Confirmation of species identification of *Medicago* NPGS germplasm using DNA barcoding and ploidy level estimation using genome size data
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Kelly P. Steele[1], Brianna Boone[1], and Martin F. Wojciechowski[2]

[1]*College of Integrative Sciences and Arts, Arizona State University Polytechnic, Mesa, AZ;* [2]*School of Life Sciences, Arizona State University, Tempe, AZ, USA; kpsteele@asu.edu*

Background:

The National Plant Germplasm System of the United States Department of Agriculture (NPGS/USDA) maintains seeds (germplasm) of nearly one million accessions of agricultural crops and their wild relatives to safeguard plant genetic diversity for the future. The value of these germplasm accessions is dependent on their accurate identification as there are consequences to incorrectly identified accessions; for example, agricultural scientists can attempt to use misidentified germplasm to cross with crop species without success. Plans for future collections depend on an accurate assessment of current accessions. **In summary, accurate taxonomic identification of germplasm is crucial to its value and management.**

Objectives:

Confirm identification of USDA accessions of species of *Medicago* section *Medicago* that comprise secondary and tertiary wild relatives of alfalfa (*Medicago sativa* L.) using DNA barcoding and ploidy level determination.

Potential results:

All samples from an accession may be from the designated species, an accession may represent a species with two ploidy levels collected from a mixed population, or the accession may in fact be different species.

Materials and Methods –

Overview: DNA sequence data from accessions can be used as a DNA barcode to compare with sequence data from individuals of known identification to confirm identity of germplasm accessions. Ploidy level can be used to aid in species identification when morphologically similar species differ in ploidy level. Ploidy level can be estimated with genome size data obtained by flow cytometry of nuclei from fresh material. Both types of data may be combined to confirm species identification.

Taxa sampled: Genome size data were obtained for currently available accessions of secondary and tertiary wild relatives of *Medicago sativa* (see Tables 1 and 2) (https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearchcwr.aspx).

Molecular markers: Sequence data of the nuclear encoded ribosomal RNA gene internal transcribed spacer (*nrDNA ITS*) region and the plastid encoded *trn*K/*mat*K and *ycf*1 genes for the above taxa are in the process of being obtained.

Genome size estimation: 50 mg of fresh plant tissue were co-macerated with 50 mg of tissue of size standards in Galbraith's buffer; propidium iodide was used as a fluorochrome. Primary standards included *Glycine max* cv. *Polanka* (estimated genome size of 2.5 picograms, pg) and *Raphanus sativus* (estimated genome size of 1.11 pg) (*Glycine* and *Raphanus* seeds were obtained from J. Dolezel and J. Bartos). *Medicago truncatula* Jemalong with an estimated genome size of 1.15 pg (Blondon et al. 1994) was used as a standard for a few samples. Standard methods of flow cytometry were performed on a BD FACSCalibur flow cytometer. With few exceptions, each accession was sampled three or more times on different days, and multiple plants grown from each accession were sampled.

Table 1 – Ploidy level estimation using genome size data

species	# of accessions	standard	mean/ SD	ploidy level consistent with chromosome #
arborea	six	radish/jemalong	3.17 / 0.04	4n
cretacea	three	radish/jemalong	2.24 / 0.02	2n
daghestanica	one	radish	1.98 / 0.04	2n
hybrida	one	radish	3.48 / 0.01	2n
marina	three	radish/jemalong	1.82 / 0.03	2n
pironae	four	radish	1.93 / 0.01	2n

Medicago sativa   **Medicago arborea**

The genus *Medicago*

The genus *Medicago* is comprised of about 88 species and includes the globally significant agriculturally important forage species, alfalfa (*M. sativa*) as well as other forage species, for example, *M. truncatula* (sect. Spirocarpae) in Australia. A few species of *Medicago* are horticultural species including the shrubby species *M. arborea* in sect. Medicago with *M. sativa.*

Table 2 – Ploidy level estimation using genome size data: apparent variation within a species

species	# of accessions	standard	mean/ SD	ploidy level
cancellata	four	Glycine	4.76 / 0.19	6n
cancellata	four	Glycine	3.32 / 0.09	4n
papillosa	four	Glycine	3.36 / 0.03	4n
papillosa	one	Glycine	1.30 / 0.00	2n
papillosa	one	Glycine	4.13 /0.06	?
prostrata	eight	radish	1.55 / 0.04	2n
prostrata	one	radish	3.07 / 1 value	4n

Results – Ploidy level:

For four species, *M. arborea, M. daghestanica, M. hybrida,* and *M. pironae*, all accessions had very similar estimated genome sizes that are consistent with published chromosome number information (Small, 2010) (Table 1). For three species there is evidence of 1) mixed populations *(M. cancellata)*, and 2) likely misidentified accessions (*M. cancellata, M. papillosa* and *M. prostrata*) (Table 2). The two different ploidy levels of *M. cancellata* were confirmed as 2n=48 and 2n=32 with chromosome counts obtained for two of the accessions (Steele and Mandakova, unpublished data).

On-going work:

Hypothesized mixed populations and misidentifications will be further tested with additional ploidy level estimation data and phylogenetic analyses of DNA barcoding data.

Literature cited:
1. Blondon, F, D Marie, S Brown and A Kondorosi. 1994. Genome size and base composition in *Medicago sativa* and *M. truncatula* species. *Genome* 37: 264-270.
2. Small, E. 2011. Alfalfa and relatives: evolution and classification of *Medicago.* NRC Research Press. Ottawa, Canada.

ASU ARIZONA STATE UNIVERSITY

College of Integrative Sciences and Arts and School of Life Sciences