Committee on the Use of Biotechnology Research in Alfalfa Improvement
Deborah A. Samac, Chair

Preamble

The 2002 report was compiled from responses received from inquiries regarding biotechnology research at laboratories around the world. The report is organized geographically. The names and addresses for a contact person at each location are listed at the end of the report. These individuals are identified by an * in the narrative. Although we attempted to contact all labs conducting alfalfa biotechnology research, we regret any omissions that may have occurred. Please inform the committee of any omissions so that the next report will be complete.

United States (compiled by Charlie Brummer)

USDA-ARS, Beltsville Agricultural Research Center

T. Austin Campbell* implemented the following research projects: (1) conducted analyses that were designed to elucidate the phylogenic relationship of *Medicago ruthenica* and *M. edgeworthii* to *M. sativa*, *M. radiata*, *M. monantha*, *M. polyceratia*, *M. medicaginoides*, *M. monspeliaca*, *M. lanigera*, *M. platycarpos*, and *M. popovii* based on sequencing of rDNA regions ITS1, 5.8S, ITS2, Seq XL (external to 18S), and a portion of 26S; (2) studied R-gene motifs in *M. edgeworthii*, *M. ruthenica*, and *M. sativa* and noted several unique R-gene analogs within the People's Republic of China species, which may be segments of new disease resistance genes that could improve disease resistance in cultivated alfalfa; (3) studied genetic diversity in *M. edgeworthii* and *M. ruthenica* germplasm based on chloroplast and mitochrondial DNA; (4) screened simple sequence repeat (SSR) molecular markers from *M. truncatula* for mapping potential in autotetraploid *M. sativa*. **Nichole O'Neill*** is determining genetic diversity within species and strains of three genera of fungi pathogenic to alfalfa: *Stemphylium*, *Phoma*, and *Leptosphaerulina*. By sequence analysis of conserved gene loci and by AFLP DNA fingerprinting, a molecular phylogeny is being developed for these groups that is predictive of biological traits including host specificity, biogeography, and virulence. **Gary Bauchan*** and his colleagues are concluding studies using Giemsa chromosome banding techniques and a computerized image analysis system to study the nine germplasm sources of tetraploid alfalfa. He has initiated a program to develop SSRs from an alfalfa genomic library for use in molecular mapping tetraploid alfalfa.

Cal/West Seeds

Jonathan Reich*, David Johnson*, Lauren Johnson*, Jay Sandman*, Eric Graham has focused on the production of transgenic alfalfa via *Agrobacterium tumefaciens* mediated transformation. Initial research focused on identification of agronomically elite dormant and nondormant alfalfa clones capable of efficient *in vitro* somatic embryogenesis and transformation. Transformation of alfalfa to enhance herbicide tolerance, delayed leaf senescence, and environmental stress tolerance were successful. Marketable improvements in delayed leaf senescence have been achieved. Laboratory, greenhouse, and field evaluations of transgenic clones, progenies and synthetic

populations are underway to study transgene inheritance, concentration, and trait expression.

Forage Genetics International

Biotechnology research is conducted under the direction of **Stephen Temple*** and **Mark McCaslin***. FGI in collaboration with Monsanto is developing Roundup Ready alfalfa. Multi-location field evaluation trials are currently being conducted and commercial release is anticipated to occur when regulatory approval has been granted. Current time line estimates suggest commercial release of Roundup Ready alfalfa will occur in 2004. Additional alfalfa biotechnology research is being carried out in collaboration with **Richard Dixon** and colleagues at the Samuel Roberts Noble Foundation. The goal of this project is to increase alfalfa forage digestibility by reducing lignin content using genetic engineering techniques.

University of Georgia

Joe Bouton* and **Wayne Parrott**, in collaboration with **Daniele Rosellini**, University of Perugia, Italy, successfully transformed alfalfa with the citrate synthetase gene in order to convey aluminum tolerance. Results show that several transgenic plants are demonstating better growth in aluminum toxic soils than checks. In other work with **Mary Sledge**, Noble Foundation, we are using 2X-4X crosses to transfer aluminum tolerant QTLs from the diploid to the tetraploid level.

Iowa State University

E. Charles Brummer*, Diane Luth and colleagues (Ken Moore, Paul Scott, Diane Luth, Heathcliffe Riday, Joseph Robins, Baldomero Alarcon-Zuniga, and Mindy Weishaar) are using a combination of traditional selection, genetic mapping, and genomics to improve biomass yield, autumn growth, and winter survival of alfalfa. They are mapping diploid and tetraploid alfalfa populations with a combination of RFLP, SSR, and AFLP markers and with putative candidate genes associated with winter survival, in collaboration with **Jeff Volenec** at Purdue University. They are mapping QTL for important agronomic traits, as well as the physiological and metabolic components that underlie them. They have also developed winter hardy germplasm from nondormant cultivars that will be used to profile gene expression and better understand the autumn dormancy-winter hardiness connection.

Kansas State University

P.C. St. Amand*, **D.C. Clark**, and **Yanmei Xiao** in the Agronomy Department are currently involved in research on marker assisted selection (MAS) in autopolyploid alfalfa for resistance to anthracnose and finding yield and disease related QTL markers.

New Mexico State University

Ian M. Ray* and colleagues are studying the association between heterosis and AFLP marker diversity in alfalfa. Genes associated with drought tolerance and salt tolerance are being isolated using several genomic techniques with the goal to develop markers for each gene, map them, and confirm their functional role under drought and salt-stress in the field.

The Samuel Roberts Noble Foundation

Rick Dixon* and his laboratory (Xian-Zhi He, Jack Blount, Lahoucine Achnine, Hideyuki Suzuki, Chang-Jun Liu, Bettina Deavours, Srinu Reddy, Fang Chen, Parvathi Kota, Deyu Xie, Shashi Sharma, Gail Shadle, and Lisa Riner) are attempting to understand how plants produce natural products (low molecular weight chemicals that often have important biological activities), and using this understanding to generate improved plants with altered natural product profiles. Three major classes of natural products are currently being studied; flavonoids/isoflavonoids, triterpene saponins, and the cell wall polymer lignin. The target species for these studies is the forage legume alfalfa, but researchers will also make use of the extensive genomics resources available at the Noble Foundation for the closely related model legume *Medicago truncatula*. The approaches are multidisciplinary, ranging from organic chemistry through biochemistry, molecular biology, genetics, genomics and structural biology. Some ongoing projects include: (1) genetic modification of lignin biosynthesis for improved forage digestibility, (2) genetic manipulation of pathways for production of isoflavonoid phytoestrogens, (3) *M. truncatula* EST sequencing, (4) molecular dissection of metabolic channeling in the phenylpropanoid and isoflavonoid pathways, (5) functional genomic approaches to triterpene saponin biosynthesis in *M. truncatula*, (6) genetic manipulation of condensed tannin biosynthesis, (7) integrated gene and metabolite profiling in elicited *M. truncatula* cell suspension cultures, and (8) structural biology of the phenylpropanoid, flavonoid and isoflavonoid pathways. **Rouf Mian*** and his colleagues are developing EST-SSRs markers for alfalfa and annual medics from the *M. truncatula* EST database. He is also working towards identifying the QTL for spotted and blue green alfalfa aphid resistance in alfalfa and annual medics using a combination of molecular markers and genomics techniques. **Mary Sledge***, in collaboration with Ian Ray at New Mexico State University, is mapping the EST-SSRs derived from the *M. truncatula* EST database in a population segregating for drought tolerance, fall dormancy, winter hardiness, and yield. In collaboration with **Joe Bouton** at the University of Georgia, she is using RFLPs to introgress two QTL for aluminum tolerance, identified in a diploid *Medicago sativa* subsp. *coerulea*, into tetraploid alfalfa. A new project is currently underway to identify genes for aluminum tolerance in *M. truncatula*, using a genomics-based approach.

Purdue University

Jeff Volenec* and his laboratory (Suzanne Cunningham, W. Kess Berg, Tommy Sors, Khaldoun Al-Hadid, Brett Winsett, and Synan Abu Qumar) are conducting research to identify and characterize physiological, biochemical, and molecular mechanisms influencing alfalfa growth and stress tolerance. This information is essential in order to identify traits and processes that control agronomic performance of alfalfa. Four research topics are currently being examined in detail: (1) Characterize mechanisms controlling synthesis and degradation of organic reserves (starches, sugars, and storage proteins) in legume roots, and understand the role of organic reserves in shoot growth and stress tolerance. (2) Understand physiological and molecular factors controlling crown bud dormancy and development, and their impact on shoot development and forage yield. (3) Determine physiological and molecular basis of fall dormancy and its association with alfalfa winter hardiness. (4) Characterize how potassium and phosphate nutrition alter

physiological and biochemical processes in roots that ultimately improve alfalfa stress tolerance and growth.

University of Minnesota and USDA-ARS, St. Paul, MN

D. A. Samac* and **H. Jung**, **USDA-ARS**, and **M. Sadowsky**, University of Minnesota, are using *Agrobacterium*-mediated transformation to generate alfalfa plants with increased tolerance to biotic and abiotic stresses and for new crop uses. Work is ongoing to increase alfalfa tolerance to aluminum in acidic soils by expression of a cDNA for a novel nodule-enhanced malate dehydrogenase. Cell-specific promoters are being used to alter the carbohydrate components in cell walls of alfalfa stems to improve forage quality. To produce plants for remediation of atrazine-contaminated soil and water, a bacterial gene for atrazine chlorohydrolase is being introduced into alfalfa and model plant species. For production of high-value products, a transit peptide from white lupin is being used to enhance secretion of proteins from roots of transgenic alfalfa. A large scale effort has been initiated to produce T-DNA insertional mutants of *Medicago truncatula* for forward and reverse genetic approaches to gene discovery in *Medicago* species. Research in **Carroll Vance***'s laboratory, USDA-ARS, is focused on the improvement of nutrient acquisition in alfalfa, particularly nitrogen and phosphorus. Genomic approaches involving *Medicago truncatula* (transcriptome profiling, macro-and microarrays, transformation, in situ hybridization) are used to determine which plant genes are specific for root nodule formation and function. Unique genes are isolated and characterized. Promoters for unique genes are isolated and controlling elements are characterized through promotes::GUS reporter constructs transformed into alfalfa. Genes with potential for improving nitrogen fixation are over expressed in alfalfa and transgenic plants characterized. In related studies genes involved in adaptation to phosphorus stress are identified and characterized from lupin and alfalfa. Genes with potential for improving nutrient phosphorus are over expressed in alfalfa in efforts to improve nutrient acquisition.

University of Wisconsin-Madison

Tom Osborn* and **Ivan Maureira** are developing a novel germplasm to complement alfalfa cultivars in hybrid combination. The nondormant Peruvian and dormant Falcata sources were selected for their genetic distinction from other alfalfa germplasms based on molecular markers, and these were intermated and random mated for several generations. Recurrent selection is being used based on test cross performance to a cultivar tester to improve the population. They are also developing a genetically engineered male-sterile for use in hybrid seed production.

Western Regional Plant Introduction Station

Richard Johnson*, **Ted Kisha*,** and **Stephanie Green*** are evaluating sampling techniques and marker systems (RAPD, AFLP, SSR, chloroplast) to develop the most efficient protocol for distinguishing and characterizing alfalfa germplasm populations. **George Vandemark*** is using quantitative PCR to assess resistance levels in alfalfa plants and populations.

Canada (compiled by Yves Castonguay)

Agri-Food Canada, Saskatoon

M. Gruber* is continuing her work on developing alfalfa with reduced bloat potential, reduced greenhouse gas production and increased protein bypass. The group is attempting to alter the expression of genes associated with secondary metabolism (tannins) in transgenic plants. They have recently isolated the world's 1st set of regulatory genes which stimulate condensed tannin biosynthesis in legume forage and in barley seed coat. They have also introduced the maize Lc regulatory gene into alfalfa and stimulated the production of anthocyanins in forage and condensed tannin enzymes in alfalfa developing seed. The "red alfalfa" requires only one or two tannin structural genes to divert anthocyanins into the tannin pathway. Transgenic alfalfa with several upper flavonoid pathway genes are also being tested for forage quality, insect resistance and disease resistance.

McGill University

Raj Dhindsa* is studying the regulation of cold tolerance at the molecular level. His research has focussed on 1) Isolation and characterization of cold acclimation-specific (cas) genes; 2) Study of the regulation and function of cas genes; and 3) Low-temperature signal transduction in plants.

University of Guelph

Steve Bowley* and his research group are continuing their work on the modification of winterhardiness, growth attributes, and quality using genetic engineering technology combined with field breeding methodologies. The emphasis is on modification of environmental stress tolerance systems and carbohydrate metabolism. The work includes: 1) Production of transgenic plants and field evaluation; 2) Pyramiding transgenes using conventional breeding; and 3) Contractual transformation and evaluation of proprietary genes. In conjunction with Czarnecka-Verner at the University of Florida they are studying the alfalfa heat shock response, characterizing an alfalfa heat shock factor and its relationship to stress tolerance. **Judy Strommer* and Reggie Lo** are testing concepts for the oral delivery of vaccines to ruminants using transgenic forages as the delivery vehicle. They are constructing chimaeric genes which encode antigenic determinants from proteins of Mannheimia haemolytica, a serious respiratory pathogen, and introducing them into alfalfa and clover so as to maximize their immunogenic potential.

University of Manitoba

Robert Hill* and Christos Doras have transformed alfalfa with the sense and antisense barley hemoglobin gene using A. rhizogenesis as a vector. The objective of this study is to determine the effect of nonsymbiotic hemoglobins on the physiology of the whole plant and more specifically on the tolerance to flooding. Plant roots constitutively expressing hemoglobin maintain root growth and ATP levels under hypoxic treatment whereas wild type and antisense lines do not. Plants constitutively expressing hemoglobin have a different phenotype than antisense lines.

Agriculture and Agri-Food Canada, Sainte-Foy, Québec

Yves Castonguay*, Serge Laberge, Paul Nadeau, Annick Bertrand and Réal Michaud have a number of projects on biotechnology and genomic research. Activities include: 1) High throughput analysis of Expressed Sequence Tags (ESTs) isolated from cDNA libraries of cold acclimated alfalfa and functional analysis of their expression on high density grids. More than 10,000 ESTs have thus far been sequenced and their expression levels is currently being studied using macroarray analysis. Identification of genes with potential applications in the improvement of persistence and forage quality is a priority. Canditates genes for cold tolerance have been identified and will be validated in transgenic plants. 2) Search of genetic markers associated to cold tolerance using DNA-based molecular markers to construct linkage maps and to identify Quantitative Trait Loci (QTL) associated with freezing tolerance in alfalfa; 3) Assessment of the adaptative value of molecular changes such as cryoprotective sugar accumulation and its potential use phenotypic selection; 4) Development of an integrated perspective on the effects of environmental parameters and management practices such as soil humidity, ice encasement or fall cutting schedule on molecular and genetic changes associated with the acquisition of freezing tolerance in alfalfa. 5) Development of alfalfa populations with improved freezing tolerance and their use for the analysis of the molecular and genetic bases of superior winterhardiness.

Medicago Inc., Sainte-Foy, Quebec

Medicago Inc, a world leader in plant-made pharmaceuticals, is specializing in the transformation of alfalfa into a living factory to produce high-value active molecules. **LouisVézina* (CSO) and his colleagues Pierre Bilodeau, Marc-André D'Aoust, and Stéphanie Aquin** are involved in several R&D projects. Medicago's effort has focused on the development of proprietary expression cassettes, alfalfa transformation technologies, biomass production capacity, recovery and quality control processes. Medicago is now entering into its second generation R&D phase. Among Medicago's priorities are the in depth knowledge of complex cellular mechanisms
controlling accumulation of heterologous proteins in alfalfa, gene silencing, folding and post-translational processing of heterologous proteins, male sterility and synthesis of secondary metabolites. Increasing resources will be needed to address issues such as automation (high throughput systems), functional genomics, proteomics, bioinformatics, etc.

Europe (compiled by Deborah Samac)

Agrobioinstitute in Kostinbrod , Bulgaria

Dr. Dimitar Djilianov and his laboratory are evaluating ecophysiology and abiotic stress tolerance working with *M. sativa* genotypes. Several lines, tolerant to osmotic stress have been selected *in vitro* after somatic embryogenesis regeneration for PEG tolerance. The lines show higher abiotic stress tolerance then the explant source genotype. Their leaves showed considerable tolerance to severe osmotic stress (40% PEG for up to 72 hours). Endogenous ABA and proline, as well as ion leakage were studied at stress conditions and after recovery. Joint investigations with the selected lines are being performed in several labs in Switzerland, Italy, Belgium and Japan. Seeds, obtained after

self-pollination, showed higher salt tolerance then those of explant source genotype. The reserch of **Dr. M.Vlahova*, A. Iantcheva** and **A. Barbulova** is focused on regeneration, genetic transformation and cellular investigation of direct somatic embryogenesis in diploid and tetraploid *Medicago.* Efficient systems for regeneration in liquid and solid media via direct somatic embryogenesis were developed for several diploid *Medicago* species – *Medicago truncatula, M. murex, M. polymorpha* and *M. littoralis*. For investigation of the process of direct somatic embryogenesis the obtained single cell embryogenic suspension cultures from *M. truncatula* and *M. falcata* were used as an experimental material. For genetic transformation of *M. falcata, gus* gene under promoters (cyc 3, cdc2a) of cell cycle regulating genes and 35S-*gfp* for *M. truncatula* were utilized. The asymmetry of the first cellular division was confirmed. The groups is also involved in engineering of herbicide resistance in economically important Bulgarian alfalfa cultivars (*Medicago sativa* L.)**.** Efficient systems for regeneration via indirect somatic embryogenesis were developed for several economically important Bulgarian alfalfa cultivars. On the basis of the established protocols for regeneration, in order to obtain herbicide resistant lines, two different methods for gene transfer were applied - leaf disk and *Agrobacterium*-vacuum infiltration of seedlings.

Istituto Sperimentale Colture Foraggere, Lodi, Italy

Carla Scotti* is using molecular markers in alfalfa breeding methodology. SSR markers originating from *M. truncatula* and *M. sativa* are being used to study the average degree of heterozygosity and genetic diversity in diallel crosses among alfalfa 2S2Syn3 and S2 Double Hybrid F2 parents which were used to produce Double and Octuple Free-Hybrids, respectively. Molecular data will be put in relation with the performance of free-hybrid progenies. In addition, a research program is being carried out in collaboration with the National Research Council, Perugia, on the detection of molecular markers possibly associated with the creeping-root character, a trait very difficult to deal with by conventional breeding methods, due to its erratic expression. Using AFLP markers, the polymorphism naturally occurring in creeping-root and non-creeping-root genotypes is being examined. Subsequently, the same markers will be used to monitor the polymorphism in plants differing for the expression of the character, and deriving from crosses between pairs of creeping and non-creeping genotypes.

Instituto di Ricerche sul Miglioramento Genetico delle Piante Foraggere of CNR, Perugia, Italy

M. Bellucci, F. Damiani, F. Paolocci, F. Pupilli and **S. Arcioni*** are developing molecular markers and conducting research aimed at improving alfalfa forage quality. The characterization of the mitochondrial genome of three somatic hybrids between alfalfa and *M. coerulea*, *M. falcata* and *M. arborea* has been carried out. Three different outcomes including the loss or gain of RFLP bands and the species-specific amplification of sub-stoichiometric DNA units were noted in the mitochondrial genome of the hybrids. To develop a method of seed certification based on molecular markers, a combination of RFLPs and AFLPs is being used to discriminate the former Italian alfalfa ecotypes using a bulked DNA-based strategy. Numerous ecotype-specific polymorphisms were identified and AFLPs were more efficient then RFLPs. With the long term goal to transfer apomixis in alfalfa via genetic engineering, molecular and genetic bases of this

mode of reproduction in the wild grass *Paspalum simplex*, taken as a model species, have been studied. A strong co-linearity of genes between the apomixis-controlling locus of *Paspalum* and the corresponding homeologous regions of rice and *Arabidopsis*, has been found. With the aim to improve forage alfalfa quality two approaches have been followed: a)increasing sulphur amino acid level and b) the induction of leaf condensed tannin biosynthesis. Maize genes coding for zeins, were transgenically expressed in birdsfoot trefoil and alfalfa. Protein expression was increased by replacing the native leader sequence with the 5'UTR of the TMV leader and by adding the KDEL motif to zein polypeptides. The accumulation of zeins in transgenic plants is possible but is too low for practical purposes that require levels of 4-5% of total leaf proteins. Alfalfa forage with moderate levels of Condensed Tannins (CT) is being pursued in order to prevent protein degradation in silage and allow the consumption of fresh forages. Using *Lotus corniculatus* as a model system, several libraries from cDNA subtraction of CT-enhanced and CT-depleted isogenic lines, have been produced. Identification of genes involved in CT synthesis is being carried out by a) evaluating the expression of selected genes by real time PCR analyses, b) by transferring the isolated sequences in sense and anti-sense orientation into plants. Transformation of alfalfa will be also performed with regulatory and structural genes already isolated from *A. thaliana*.

Department of Plant Biology and Agro-environmental Biotechnology at the University of Perugia, Italy

Fabio Veronesi, Daniele Rosellini*, Emidio Albertini, and **Pierluigi Barone** are focusing on identifying genes for female sterility, developing choroplast transformation, and developing molecular markers. Cytological investigations have shown that a female-specific meiotic block may occur in female sterile mutants. In these plants, callose is deposited early during ovule development, and invades the nucellus cell walls. The megaspore mother cell is formed but never undergoes meiosis. Male fertility is unaffected. Since the genetic regulation of female meiosis in plants is not known, we are searching differentially expressed genes by differential display. Chloroplast transformation is being devleoped in collaboration with Wayne Parrott, University of Georgia, Athens. Two alfalfa-specific vectors based on spectinomycin and kanamycin resistance genes have been constructed for biolistic transformation. Recently a new molecular marker technique based on the *Tms1* LTR retrotransposon (LTR S-SAP) (Porceddu et al., 2002. Mol.Genet.Genomics 267: 107-114) was developed. The LTR S-SAP showed a marker system utility, assay efficiency, and marker index higher than that of AFLP and SAMPL markers. We plan to apply this technique alongside other molecular markers, such as AFLP, SAMPL, SSR, ISSR, in genetic linkage analysis, phylogenetic relationship investigations, and biodiversity studies in *Medicago*.

Asia and Australia

Tropical Plant Protection, CRC, Australia

John Irwin* and **Julie Mackie** are conducting a project on the use of molecular markers to facilitate lucerne improvement by breeding. The aims of the project are to use molecular markers to assess the overall genetic diversity in nine Australian grown lucerne cultivars and to identify new sources of genetic diversity, in particular from highly winter

active material from North Africa, and oases in Saudi Arabia and adjacent countries, for introgression into Australian breeding programs. The project also aims to develop and apply molecular marker technology in lucerne to facilitate delivery of improved cultivars to industry by: (1) developing an autotetraploid genetic map of lucerne using molecular markers and phenotypic markers important to Australian production areas, (2) identifying molecular markers that are linked to genes for resistance to Phytophthora root rot, Colletotrichum crown rot, *Stagonospora* and lucerne aphids (spotted and blue-green), and (3) integrating molecular marker technology into on-going lucerne breeding programs by developing robust markers for use in identifying clones with multiple resistance.

List of Contacts

Dr. Sergio Arcioni
Istituto di Ricerche sul Miglioramento
Genetico delle Piante Foraggere del CNR
Via della Madonna Alta n. 130
06128 Perugia, Italy
Tel: +39 075 5014 853
Fax: +39 075 5014 869
E-mail: S.Arcioni@irmgpf.pg.cnr.it

Gary R. Bauchan
USDA/ARS/PSI/SGIL
Bldg. 006,Room 14, BARC-West
10300 Baltimore Avenue
Beltsville MD 20705 - 2350
Tel: (301) 504-6649
Fax: (301)504-5728
E-mail : bauchang@ba.ars.usda.gov

Joe Bouton
Dept of Crop and Soil Sciences
3111 Plant Sciences Building
University of Georgia
Athens GA 30602-7272
Tel: (706) 369-5808
Fax: (706) 542-0914
E-mail: jbouton@arches.uga.edu

E. Charles Brummer
1204 Agronomy Hall
Iowa State University
Ames IA 50010
Tel: (515) 294-1415
Fax: (515) 294-6505
E-mail: brummer@iastate.edu

Steve Bowley
Department of Plant Agriculture,
Crop Science Building,
University of Guelph,
Guelph (ON)
Canada N1G 2W1
Tel : (519) 824-4120 ext 8704
Fax : (519) 763-8933
Email : sbowley@uoguelph.ca

Yves Castonguay
Agriculture and Agri-Food Canada
Soils and Crops Research and Development Center
2560 Hochelaga Blvd.
Sainte-Foy (QC)
Canada G1V 2J3
Tel : (418) 657-7980 ext 231
Email : castonguayy@agr.gc.ca

T. Austin Campbell
USDA/ARS/PSI/SGIL
Bldg. 002,Room 12, BARC-West
10300 Baltimore Avenue
Beltsville MD 20705 - 2350
Tel: (301) 504-5638
Fax: (301)504-5167
E-mail: campbelA@ba.ars.usda.gov

Rajinder S. Dhindsa
Department of Biology
1205 Docteur Penfield Avenue
Montreal (QC)
Canada H3A 1B1
Tel : (514) 398-6423
Fax : (514) 398-5069
Email : rdhindsa@bio1.lan.mcgill.ca

Rick Dixon
Plant Biology Div.
Samuel Roberts Noble Foundation
P.O. Box 2180
Ardmore OK 73402
Tel: (405) 223-5810
Fax: (405) 221-7380
E-mail: radixon@noble.org

Stephanie Greene
USDA/ARS/WRPIS
W.S.U. Irrigated Ag. Research
& Ext. Center
Prosser WA 99350
Tel: (509) 786-9265
Fax: (509) 786-9370
E-mail: greenes@wsu.edu

Margie Gruber
Room ML 16.2
107 Science Place
Saskatoon (SK)
Canada S7N 0X2
Tel : (306) 956-7263
Fax : (306) 956-7247
Email : gruberm@agr.gc.ca

Robert D. Hill
Department of Plant Science
The University of Manitoba
Winnipeg (MB)
Canada R3T 2N2
Tel : (204) 474-6087
Fax: (204) 474-7528
Email: rob_hill@umanitoba.ca

J.A.G. Irwin
Botany Department
Univ. of Queensland
Brisbane QLD 4072
AUSTRALIA
Tel: 61-7-3652790
FAX: 61-7-3654771
E-mail: J.Irwin@botany.uq.edu.au

David Johnson
Cal/West Seeds
RR1,Box 70 (Country Rd M Co.)
West Salem WI 54669
Tel: (608) 786-1554
Fax: (608) 786-0565
E-mail: d.johnson@calwestseeds.com

Lauren Johnson
Cal/West Seeds
P.O. Box 1428
Woodland CA 95776
Tel: (530) 666-3331
Fax: (530) 666-1464
E-mail: l.johnson@calwestseeds.com

Richard Johnson
USDA-ARS
59 Johnson Hall
Washington State University
Pullman WA 99164
Tel: (509) 335-3771
Fax: (509) 335-6654
E-mail: rcjohnson@wsu.edu

Ted Kisha
USDA-ARS
59 Johnson Hall
Washington State University
Pullman WA 99164
Tel: (509) 335-6898
Fax: (509) 335-6654
E-mail: kisha@wsunix.wsu.edu

Mark McCaslin
Forage Genetics
P.O. Box 415
18591 Mushtown Rd.
Prior Lake MN 55372
Tel: (952) 226-3930
E-mail:mccaslin@foragegenetics.com

Rouf Mian
Plant Biology Div.
Samuel Roberts Noble Foundation
P.O. Box 2180
Ardmore OK 73402
Tel: (580) 224-6841
E-Mail: rmmian@noble.org

Nichole R. O'Neill
USDA/ARS Molecular Plant Pathology Lab.
Bldg. 004, Rm. 116, BARC-West
10300 Baltimore Avenue
Beltsville MD 20705 - 2350
Tel: (301) 504-5331
Fax: (301) 504-5728
Email: OneillN@ba.ars.usda.gov

Tom Osborn
Dept. of Agronomy
University of Wisconsin-Madison
1575 Linden Drive
Madison WI 53706
Tel: (608) 262-2330
Fax: (608) 262-5217
E-mail: tcorborn@facstaff.wisc.edu

Jonathan Reich
Cal/West Seeds
P.O. Box 1428
Woodland CA 95776
Tel: (530) 666-3331
Fax: (530) 666-1464
E-mail: j.reich@calwestseeds.com

Deborah A. Samac
USDA-ARS
1991 Upper Buford Circle
495 Borlaug Hall
St. Paul MN 55108
Tel: (612) 625-1243
Fax: (651) 649-5058
E-mail: dasamac@tc.umn.edu

Carla Scotti
Istituto Sperimentale Colture Foraggere
Viale Piacenza, 29 – 26900 Lodi
ITALY
Tel: +39/371/40471
Fax: +39/371/31853
E-mail: iscfbiol@telware.it, iscfbred@telware.it

Paul C. St. Amand
2004 Throckmorton Hall
KSU Agronomy Dept.
Manhattan,KS 66506 - 5501
Tel: (785) 532-7746
FAX: (785) 532-6094
E-mail: pst@ksu.edu

Stephen Temple
Forage Genetics
N5292 S. Gills Coulee Rd.
West Salem WI 54669
Tel: (608) 786-2121
Fax: (608) 786-2193
E-mail: stemple@foragegenetics.com

Ian M. Ray
Dept. of Agronomy & Horticulture
New Mexico State University
Gerald Thomas Hall
Las Cruces NM 88005
Tel : (505) 646-3819
E-mail: iaray@nmsu.edu

Daniele Rosellini
Sezione Genetica e Miglioramento Genetico
Universita' degli Studi di Perugia
Borgo XX giugno, 74
06121 Perugia, ITALY
Tel: +39 075 5856211
Fax: +39 075 5856224
E-mail:roselli@unipg.it

Jay Sandman
Cal/West Seeds
Rt. 1, Box 70
West Salem WI 54669
Tel: (608) 786-1554
Fax: (608) 786-0565
E-mail: j.sandman@calwestseeds.com

Mary Sledge
Noble Foundation, Inc.
2510 Sam Noble Parkway
Ardmore OK 73401
Tel: (580) 221-7344
Fax: (580) 221-7490
E-mail: mksledge@noble.org

Judy Strommer
Department of Plant Agriculture,
Crop Science Building,
University of Guelph,
Guelph (Ontario)
Canada N1G 2W1
Tel : (519) 824-4120 ext 2759
Fax : (519) 767-0755
Email : jstromme@uoguelph.ca

Carroll Vance
USDA-ARS
1991 Upper Buford Circle
411 Borlaug Hall
St. Paul MN 55108
Tel: (612) 625-5715
Fax: (651) 649-5058
E-mail: vance004@tc.umn.edu

George Vandemark
USDA/ARS
24106 N. Bunn Rd.
Prosser WA 99350
Tel: (509) 786-9218
Fax: (509) 786-9218
E-mail: gvandema@tricity.wsu.edu

LouisVézina
Medicago inc.
1020, route de l'Église, bureau 600
Sainte-Foy (QC)
Canada G1V 3V9
Tel: (418) 658-9393
Fax: (418) 658-6699
Email: lovezina@medicago.com