and soil conditions can strongly influence the yield in the field (4). Alfalfa seed yields have significantly increased during the last 30 years. For instance, the French average yield increased from 200 kg ha^{-1} to 500 kg ha^{-1}, with big fluctuations depending on year and climate. Both seed yield and quality (purity 98%, viability minimum 80%, without weed seeds) have been improved in a context of a sustainable agriculture that requires less chemicals because they are dangerous for the environment and the pollinating insects. The main agronomic improvements aim at avoiding lodging and providing the best conditions for flowering. There are several steps that need to be considered, which are discussed below.

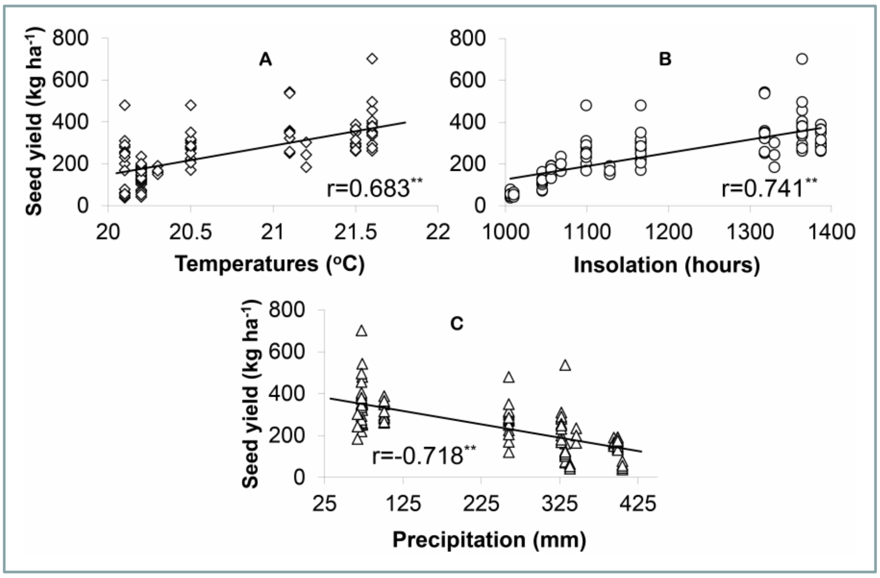

Figure 1. Correlation between alfalfa seed yield and air temperatures (A), solar radiation (B) and precipitation (C) during the seed crop period (May-August) in the period 1999-2001 (6)

Site selection and agro-climatic factors

Suitable areas for alfalfa seed production must combine good edaphic properties and weather conditions. Soils that guarantee a deep root development, rhizobium activity and nutrient requirements are suitable for alfalfa seed production. In general, favourable sites possess calcareous clayey and well-drained soils with a pH value between 7 and 8, and high water-holding capacity (to escape drought periods in the absence of irrigation) (4, 6, 8). Rainfall and low temperature during the pollination period result in an excessive vegetative growth and low pollinator activity. This is why seed production must be restricted to sites in which dry and warm weather prevails during the peak of the flowering and harvesting periods (Fig. 1).

Crop establishment and weed control

The field should be free from aggressive weeds and other weeds species whose seed is prohibited or restricted under rules for certified seed production. At least 5 years are necessary between two alfalfa crops on the same field, growing cereals or other winter and spring crops during this interval to reduce diverse aggressive weeds (2).

The most common option for establishing alfalfa, particularly in France, is with sunflower as a cover crop. Other cover crops are also possible, such as cereals. Another option is a direct sowing without a cover crop in the late summer, or in the spring (with irrigation if necessary).

The row spacing depends on the site climate: 30 cm is usually enough, with 50-60 cm in drier conditions. The sowing rate is usually about 3 kg ha^{-1}, with additional 1 or 2 kg ha^{-1} if sown with a companion crop (8).

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Weeds are easy to control when they are young and small, with mechanical or chemical methods. New herbicides are recommended in established crops, mainly during alfalfa resting period in winter and according to the country legislation (8).

Cutting schedule

Cutting is the most effective practice to limit the luxuriant growth and to obtain a flowering period synchronized with maximum pollinator activity (7). When cutting is too early, lodged plants are not suitable for pollination. However, if cutting is too late, the available soil water is not sufficient in the period of regrowth, leading to short plants and reducing the number of fertile stems (4). Cutting time is probably one of the most determining decisions for seed yield.

Pests and diseases control

Different pest species must be controlled in each region, for instance alfalfa weevil larvae, alfalfa pod weevils and seed moth in France, mirid bugs, aphids or *Diptera* (gall midges) in other countries (5, 8).

Using sweep netting and a network advising system is a good method to monitor the evolution of insect populations. A threshold for each pest is used to decide whether to treat or not.

The main foliar diseases are alfalfa rust (*Uromyces striatus*), *Phoma medicaginis* and *Pseudopeziza medicaginis*, especially when summer is wet. Efficient fungicides are available, but their use is less frequent than that of insecticides.

Pollination and seed set

Insect pollination is obligate for alfalfa. The most efficient species are alkali bees (*Andrena, Eucera, Halictus, Melitta, Melliturga, Rophites*...) and bumble bees, which feed on flowers nectar and pollen (3). In Europe, the main pollinators are wild species. If native populations of alkali bees are not present, alfalfa leaf cutting bees (*Megachile rotundata*) colonies can be added in the seed production fields (a common practice in North America).

It is very important to insure the best conditions during the flowering period and to conserve native pollinators, paying special attention to their natural habitat and to pest and disease treatments that can kill these insects. Seed growers must avoid chemical treatments during flowering or restrict spraying until late evening.

Seed set requires adequate temperature, water supplies and nutrients. Nevertheless, a large proportion of pollinated flowers will not lead to pods with seeds, probably as a part of a necessary regulation of the plant.

Harvest and postharvest

Losses may occur due to various reasons, such as diverse timing of seed ripeness, lodging, seed shattering, seed sprouting, or mechanical harvesting or separation. All these factors may also result in poor seed quality.

The optimum stage of ripeness is about 85% of brown pods, but the crop may be very heterogeneous. Lodging amount is also important. In this respect, cultivars which are sensitive to lodging also tend to have lower seed yield (7).

Pre-cutting or desiccation using an chemical desiccant (diquat) are two methods to reduce vegetative bulk, but either option requires good timing in relation to crop maturity and expected weather conditions. Precise adjustments for the combine harvester are necessary. For alfalfa, seed drying is not useful, except after especially wet conditions for harvesting. Usually, seed pre-cleaning (on the farm or in the factory) is sufficient to obtain a seed lot with moisture content below 12%.

Even under optimal management, alfalfa seed yields in the same area can vary largely between years, because of unpredictable climate variation. For example, seed yields in Serbia may vary from 50 kg ha^{-1} to 1200 kg ha^{-1} under the same crop management depending on weather condition, a reasonable target being represented by an average yield of 700 kg ha^{-1} - 800 kg ha^{-1} of high quality seed. ■

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Alfalfa in ruminant diets

by René BAUMONT[1, 2*], Valérie HEUZÉ[3], Gilles TRAN[3] and Maryline BOVAL[4]

Abstract: Alfalfa can be used in ruminant diets as hay, silage, dehydrated, or grazed. Alfalfa is of high interest for ruminant nutrition as it is generally well ingested and well suited to improve crude protein and mineral content of diets. Compared to grasses, alfalfa has similar digestibility but higher protein content, and allows for high levels of voluntary intake. Hay or dehydrated alfalfa also brings structural fibre in the diet. The best stage to combine alfalfa biomass production and nutritive value for conservation is bud stage or beginning of flowering.

Key words: cell-wall, crude protein, digestibility fresh forage, intake, hay, silage

Alfalfa (*Medicago sativa* L.) can be grazed, fed as green forage, offered as hay or silage, or dehydrated. The high dry matter (DM) yield, protein and calcium content of alfalfa, as well as its high palatability and voluntary intake, make it a suitable forage for all classes of ruminants.

Alfalfa yields more protein per unit area than soybean, and has a well-balanced amino acids profile for ruminants that compares favourably with that of soybean. Alfalfa provides higher amounts of minerals (mainly calcium, but also magnesium, potassium, sulphur, iron, cobalt, manganese, and zinc) and vitamins (beta-carotene) than other fodders (4). The energy content of alfalfa, though slightly lower than that of some forage grasses, should not be overlooked (2). A recent synthesis on alfalfa utilisation for animal nutrition was achieved in the Feedipedia project (5).

Grazed or fresh alfalfa

Rotational grazing is the most suitable management for alfalfa-based pastures. The stocking rates should be high enough to remove the herbage within 7-10 days. The rest period between grazing should be long enough to let alfalfa reach the bud-to-early-flower stage before the next grazing. In the USA, several trials reported that cows could graze 20 kg DM day^{-1} of alfalfa pasture and yield about 25 kg day^{-1} milk without supplementation (5). Grazed or fresh alfalfa at vegetative or mid-bud stage may cause bloat in sheep and cattle. Supplementing cattle and sheep with grass, cereal grains or anti-foaming agents (such as poloxalene) can alleviate this problem, and is essential in intensively grazed situations (4).

Fresh alfalfa can also be fed indoors. It is the ideal complement to maize silage to enrich the diet in protein and minerals and to provide fibre for chewing behaviour. An inclusion level of 50 % of the diet results in overall higher DM intake.

Conservation of alfalfa

Alfalfa may be cut for conservation several times a year (up to 12 in irrigated subtropical environments). The best stage for cutting is at bud stage or beginning of flowering as the nutritive value declines after that with the decrease of the % of leaves in the aerial biomass (see Scotti and Julier in the same issue). After the first cut, it is advisable to wait for the young shoots to be 35 to 50 cm long before the next cut (10).

Hay. Best quality hay is obtained by cutting during a dry period so that the swathe dries quickly. Raking has to be done at 60 % DM. Baling should be done at 82 % DM. The main difficulty in hay making for alfalfa is to preserve the leaves that are the most nutritive parts of the plant, especially in terms of proteins (10).

Alfalfa hay is particularly valuable for ruminants. The high content of structural fibre is rapidly digested by rumen microflora and helps prevent acidosis. This effect is due to its intrinsic buffering effect and to the stimulation of ruminative chewing and salivation which also results in rumen buffering (7). Alfalfa hay may be finely chopped, or coarse with long fibre.

Dehydrated alfalfa. Dehydration is the best way to conserve alfalfa, as it dries and stabilizes the forage while preserving its high protein content, vitamins and overall nutritive value. Dehydration requires prewilting and chopping in the field, transportation of the plant and drum-drying (between 600°C and 800°C) down to 10 % moisture. After drying, dehydrated alfalfa may be compacted into big square bales, ground to make alfalfa meal, or ground and passed through a screw die to make pellets. Pellets are often standardised to a certain protein content (such as 17% or 18 %).

Dehydrated alfalfa can partially replace concentrates in dairy cow diets. Providing 3 kg of dehydrated alfalfa to cows fed on grass silage, maize silage, barley grain and soybean meal, resulted in similar animal performance, higher feed intake (+2.5 kg DM day^{-1}), higher milk protein yield (+1 g kg^{-1}) and lower soybean meal inclusion (-350 g day^{-1}) (8).

Silage. Given its low soluble carbohydrate content, alfalfa has to be supplemented with carbon sources, such as ground cereal grains, or conservation additives such as organic acids (formic acid, formic acid + formaldehyde, propionic acid) to improve the preservation of alfalfa when ensiled at low DM content. Wilted silage at 30 % DM, or wrapped bale silages at more than 40% DM (3) are also suitable to conserve alfalfa, as they reduce the risk of bad preservation and consequent high protein degradability in the rumen. Mixed-crop silages combining alfalfa with a forage grass are also a way to achieve a conserved forage of high nutritive value.

Factors of variation of alfalfa's feed value

Updates in nutritive value of alfalfa were provided for dehydrated alfalfa and different types of alfalfa forages (2). The main factors affecting the feed value of alfalfa are discussed below.

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Table 1. Effect of growth stage and harvest on the feed value of fresh alfalfa (2)

	Dry matter (%)	Crude protein (g kg $^{-1}$ DM)	NDF (g kg $^{-1}$ DM)	OM digestibility (g g^{-1})	Voluntary intake in cow (g kg^{-1} LW$^{0.75}$)
Growth stage in 1st cycle					
Vegetative (30 cm)	14.4	246	423	0.77	156
Vegetative (60 cm)	15.6	225	449	0.72	152
Bud	17.6	193	488	0.66	143
Early flowering	18.9	178	513	0.63	140
Age at second harvest					
5 weeks	19.3	222	487	0.68	161
9 weeks	22.5	179	512	0.59	136
Age at third harvest					
5 weeks	21.0	241	468	0.69	147
9 weeks	24.9	204	476	0.62	140

Table 2. Effect of conservation type on alfalfa feed value assessed for the same fresh forage harvested at 7 weeks in the second vegetation cycle (2)

	Dry matter (%)	Crude protein (g kg $^{-1}$ DM)	NDF (g kg $^{-1}$ DM)	OM digestibility (g g^{-1})	Voluntary intake in cow (g kg^{-1} LW$^{0.75}$)
Fresh forage	19.7	198	513	0.65	144
Silages	Dry matter (%)	Crude protein (g kg $^{-1}$ DM)	NDF (g kg $^{-1}$ DM)	OM digestibility (g g^{-1})	Voluntary intake in cow (g kg^{-1} LW$^{0.75}$)
- Direct cut with preservative	21.0	187	466	0.64	135
- Wilted	33.5	198	488	0.62	139
- Wrapped bale	55.0	183	504	0.61	133
Hays	Dry matter (%)	Crude protein (g kg $^{-1}$ DM)	NDF (g kg $^{-1}$ DM)	OM digestibility (g g^{-1})	Voluntary intake in cow (g kg^{-1} LW$^{0.75}$)
- Barn dried hay	85	181	539	0.61	144
- Field dried, sunny weather	85	177	555	0.60	136
- Field dried, rainy weather	85	172	585	0.55	128

Table 3. Feed value of dehydrated alfalfa as a function of its crude protein content (9)

Crude protein content (% DM)	Dry matter (%)	Crude protein (g kg^{-1} DM)	NDF (g kg^{-1} DM)	OM digestibility (g g^{-1})
< 16	91.4	151	503	0.60
17-18	90.6	175	474	0.62
18-19	90.6	184	461	0.63
22-25	89.8	233	379	0.70

Effect of growth stage and harvest. Cell wall content increases while crude protein, organic matter (OM) digestibility and voluntary intake decreases with alfalfa aging in each growing cycle (Table 1). Compared to temperate grasses like perennial ryegrass or cocksfoot, the cell wall content of alfalfa is lower, but the OM digestibility is similar as a result of lower cell wall digestibility in alfalfa. In contrast, the voluntary intake of alfalfa is always higher than that of grasses, as a result of the lower cell wall content and lower rumen fill effect.

The highest digestibility is obtained at the beginning of the first vegetation cycle. The feed value of alfalfa regrowths decreases with age at about the same rate during the second cycle as during the first cycle (Table 1). After the second cut, regrowths become leafier, resulting in a lower decrease of feed value with age.

Effect of the conservation as silage or hay. Silage preserves chemical composition of fresh alfalfa and decreases slightly the OM digestibility and the energetic value (Table 2). Cell-wall content can even be slightly decreased due to partial hydrolysis of hemicelluloses during silage fermentation. However, voluntary intake of silage is lower than that of fresh alfalfa, because of the difficulty to obtain well-preserved silages, especially at low DM content.

Hay making has a negative effect on alfalfa digestibility, owing to the loss of leaves during raking and baling (Table 2). Alfalfa hays are very well consumed by animals when they are barn-dried or field-dried under good weather conditions. When made under unfavourable weather conditions, however, they show low digestibility and voluntary intake, making wrapped bale silage preferable (Table 2).

Feed value of dehydrated alfalfa. The feed value of dehydrated alfalfa increases with its crude protein content, which is associated with increased OM digestibility as a consequence of decreased cell-wall content (Table 3).

Conclusion and prospects

Alfalfa has high interest for ruminant nutrition because of its feed quality and its flexibility of utilization. However, alfalfa proteins are highly and rapidly degraded in the rumen. This can lead to poor nitrogen utilization and excessive nitrogen losses in urine. Decreasing the rate of alfalfa protein degradation in the silage and the rumen is a challenge to further improve its high-protein crop value. Prospects of using polyphenol-oxidase present in red clover or tannins present in other legumes like sainfoin (1) are under investigations. Saponins present in alfalfa might contribute to reduce methane emissions during rumen fermentation, but results on this issue are still controversial (6). ■

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Alfalfa non-feed uses

by JoAnn F. S. LAMB[1]*, Hans-Joachim G. JUNG[2], Michael P. RUSSELLE[1], Craig C. SHEAFFER[2], Deborah A. SAMAC[1] and Carroll P. VANCE[1]

Abstract: Non-feed uses for alfalfa such as biomass energy and phytoremediation could increase alfalfa acreage and improve farm profitability. The new bio-energy alfalfa and production system increased forage yield and ethanol production. New alfalfas with enhanced nitrogen cycling capacities would protect water quality and enhance alfalfa's value in crop rotation systems.

Key words: bio-energy, ethanol production, leaf crude protein, nitrate uptake, nitrogen (N) cycling, phytoremediation, symbiotic N_2 fixation

Introduction

Alfalfa plays an essential role in sustainable agriculture. Its perennial growth habit and extensive root system permits the crop to aerate soil, access deep water resources, capture nutrient run-off and mitigate soil erosion. Using alfalfa in rotation interrupts pest and pathogen cycles and through symbiotic N_2 fixation with soil bacteria, provides up to 100% of the N nutrient requirement of the subsequent crop. Non-feed uses are needed to increase and diversify revenue streams for growers and allow alfalfa a greater role in sustainable agricultural systems. New uses include alfalfa as a renewable biomass feedstock that can help meet the energy demands of the future (2), and as an inexpensive and efficient remediation tool to remove sub-soil nitrate from polluted sites (2).

Alfalfa biomass energy system

A biomass energy production system using alfalfa would separate leaves from stems creating two products. The stems would be processed to produce electricity (combustion) or biofuel [ethanol (fermentation) or bio-oil (pyrolysis)], and the leaves would generate a secondary income as valuable protein feed for livestock (Fig. 1). Therefore, the key traits of interest for an alfalfa bio-energy germplasm would include concentrations and seasonal yields of leaf protein, stem biomass, and/or stem cell wall polysaccharides, depending on which the energy conversion platform is being used. Increasing stem yield in alfalfa can be as simple as harvesting forage at a later maturity than early bud, which is typically used in hay production.

An experimental bio-energy alfalfa population was created through selection for large, erect, non-lodging stems when the alfalfa was in bloom. Management protocols have also been modified to (i) reduce population stand density to allow the development of larger crowns and decrease light competition to keep leaves from senescing and (ii) harvest at later maturity stages to maximize both leaf and stem yield. The bio-energy alfalfa under bio-energy management harvested at the green pod maturity stage increased stem yield by 56% and doubled the ethanol yield compared to commercial cultivars grown under standard hay management practices. Leaf crude protein yields were similar between the hay and bio energy production systems for two of three of the site-year combinations (4). In another comparison between the bio-energy and high quality hay cultivars under the two management schemes, stem yields increased 40%, and potential ethanol yield increased between 50% and 97% when harvested at full bloom compared to early bud stage (1). The bio-energy alfalfa under the bio-energy production system shows great potential for helping meet the energy demands of the future.

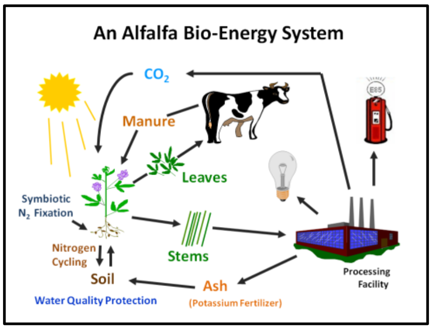

Figure 1. The alfalfa bio-energy system with two product streams, stems for energy and leaves for animal feed as well as value added products with enhanced nutrient cycling

Phytoremediation

Nitrogen laden ground water is a well documented human health risk and practical remediation methods are needed when over-fertilization of annual crops with inorganic N threatens ground water quality. Although alfalfa can obtain most of the N required for growth through symbiotic N_2 fixation, it is also very effective at removing nitrate-N from the sub-soil (Fig. 1) (2, 5).

At the site of an anhydrous ammonia spill where nitrate-N concentration of the ground water exceeded drinking water standards, a conventional cultivar and an ineffectively nodulated population (incapable of symbiotic N_2 fixation) were irrigated with the N containing ground water for three years. Cumulative N removal from the site was over three times greater by the ineffectively nodulated alfalfa than would be expected with annual cereal grains (5), and soil nitrate-N concentrations were rapidly reduced to low and stable levels. With these results in mind, we successfully created a selection protocol that produced new, contrasting cultivars that can either reduce N losses to ground water by increased nitrate-N uptake capacity, or decrease fertilizer N requirements in production systems through enhanced symbiotic N_2 fixation (3). These new cultivars could strengthen the crop's role in sustainable agriculture by protecting water quality and enhancing its value in crop rotation systems. ■

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EGF at 50: the Future of European Grasslands

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http://www.egf2014.org/

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Grassland and Forages in High Output Dairy Farming Systems

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