CSIRO PUBLISHING
Crop & Pasture Science, 2012, **63**, 734–738
http://dx.doi.org/10.1071/CP12127

Review

An overview of the role of lucerne (*Medicago sativa* L.) in pastoral agriculture

J. H. Bouton

Forage Improvement Division, The Samuel Roberts Noble Foundation, 2150 Sam Noble Parkway, Ardmore, OK 73401, USA. Email: jhbouton@noble.org

Abstract. Pastoral agriculture is unique among the world's agricultural production systems. Lucerne (also known as alfalfa), *Medicago sativa* L. subsp. *sativa*, has a long history of playing a very important role in pastoral agriculture. That role is expanding outside traditional hay and grazing production systems into sprouts for salads, nutritional supplements, and bioenergy feedstock. It is also the forage legume of choice for delivery of new traits via biotechnologies. The use of biotechnologies in lucerne improvement will cause re-examination of research methods and will require unique collaborations that are both interdisciplinary and even cross-institutional. The Consortium for Alfalfa Improvement (CAI) is discussed as a model for this type of collaboration. Breeding programs will continue development of cultivars with the proper fall (autumn) dormancy, a broad genetic base for pest resistance, increased local adaptation, persistence, and yield, while also adding new complex traits to these base traits. Increasing nutritional quality via down-regulation of lignin genes and increasing persistence via grazing tolerance, drought tolerance, and tolerance to acid, aluminium-toxic soils are discussed as examples of the potential impacts and challenges surrounding incorporation of complex traits. However, it is the potential for lucerne to become a major part of tropical or subtropical production systems or even an important adjunct to overcome deficiencies in the widely used perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) temperate systems that begs further attention.

Additional keywords: acid-aluminum soils, biotechnologies, CAI, drought, new uses, lignin reduction, persistence.

Received 2 April 2012, accepted 17 September 2012, published online 10 December 2012

Introduction

There are several general aspects of pastoral agriculture known to all forage agronomists that makes it unique among agricultural production systems:

1. Although some crops are intensively managed as monocultures within pastoral agriculture, not unlike the major grain crops in that respect, most acreage is dominated worldwide by native range and low-input, extensively managed systems which are mainly polycultures.
2. There is a diversity of species with use within pastoral agriculture and literally thousands of cultivars among these species are sold worldwide.
3. The economic system associated with pastoral agriculture is complicated and undervalued, due mainly to the indirect value of the final saleable products and because most production systems are on lower priced land containing poorer soils.
4. The choosing of new species for pastoral agriculture, as well as developing cultivars within those species, requires an assessment of the effect of the plants on the animals, and importantly, an assessment of the effect of the animal on the plant.
5. Forage breeding objectives for a target species are structured around a specific region's animal production and economic systems, climate, soils, and, especially, accepted forage management practices. This is simply an extrapolation of one of the first things that all plant breeders learn in any entry-level breeding class: 'what are your reference population of environments and reference populations of species and genotypes?'
6. In addition to plant breeding and genetics principles, understanding proper forage management and underlying crop physiological principles is critical for both cultivar development and on-farm economic success.
7. Perennial species are used where risk can be minimised at the expense of animal performance (e.g. beef cow–calf systems), and annual systems used where short-term animal production and performance requirements are high and more risk can be accepted (e.g. high-producing dairy cows). Lucerne (also known as alfalfa) is unique in that it is a perennial that can be used for both of these production outcomes.
8. Grasses supply the persistence base in pastoral agriculture, whereas the legume component simply needs enough persistence, either on a short-term or long-term basis, or on a farm or pasture basis, so it can fulfil one of its main roles of replacing the use of nitrogen (N) fertiliser or supplementing the overall forage supply with its own dry matter yield, protein, and energy.

There are now some significant challenges to pastoral agriculture due to global economic and energy issues. These include, but are not limited to, significant increases in costs for three things commonly used in forage-livestock production: feed, fuel, and fertiliser ('the three Fs'). In the past, a livestock producer needing to supplement his forage feed supply could buy feed grains at a reasonable price; if he needed to harvest and store or even plant his own forages, he could depend on cheap energy and fuel; if he wanted to fertilise the grass base, he could depend on cost-effective N fertiliser. Although still available, the cost of the three Fs is becoming less sustainable and driving up input costs to levels that cannot be sustained economically for pastoral farmers.

This begs the question: what is lucerne's current and future role in pastoral agriculture? First, it is unique among the world's forage and pasture crops in that its overall acreage, commercial value, yields, and management inputs in many countries mimics that of crops such as maize and wheat. However, its use and history as a perennial are firmly entrenched in pastoral agriculture where traits such as yield, nutritive quality, dependability, and persistence are paramount. Second, since the roles of the legume in any pastoral production system are to reduce N fertiliser costs and supplement nutrition (protein and digestible energy) for the grass base, lucerne is unique in that it is a high-yielding perennial that can be used for both of these production outcomes. Finally, lucerne has recently become the forage crop of choice for delivery of traits via biotechnologies (Bouton 2001). It is the only forage legume where such a trait, Roundup Ready, has been successfully commercially deployed (Anon. 2011). All this makes lucerne's role very important in assisting farmers to overcome the costs of the 3 Fs, and solidifies its position in the future of pastoral agriculture.

This review is brief for such a broad topic and emphasises mainly lucerne improvement. As such, there is no in-depth treatment of the crop's physiological research, which has been very substantial. Lucerne's very specific role in Australian pastoral agriculture (especially in the context of dryland grazing systems) is likewise not discussed in depth. However, that topic was reviewed by the author as part of a recent Farrer review (Bouton 2012). It is therefore suggested that Farrer review be read in conjunction with this brief paper.

History, acreage, uses

Lucerne has been spreading worldwide as an important forage crop since the days of the Roman Empire. In fact, reference to the crop is recorded by Virgil: 'But let him who longs for milk bring with his own hand lucerne and lotus in planting and salted herbage to the stalls'. From its centre of origin in the Caucasus, north-western Iran, and north-eastern Turkey, it spread first throughout Europe and Asia and then finally into the Americas and Australasia.

The crop was estimated during the late 1980s to be cultivated worldwide on ~32 Mha, mostly within the temperate regions of both the Northern and Southern Hemispheres (Michaud *et al*. 1988). The USA, Argentina, and the southern portion of the former USSR (central Asia, Transcaucasus, and Ukraine) comprised the majority of the acreage, with next greatest amount found in France, Italy, Canada, and China. World acreage areas have not been estimated recently. However, in 2001, decreases in traditional growing areas were noted, but these losses were estimated to be offset with increases in China and Australia, leaving 32 Mha as probably still the best estimate for world production at that time (Bouton 2001).

Important developments for lucerne use during the past century were summarised as follows (Bouton 2001):

- Local varieties and landraces were used as base populations for cultivar improvement that capitalised on the very important roles of natural selection and adaptation in the success that modern lucerne cultivars enjoy today.
- Intensive research was conducted on the management and physiology of the crop.
- A major seed industry developed worldwide.
- Since insect and disease pests are numerous in lucerne, development of cultivars with the proper fall (autumn) dormancy and a broad genetic base for pest resistance provided increased adaptation, persistence, and yield.
- Introduction of more complex genetic traits into these multiple-pest resistant, dormancy-specific cultivars is the latest trend.
- Lucerne has become the main forage legume species for delivery of traits via biotechnologies.

A major development that enhanced the role of lucerne was the formation of the North American Alfalfa Improvement Conference (NAAIC; www.naaic.org). This is a venue for meeting and for sharing research, outreach, and commercial information (McCaslin 2010). Its proceedings are published online at the web address and remain the best current information. In some cases, results are available even before publication in scientific journals. A major development made possible by the NAAIC was the use by lucerne breeders worldwide of the standard tests for screening and selection for various diseases, insects, and nematode pests; in addition, agronomic tests for fall dormancy, salt tolerance, winter hardiness, relative feed value, and grazing tolerance were also a major development made possible through the conference (NAAIC 2004). These standard tests, along with seed for the differential checks, are used extensively to develop commercially successful cultivars.

New roles for tomorrow's lucernes

The main uses for lucerne continue to be hay and silage production and pasture for livestock, but its ability to fix atmospheric N_2 enhances its ability to be a companion legume in mixed pastures, or part of crop rotations to enhance productivity of subsequent grain crops. Inter-seeding lucerne into bermudagrass (*Cynodon dactlyon* L.), the main perennial grass hay crop of the southern USA, is a good example of an expanded role as a companion legume for perennial grass stands to offset N costs and increase the nutritive quality of the hay. Other large-scale hay production systems elsewhere that are grass–N-fertiliser based should also examine this approach. Finally, the sod seeding of Roundup Ready lucerne into tall fescue (*Festuca arundinacea* Schreb) or perennial ryegrass (*Lolium perenne* L.) paddocks containing toxic endophyte should result in a method for maintaining a good forage supply while at the same time killing the toxic grass base with Roundup herbicide. This will allow later reseeding with the same grasses containing nontoxic endophytes with little land disturbance.

Lucerne's traditional role of hay, silage, and pasture crop has also expanded to include uses such as sprouts for salads, nutritional supplements for human diets, as a bio-remediation system for removal of harmful nitrates, a source of pulp for paper manufacturing, a 'factory' for production of industrial enzymes, and a bioenergy feedstock (Bouton 1996).

From recent NAAIC proceedings (www.naaic.org/Meetings/National/index.html), it is apparent that new traits receiving emphasis are herbicide tolerance; drought tolerance; resistance to disease and insect pests heretofore not easily screened; tolerance to acid, aluminium (Al)-toxic, and/or saline soils; tolerance to cold or freezing injury; expression of plant genes controlling nodulation and N_2 fixation; increasing nutritional quality of alfalfa via down-regulation of lignin genes; flowering control; increased biomass yield; and reducing bloat and bypass protein via incorporation of genes to express condensed tannins. These traits will all have high impact in both enhancing and expanding lucerne's current important roles in pastoral agriculture.

The new biotechnologies are also at the centre of the main tools being employed to enhance these high-impact traits. Their use in crop improvement therefore presents both an opportunity and challenge for lucerne improvement and use that will surely cause re-examination of research priorities, methods, and collaborations (McCaslin 2010). It will require collaborations that are both interdisciplinary and cross-institutional, with structural challenges in the treatment of confidential information and intellectual property. The CAI is a partnership model of government, private non-profit, and private profit entities needed to advance long-term, high-risk science that potentially will develop large payoffs for ruminant livestock producers (Bouton 2008; Martin 2010). Briefly, CAI members—USA Dairy Forage Research Center/ARS-USDA, The Samuel Roberts Noble Foundation, Inc., and Forage Genetics International—meet on a regular basis to identify key research projects. They then prioritise research efforts, coordinate scientific resources, and develop potential collaborations, both within CAI and external to CAI (www.agweb.com/assets/import/files/20CIAPressRelease.pdf). Finally, although CAI has a main goal of developing cultivars with improved yield and nutritional characteristics, its members are free to publish research findings in major scientific journals.

Lignin down-regulation to improved ruminant and biofuel use

The CAI is initially committed to redesigning lucerne by reduction of cell wall lignin via down-regulation of genes in the lignin pathway. During proof-of-concept studies, reduced-lignin transgenic lucerne hay fed in total mixed diets with corn silage demonstrated increased fibre digestibility in both lactating dairy cows and rapidly growing lambs (Martin 2010). One transgenic event was found to increase 3.5% fat corrected milk production over the null controls by 2.86 lb (1.30 kg) head^{-1} day^{-1}. Late-harvested COMT and CCOMT down-regulated lines also demonstrated the same neutral detergent fibre digestibility as their control populations harvested 8–12 days earlier. This means that producers should be able to delay harvest while maintaining nutritive quality, with a potential to reduce the number of annual harvests while increasing annual yield (Martin 2010).

It was also demonstrated that lucerne lines containing the lignin down-regulated trait showed a two-fold improvement in enzymatic hydrolysis efficiency in production of ethanol for biofuels (Chen and Dixon 2007). This efficiency could therefore eliminate the requirement for costly chemical pre-treatment for sugar production. More ethanol should then be able to be produced per tonne of this low-lignin lucerne, thereby making it a very efficient biofuel feedstock. This efficiency, in turn, would allow biorefineries to pay substantially more for the delivered lucerne feedstock due to the high alcohol yield per tonne, along with the concurrent need to store lesser amounts of the feedstock at the biorefinery.

Increased persistence

In general, after the breeding targets of adaptation, fall dormancy, and pest resistance are met, three of the main persistence-limiting traits on a global basis are grazing tolerance to allow an expanded use of the crop into intensively grazed pasture conditions; tolerance to acid, Al-toxic soils; and drought tolerance to mitigate the heat- and water-related problems projected due to climate change (Bouton 2012). It is also interesting that the breeding approaches used to improve these traits included traditional selection approaches being successfully used to develop and deploy grazing-tolerant cultivars, and protocols that moved from traditional to more biotechnology-based protocols being used for tolerance to acid, Al-toxic soils and for drought tolerance.

Grazing tolerance

The development and use of the grazing-tolerant cultivar 'Alfagraze' demonstrated that a high level of grazing tolerance could be achieved with good yield ('dual-purpose'), proper fall dormancy, and pest resistance (Bouton *et al*. 1991). This one event expanded lucerne's ability to be used in more intensively grazed situations. Its selection protocol serves as a vehicle that increased the development of new cultivars (Moutray 2000) as well as the interest in and use of lucerne for all grazing situations (Henning 2000; Smith *et al*. 2000). Other researchers reported that prostrate types demonstrated the best tolerance under grazing in Australia (Humphries *et al*. 2001) and Italy (Pecetti *et al*. 2008), but again, some tolerance could be found among upright, even winter-active, types. Another similarity among all these studies was the positive role that 'adaptation' to the target environment played in achieving greater results that should be useful for targeting grazing-tolerant cultivars for all regions.

The grazing tolerance trait has also been extrapolated to give better 'traffic tolerance' with better stands found after stresses imposed by the wheels of heavy harvesting equipment in intensively managed commercial hay fields (Lawton 2002). The production and current commercialisation of two new, Alfagraze-type cultivars, Alfagraze 300RR and Alfagraze 600RR, each containing the Roundup Ready trait, an expanded pest resistance package, and in the fall dormancy categories 3 and 6, respectively, is also noteworthy. They solidify the place of grazing-tolerant cultivars among modern lucerne cultivars (Bouton *et al*. 2006).

Acid, aluminium-toxic soils

Of the problems facing lucerne growers, acid, Al-toxic soils are the most widespread and limiting of all because lucerne is highly sensitive to these conditions and acid, Al-toxic soils are found in large areas on every continent. It is also one of the main reasons lucerne is not grown in the tropics and subtropics. Since genetic selection for acid soil and Al tolerance offered an avenue for increasing lucerne's productivity and reducing its production costs, plant breeding programs were, and are currently, being pursued. Their objective was to develop lucerne cultivars and management systems tolerant to these conditions (Bouton and Radcliffe 1989; Hartel and Bouton 1991; Zhang *et al*. 2007).

However, due to genetic complexity, as well as no tolerant cultivars being produced for on-farm use via conventional selection, breeding efforts evolved into the use of biotechnologies. Tolerance genes were found in the secondary diploid gene pool, and molecular markers are now being used to transfer them into cultivated types (Sledge *et al*. 2002; Narasimhamoorthy *et al*. 2007; Khu *et al*. 2010).

Drought

Drought tolerance is an inherent, positive trait in lucerne, and one that has historically been associated with the crop. This view was supported recently during the 2006–07 droughts in South Australia where established lucerne stands showed some stand decline but were still able to maintain acceptable ground cover (Marshall *et al*. 2008). An even more dramatic response was seen after the record summer heat wave in 2011 and corresponding drought in the southern Great Plains area of the USA where lucerne paddocks showed complete autumn recovery while the adjacent tall fescue paddocks did not (Bouton 2012). Therefore, although lucerne is a drought-tolerant species, some of its acreage is produced under irrigation to achieve economic yields or under dryland conditions where lack of water commonly limits productivity.

Several research organisations are currently investigating ways to increase lucerne use in dryland conditions as well as improving drought tolerance and water-use efficiency. Gene candidates for drought tolerance are now being expressed in lucerne. One of these is the WXP1 transgene discovered by scientists at the Samuel Roberts Noble Foundation, where 'proof-of-concept' experiments demonstrated that insertion of WXP1 increased lucerne's ability to be productive, or even recover more quickly, after periods of limited water (Zhang *et al*. 2005).

In a related genomics project, Noble Foundation scientists are collaborating with others at New Mexico State University to identify genetic mechanisms associated with drought tolerance within cultivated lucerne. Molecular markers associated with yield under drought conditions were initially identified that can be used in selecting genotypes for the production of new drought-tolerant cultivars (Han *et al*. 2008). This approach also brings the power of genomics technologies to bear in the process without the regulatory and public perception problems associated with transgenics.

Conclusions

It is apparent that lucerne plays a very important role in current pastoral agriculture. This is now expanding to include new roles outside traditional hay and grazing production systems, as well as lucerne being the main forage legume to commercially deliver traits via biotechnology. However, the crop could assume an ever larger role if producers and experts are able to stand back and examine some of the limitations of their current traditional systems and lucerne's potential to help.

For example, one important question remains (Leach and Clements 1984): can lucerne become a viable crop in the tropics and subtropics? To be successful at producing a tropical lucerne, it was speculated that the following factors need to be capitalised upon: (*1*) the availability of very non-dormant, winter-active cultivars which allow forage production on a year-round basis; (*2*) the wide availability of pest-resistant germplasm and screening and selection methods for the many disease, insect, and nematode pests; (*3*) the increasing interest and need in dairy production and high-quality grazing in the subtropics and tropics; and (*4*) the advances in research which may allow the crop to be grown in problem acid, Al-toxic soils (Bouton 2001). An ability to provide a non-dormant, acid soil tolerant, high pest resistance cultivar would be a big step towards establishment of widespread 'tropical lucerne' systems.

The perennial ryegrass–white clover (*Trifolium repens* L.) system is a good base production system indeed. In fact, livestock producers in all of the world's geographies would love to have this as their main production base. But, have countries that currently employ perennial ryegrass and white clover as their main base system, especially their dairy industry where daily production is paramount, become too reliant on it to supply most of their current and future needs? Are breeders of these current major species up against the biological and genetic limits of these species to dramatically improve them for persistence problems, especially in a future of increased temperature and drought as predicted due to climate change? Can a drought-tolerant species like lucerne be considered to add to this base and increase high-quality forage during the base system's off-season or during times of environmental stress? On a personal note, I remember standing in a lucerne field in the Canterbury Plains right outside Christchurch, New Zealand, during a dry period. All the ryegrass–clover pastures as far as the eye could see were dry and brown, yet the lucerne was up to my knees in green growth with lambs happily grazing. I asked my companion, a very well-respected New Zealand pastoral scientist: 'Why, in this context, is there not more lucerne used in New Zealand?' His answer was: 'In this context, I cannot explain it!'

In the USA and Argentina, lucerne is an integral part of their dairy production systems. Why? Because it is a nitrogen-fixing, drought-tolerant legume capable of very high yields of quality forage over a very long growing season. Lucerne is, simply, in these two countries a major crop, right up there in acreage with maize and soybeans (*Glycine max* L.), with a long history of intensive and productive research, including breeding in both the public and private sectors, with a major transgene (Roundup Ready) even successfully deployed commercially, and an estimated direct annual impact value to USA agriculture alone of US$8.1 billion (www.naaic.org/Alfalfa/Importance.html). So, the question of why lucerne is not expanding its role in all countries will remain a pertinent one for the foreseeable future.

References

Anon. (2011) Roundup ready alfalfa. Available at: www.monsanto.com/newsviews/Pages/roundup-ready-alfalfa-supreme-court.aspx.

Bouton JH (1996) 'New uses for alfalfa and other "old" forage legumes.' In 'Progress in new crops'. (Ed. J Janick) pp. 251–259. (ASHS Press: Alexandria, VA)

Bouton JH (2001) Alfalfa. In 'Proceedings XIX International Grassland Congress'. (Eds JA Gomide, WRS Mattos, SC d'Silva) pp. 545–547. (FEALQ, Piracicaba: Sao Pedro, SP, Brazil)

Bouton J (2008) Molecular breeding to improve forages for use in animal and biofuel production systems. In 'Molecular breeding of forage and turf'. (Eds T Yamada, G Spangenberg) pp. 335–340. (Springer Sciences: New York)

Bouton JH (2012) Breeding lucerne for persistence. *Crop & Pasture Science* **63**, 95–106. doi:10.1071/CP12009

Bouton JH, Radcliffe DE (1989) 'Effects of acid soil selection on agronomically important traits in alfalfa.' In 'Proceedings XVI International Grassland Congress'. Nice, France, 4-11 October 1989. pp. 377–378. (Association Française pour la Production Fourragère: Versailles, France)

Bouton JH, Smith SR Jr, Wood DT, Hoveland CS, Brummer EC (1991) Registration of 'Alfagraze' alfalfa. *Crop Science* **31**, 479. doi:10.2135/cropsci1991.0011183X003100020052x

Bouton J, McCaslin M, Fitzpatrick S, Temple S (2006) Roundup Ready® Alfagraze. In 'Proceedings 40th North American Alfalfa Improvement Conference'. Bloomington, MN, 16–19 July 2006. (NAAIC) Available at: www.naaic.org/Meetings/National/2006meeting/procedings/Bouton (oral).PDF

Chen F, Dixon RA (2007) Lignin modification improves fermentable sugar yields for biofuel production. *Nature Biotechnology* **25**, 759–761. doi:10.1038/nbt1316

Han Y, Ray IM, Sledge MK, Bouton JH, Monteros MJ, Han YH (2008) Drought tolerance in tetraploid alfalfa. In 'Multifunctional grasslands in a changing world. Volume II: XXI International Grassland Congress and VIII International Rangeland Congress'. Hohhot, China, 29 June–5 July 2008. p. 419. (Guangdong People's Publishing House: Guangzhou)

Hartel PG, Bouton JH (1991) *Rhizobium meliloti* inoculation of alfalfa selected for tolerance to acid, aluminum-rich soils. In 'Plant–soil interactions at low pH'. (Eds RJ Wright, VC Baligar, RP Murrmann) pp. 597–601. (Kluwer Academic Publishers: Dordrecht, The Netherlands)

Henning JC (2000) On-farm use of alfalfa for grazing. In 'Proceedings/Reports of the American Forage and Grassland Council, 37th North American Alfalfa Improvement Conference'. Madison, WI, 16–19 July 2000. pp. 359–363. (NAAIC)

Humphries AW, Auricht GC, Kobelt ET (2001) Developing grazing tolerant lucerne. In 'Proceedings 10th Australian Agronomy Conference'. Hobart, January 2001. (Australian Society of Agronomy/The Regional Institute Ltd: Gosford, NSW) Available at: www.regional.org.au/au/asa/2001/6/a/humphries2.htm

Khu D-M, Reyno R, Brummer EC, Bouton JH, Monteros MJ (2010) Identification of aluminum tolerance QTL in tetraploid alfalfa. In 'Proceedings 42nd North American Alfalfa Improvement Conference'. Boise, ID, 27–30 July 2010. (NAAIC) Available at: www.naaic.org/Meetings/National/2010meeting/DongManKhu.pdf

Lawton K (2002) Traffic tolerance. Farm Industry News, 1 April 2002. Available at: http://farmindustrynews.com/traffic-tolerance

Leach GJ, Clements RJ (1984) Ecology and grazing management of alfalfa pastures in the subtropics. *Advances in Agronomy* **37**, 127–154. doi:10.1016/S0065-2113(08)60453-8

Marshall EM, Humphries AW, Kobelt ET, Rowe TD, Auricht GC (2008) Drought response of lucerne in South East & Mallee environments in SA. Report for South Australian Research and Development Institute, Adelaide. Available at: www.sardi.sa.gov.au/__data/assets/pdf_file/0003/116553/Persistence_Drought_response_of_Lucerne.pdf

Martin N (2010) A comprehensive vision of increasing the value and uses of alfalfa and forages. In 'Proceedings 42nd North American Alfalfa Improvement Conference'. Boise, ID, 27–30 July 2010. (NAAIC) Available at: www.naaic.org/Meetings/National/2010meeting/Martin.pdf

McCaslin M (2010) An industry perspective on investing in alfalfa improvement. In 'Proceedings 42nd North American Alfalfa Improvement Conference'. Boise, ID, 27–30 July 2010. (NAAIC) Available at: ??

Michaud R, Lehman WF, Rumbaugh MD (1988) World distribution and historical development. In 'Alfalfa and alfalfa improvement'. (Eds AA Hanson, DK Barnes, RR Hill) pp. 25–91. (American Society of Agronomy: Madison, WI)

Moutray JB (2000) Future of alfalfa as a grazing crop: grazing tolerance. In 'Proceedings/Reports of the American Forage and Grassland Council, 37th North American Alfalfa Improvement Conference'. Madison, WI, 16–19 July 2000. pp. 345–350. (NAAIC)

NAAIC (2004) 'Standard tests to characterize alfalfa cultivars.' 3rd edn. North American Alfalfa Improvement Conference. Available at: www.naaic.org/stdtests/index.html

Narasimhamoorthy B, Bouton JH, Olsen KM, Sledge MK (2007) Quantitative trait loci and candidate gene mapping of aluminum tolerance in diploid alfalfa. *Theoretical and Applied Genetics* **114**, 901–913. doi:10.1007/s00122-006-0488-7

Pecetti L, Romani M, De Rosa L, Piano E (2008) Selection of grazing-tolerant lucerne cultivars. *Grass and Forage Science* **63**, 360–368. doi:10.1111/j.1365-2494.2008.00640.x

Sledge MK, Bouton JH, Dall'Agnoll M, Parrott WA, Kochert G (2002) Identification and confirmation of aluminum tolerance QTL in diploid *Medicago sativa* subsp *coerulea*. *Crop Science* **42**, 1121–1128. doi:10.2135/cropsci2002.1121

Smith SR Jr, Bouton JH, Singh A, McCaughey WP (2000) Development and evaluation of grazing-tolerant alfalfa cultivars: A review. *Canadian Journal of Plant Science* **80**, 503–512. doi:10.4141/P99-048

Zhang J-Y, Broeckling CD, Blancaflor EB, Sledge MK, Sumner LW, Wang Z-Y (2005) Overexpression of WXP1, a putative *Medicago truncatula* AP2 domain-containing transcription factor gene, increases cuticular wax accumulation and enhances drought tolerance in transgenic alfalfa (*Medicago sativa*). *The Plant Journal* **42**, 689–707. doi:10.1111/j.1365-313X.2005.02405.x

Zhang X, Humphries A, Auricht G (2007) Genetic variability and inheritance of aluminium tolerance as indicated by long root regrowth in lucerne (*Medicago sativa* L.). *Euphytica* **157**, 177–184. doi:10.1007/s10681-007-9409-3