Development of WISFAL-2 *Medicago sativa* subspecies *falcata* Tetraploid Germplasm

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ABSTRACT

WISFAL-2 *Medicago sativa* subsp. *falcata* germplasm (PI 659349) is a tetraploid developed from diploid *M. falcata* at the Wisconsin Agricultural Experiment Station, Madison WI. WISFAL-2 was placed in the National Plant Germplasm System in 2010 and designated PI 659349. Conversion of diploids to tetraploids permits efficient transfer of *M. falcata* germplasm to alfalfa for breeding purposes. The objective in the development of WISFAL-2 was to add germplasm of two important diploids, PI 231731 and PI 258754, to the tetraploid genetic base of WISFAL-1, PI 560533. The method was sexual polyploidization in 2x-4x crosses. The 2x-4x crossing strategy screens for unreduced eggs and results in tetraploid progeny that were used to produce WISFAL-2. WISFAL-2 contains new germplasm at the tetraploid level for use in alfalfa breeding and genomic research.

Many accessions of *Medicago sativa* subsp. *falcata*, here after *M. falcata***,** are diploids**,** 2n=2x=16, whereas alfalfa, *M. sativa* subsp. *sativa* is a tetraploid, 2n=4x=32. Transfer and utilization of diploid *M. falcata* germplasm is more efficient at the tetraploid level. WISFAL-2 was developed by crossing tetraploidy into diploid *M. falcata* plant introductions PI 231731 and PI 258754. Both plant introductions have potential value in alfalfa breeding. PI 231731 was the source of a parent clone of the cultivar 'Weevlchek' (Farmers Forage Res. Coop. 1971), and a parent clone of the cultivar 'WL 216' (Waterman-Loomis Co. 1972), Barnes, et al. 1977. The respective parent clones, which were independently selected, were from 2x-4x outcrosses with alfalfa of unknown origin during the seed increase of PI 231731. Interestingly, only two gametes of PI 231731 were involved, and both delivered germplasm to alfalfa cultivars. However, only a fraction of the potential of PI 231731 has been exploited.

Medicago falcata PI 258754 is remarkable for its persistence and bacterial wilt resistance (R. P. Murphy, personal communication). The persistence of PI 258754 has been confirmed in perennial nurseries at Madison and Arlington, WI, over the past three decades. Also, PI 231731 and PI 258754 are higher in herbage yield than the diploid *M. falcata* plant introductions that were used in the development of WISFAL-1, Woodfield et al. 2006. Both plant introductions are native to Asia Minor, but both have been grown in perennial nurseries, and advanced five sexual generations in Wisconsin over the past 40 years. Thus, both *M. falcata* lines are relatively naturalized. The objective in the development of WISFAL-2 was to make this germplasm available for research at the tetraploid level.

Materials and Methods

Conversion of diploid germplasm to tetraploid was accomplished by 2x-4x crosses that screen for eggs with the unreduced chromosome number in the 2x parents, Bingham, 1990. Such eggs are rare, circa 1/1000, but there are ten eggs in the pod of every flower, and it is only necessary to pollinate about 500 diploid flowers with pollen from a tetraploid to ensure one or more tetraploid progeny. Hence, the diploid plant introductions were used as seed parents, and crossed by hand with pollen from tetraploid *M. falcata* 'WISFAL-1', Bingham 1993. The selected WISFAL-1 pollen parents had yellow/orange flower color and sickle pods. Sufficient 2x-4x crosses were made to produce six new tetraploid hybrids of PI 231731 X WISFAL-1, and eight tetraploid hybrids of PI 258754 X WISFAL-1. The 14 new tetraploids were intercrossed by hand in isolation to produce Syn. 0 seed of WISFAL-2, which was again intercrossed by hand to produce the Syn.1 generation for this release.

Flower color was assigned using the classification of Barnes, 1972. Fall dormancy was determined according to methods of Teuber, et al. 1998.

Characteristics

Half of the germplasm in WISFAL-2 is from PI 231731 and PI 258754, and half from WISFAL-1. Flower color and pod shape of WISFAL-2 closely resembles the yellow/orange flower color and sickle pod shape of the wild *M.falcata*. The flower color corresponds to the 4.3/4.4 flower color code of Barnes, 1972. In contrast, WISFAL-1 plants have flower colors ranging from light yellow (4.1) to yellow/orange (4.4), and pods that range from a full coil to sickle shaped. Thus, flowers and pods of WISFAL-2 more closely resemble the diploid plant introductions than does WISFAL-1.

WISFAL-2 plants are fall dormant (FDC 1), with all plants very decumbent by late-October in Wisconsin. WISFAL-2 tends to produce a heavy first crop of herbage, and

recovers slowly for a second crop, typical of *M. falcata*. Thus, it is a two-cut type, if used for hay in the North Central Region of the USA. No tendency to spread from the crown has been observed, and the potential use in pastures has not been studied.

Discussion

Development of WISFAL-2 broadens the *M. falcata* germplasm available for breeding and genomic research at the tetraploid level, where it is directly crossable with alfalfa. Importantly, half of the germplasm in WISFAL-2 is from two plant introductions with a proven potential in alfalfa breeding.

WISFAL-2 is useful for backcrossing tetraploidy into other plant introductions of diploid *M. falcata*. WISFAL-2 also should be useful in research that previously used WISFAL-1. This research includes characterization of germplasm sources in alfalfa, Kidwell et al. 1994, Musial et al. 2002, Riday et al. 2003, Segovia-Lerma et al. 2004, and Ariss and Vandemark 2007; breeding theory, Mauriera et al. 2004, 2007, and several aspects of breeding, heterosis, and trait mapping, Brummer et al. 2000, Riday and Brummer 2002a, 2002b, Riday et al. 2002, Riday and Brummer 2004a, 2004b, Mackie et al. 2005, Robins et al. 2007, and Li and Brummer 2009. Several additional studies using WISFAL-1 are cited in Robins and Brummer 2010. Forage yield and fertility of WISFAL-1 and alfalfa cultivars was reported by Holland and Bingham 1993, and Woodfield et al. 2007.

Survival of WISFAL-1 for 10 years in a pasture rotation was reported by Smith 2004. Remarkably, yellow-flowered WISFAL-1 plants, along with outcross plants with variegated flowers, are still in the grass-legume pasture stand in 2014, after 20 years. Seed of yellow-flowered plants has been collected to preserve and study this material.

The yellow flower color of WISFAL-1 was used along with cream and purple in a study of pollinator effects on crossing and genetic shift in alfalfa populations, Steiner et al. 1992. Stuteville 2002, reported the occurrence of rust on WISFAL-1 in a survey of alfalfa germplasm. The ease tetraploid *M. falcata* intercrosses with alfalfa is shown in the study by Steiner et al. 1992. Hence, WISFAL-2 should be increased in isolation to ensure purity.

Seed of WISFAL-2 is available from the author (request via email: ebingham@wisc.edu) until the supply is depleted, and thereafter from the National Plant Germplasm System. Samples of 100 seeds are available for all research and breeding purposes. Plant variety protection has not been requested. We request that appropriate recognition be made when WISFAL-2 is used in research publication or cultivar development.

References begin on next page.

References

Ariss, J.J., and G. J. Vandemark. 2007. Assessment of genetic diversity among nondormant and semidormant alfalfa populations using sequence-related amplified polymorphisms. Crop Sci. 47:2274-2284.

Barnes, D.K. 1972. A system for visually classifying alfalfa flower color. USDA Agric. Handbook no. 424. ARS USDA, Beltsville.

Barnes, D.K., E.T. Bingham, R.P. Murphy, D.J. Hunt, D.F. Beard, W.H. Skrdla, and L.R.Tueber. 1977. Alfalfa germplasm in the United States: Genetic vulnerability, use, improvement and maintenance. USDA Tech. Bull. 1571.

Bingham, E.T. 1990. Backcrossing tetraploidy into diploid *Medicago falcata* L. using 2n eggs. Crop Sci. 30:1353-1354.

Bingham, E.T. 1993. Registration of WISFAL (*Medicago sativa* subsp. *falcata*) germplasm derived from diploids. Crop Science 33:217-218.

Bingham, E.T. 2004. History of Wisconsin *Medicago falcata* (WISFAL). Medicago Genetic Reports, Vol. 4. www.medicago-reports.org

Brummer, E.C., M. Maroof Shah, and D. Luth. 2000. Reexamining the relationship between fall dormancy and winter hardiness in alfalfa. Crop Sci. 40:971-977.

Holland, J.B., and E.T. Bingham. 1994. Genetic improvement for yield and fertility of alfalfa cultivars representing different eras of breeding. Crop Sci. 34: 953-957.

Kidwell, K. K., D.F. Austin, and T.C. Osborn. 1994. RFLP evaluation of nine *Medicago* accessions representing the original germplasm sources for North American alfalfa cultivars. Crop Sci. 34:230-236.

Li, X., and E.C. Brummer. 2009. Inbreeding depression for fertility and biomass in advanced generations of inter- and intraspecific hybrids of tetraploid alfalfa. Crop Sci. 49: 13-19.

Musial, J.M., K.E. Basford, and J.A.G. Irwin. 2002. Analysis of genetic diversity within Australian lucerne cultivars and implications for future genetic improvement. J. Agric. Res. 53: 629-636.

Mackie, J.M., P. Pepper, K.F. Lowe, J.M. Musial, and J.A.G. Irwin. 2005. Potential to increase yield in Lucerne (*Medicago sativa* subsp. *sativa*) through introgression of *M.*

sativa subsp. *falcata* into Australian adapted material. Aust. J. Agric. Res. 56: 1365-1372.

Maureira, I. F. Ortega, H. Compos, and T. Osborn. 2004. Population structure and combining ability of diverse *Medicago sativa* germplasms. Theor. Appl. Genet. 109: 775-782.

Maureira-Butler, I., J. Udall , and T. Osborn. 2007. Analysis of a multi-parent population derived from two diverse alfalfa gerplasms: Testcross evaluations and phenotype DNA. Theor. Appl. Genet. 115: 859-867.

Riday, H. and E.C. Brummer. 2002a. Forage yield heterosis in alfalfa. Crop Sci. 42: 716-723.

Riday, H. and E.C. Brummer. 2002b. Heterosis of agronomic traits in alfalfa. Crop Sci. 42: 1081-1087.

Riday, H. and E.C. Brummer, and K.J. Moore. 2002. Heterosis of forage quality in alfalfa. Crop Sci. 42: 1088-1093.

Riday, H., E.C. Brummer, T.A.Cambell, D. Luth and P.M. Cazcarro. 2003. Comparisons of genetic and morphological distance with heterosis between *Medicago sativa* subsp. s*ativa* and subsp. *falcata.* Euphytica 131: 37-45.

Riday, H., and E.C. Brummer. 2004. Performance of intersubspecific hybrids in sward versus space planted plots. Euphytica 138: Number 2 / July 2004

Riday, H., and E.C. Brummer. 2004. Relationships among biomass yield components within and between subspecies of alfalfa. Medicago Genetic Reports Vol. 4. www.medicago-reports.org

Riday, H., and E.C. Brummer. 2007. Registration of two improved yellow-flowered alfalfa germplasms. Journal of Plant Registrations 1: 131-133.

Robins, J.G., G.R. Bauchan , and E.C. Brummer. 2007. Genetic mapping forage yield, plant height, and regrowth at multiple harvests in tetraploid alfalfa (*Medicago sativa L.). Crop Science 47: 11-18.*

Robins, J.G. and E.C. Brummer. 2010. QTL underlying self-fertiltiy in tetraploid alfalfa. Crop Sci. 50: 143-149.

Segovia-Lerma, A., L.W.Murray, M.S. Townsend, and I.M. Ray. 2004. Population-based diallel analyses among nine historically recognized alfalfa germplasms. Theor. Appl. Genet. 109: 1568-1575.

Smith, L. 2004. History of Wisconsin *Medicago falcata* (WISFAL) at Larry Smith Farms. Medicago Genetic Reports Vol.4 www.medicago-reports.org

Steiner, J.J., P.R. Beuselinck, R.N. Peaden, W.P. Kojis, and E.T. Bingham. 1992. Pollinator effects on crossing and genetic shift in a three-flower-color alfalfa population. Crop Sci. 32: 73-77.

Stuteville, D.L. 2002. First report of Uromyces on white sweetclover in Kansas. Plant Disease 86: 1404.

Teuber,L.R., K.L. Taggard, L.K. Gibbs, M.H. McCaslin, M.A. Peterson, and D.K. Barnes. 1998. Fall dormancy. In: Standard Tests To Characterize Alfalfa Cultivars. www.naaic.org

Woodfield, D.R., J.B. Holland, W.P. Kojis, and E.T. Bingham. 2006. Growth analysis of *Medicago sativa* subsp*. sativa* and *M. sativa* subsp*. falcata* at two ploidy levels. Medicago Genetic Reports. Vol.6. www.medicago-reports.org