Evaluation Of Stratified Sampling Strategies to Access Maximal Genetic Diversity from Crop Plant Genebank Collections.

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Purpose:

Potato (*Solanum tuberosum*) is the fourth most important food crop in the world and is the single most important dicot food crop (http://apps.fao.org/). More than 60 diseases of current economic importance affect potato (Stevenson et al. 2001).

Wild germplasm is a frequently accessed source of genes for crop improvement. Late blight, caused by the oomycete *Phytophthora infestans,* is among the most devastating potato diseases. The wild diploid species *S. bulbocastanum*, a native of central and southern Mexico and Guatemala, is a noted source of potent late blight resistance genes (Helgeson et al. 1998; Naess et al. 2000, 2001) (Fig1). In field trials in Minnesota in 2002 and 2003, we also identified *S. bulbocastanum* as a source for resistance to *Verticillium* wilt (unpublished; Fig 1). The USDA Potato Genebank maintains 60 accessions of *S. bulbocastanum* including three morphologically defined subspecies: ssp. *bulbocastanum,* ssp. *partitum,* and ssp. *dolichophilum*

One challenge facing researchers utilizing wild germplasm for crop improvement is efficiently screening genotypes for a desired trait while maximizing overall genetic diversity. In support of our ongoing efforts to characterize and isolate disease resistance genes for potato protection, we seek to develop a sampling strategy for screening genebank accessions that maximizes genetic diversity while minimizing resource input. Simply put, we ask the question: "Given limited research dollars, am I better off screening a few genotypes from a lot of accessions (populations), or should I screen a lot of genotypes from a few accessions?"

Figure 1:

Fig. 1a: *S. bulbocastanum* can be readily distinguished from other potato species by its simple leaves and star-shaped flowers.

Fig. 1b: *S. bulbocastanum* growing in *Verticillium* –infested soil at UM's North Central Research and Outreach Center, Grand Rapids, MN

Fig 1c: Resistance genes derived from *S. bulbocastanum* impart late blight resistance to cultivated potato; compare with susceptible cultivars (Hancock, WI; courtesy J. Helgeson)

Fig. 1d: 'Katahdin' is a late blight susceptible potato cultivar. Unchallenged Katahdin (right); Katahdin 6 dpi with *P. infestans* (center); Katahdin + *S. bulbocastanum* resistance gene *RB* 6 dpi (left). (courtesy J. Helgeson)

Materials and Methods:

▪Two Amplified Fragment Length Polymorphism (AFLP) data sets were generated. Each data set was representative of the three morphologically-defined subspecies, the entire geographic distribution, and included multiple (2-5) genotypes for each population:

- ▪a primary set included 42 *S. bulbocastanum* populations examined with one AFLP primer pair; 31 polymorphic bands were scored (Fig 2; Table 1).
- ▪a secondary set consisted of 17 *S. bulbocastanum* populations examined using three AFLP primer pairs; 89 polymorphic bands were scored.

▪Jaccard coefficients of similarity calculated from AFLP data were used to generate dendrograms via UPGMA in NTSys Version 2.1 (Fig 3). Conclusions concerning correlations between genetic distance and geographic distribution, subspecies classification, or inter- vs. intra-population diversity were drawn from dendrogram topology and cophenetic comparisons.

Figure 2:

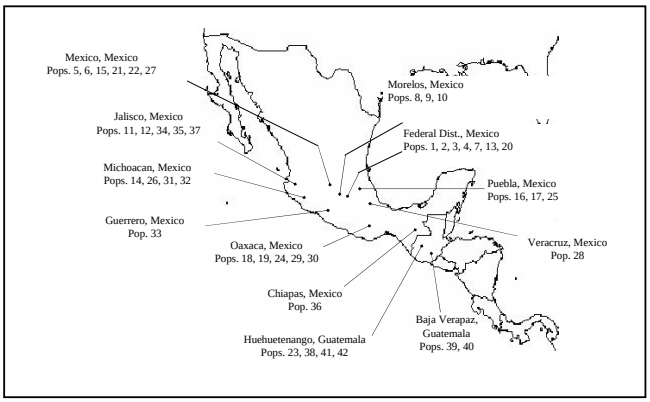

Fig 2. The disease resistant wild potato, *S. bulbocastanum*, is distributed throughout central and southern Mexico and Guatemala. Geographic origin of populations examined in this study are indicated; population numbers are as specified in the adjacent table.

Table 1:

Population	PI Number	Genotypes	Genebank PI	Species/Subspecies
1	031-0001	A,B,C,D,E	243345	*S. bulbocastanum ssp. bulbocastanum*
2	031-0002	A,B,C,D,E	243504	*S. bulbocastanum ssp. bulbocastanum*
3	031-0004	A,B,C,D,E	243506	*S. bulbocastanum ssp. bulbocastanum*
4	031-0005	A,B,C,D,E	243507	*S. bulbocastanum ssp. bulbocastanum*
5	031-0006	A,B,C,D,E	243508	*S. bulbocastanum ssp. bulbocastanum*
6	031-0007	A,B,C,D,E	243509	*S. bulbocastanum ssp. bulbocastanum*
7	031-0008	A,B,C,D,E	243510	*S. bulbocastanum ssp. bulbocastanum*
8	031-0010	A,B,D	243512	*S. bulbocastanum ssp. bulbocastanum*
9	031-0011	A,B,C,D,E	243513	*S. bulbocastanum ssp. bulbocastanum*
10	031-0012	A,B,C,D,E	253210	*S. bulbocastanum ssp. dolichophyllum*
11	031-0013	A,B,C,D,E	255516	*S. bulbocastanum ssp. dolichophyllum*
12	031-0014	A,B,C,D,E	255518	*S. bulbocastanum ssp. dolichophyllum*
13	031-0015	A,B,C,D,E	275185	*S. bulbocastanum ssp. bulbocastanum*
14	031-0017	A,B,C,D,E	275187	*S. bulbocastanum ssp. bulbocastanum*
15	031-0018	A,B,C,D,E	275188	*S. bulbocastanum ssp. bulbocastanum*
16	031-0020	A,B,C,D,E	275190	*S. bulbocastanum ssp. bulbocastanum*
17	031-0021	A,B,C,D,E	275191	*S. bulbocastanum ssp. bulbocastanum*
18	031-0024	A,B,C,E	275194	*S. bulbocastanum ssp. bulbocastanum*
19	031-0025	A,B,D,E	275195	*S. bulbocastanum ssp. bulbocastanum*
20	031-0027	A,B,C,D,E	275197	*S. bulbocastanum ssp. bulbocastanum*
21	031-0028	A,B,C,D,E	275198	*S. bulbocastanum ssp. bulbocastanum*
22	031-0029	B,C,D,E	275199	*S. bulbocastanum ssp. bulbocastanum*
23	031-0030	A,B,C,D,E	275200	*S. bulbocastanum ssp. partitum*
24	031-0031	B,C,D,E	283096	*S. bulbocastanum ssp. bulbocastanum*
25	031-0032	B,C,D,E	310960	*S. bulbocastanum ssp. bulbocastanum*
26	031-0033	A,B,C,D,E	347757	*S. bulbocastanum ssp. bulbocastanum*
27	031-0034	A,B,C,D,E	347758	*S. bulbocastanum ssp. bulbocastanum*
28	031-0035	A,B,C,D,E	365379	*S. bulbocastanum ssp. bulbocastanum*
29	031-0036	A,C,D	498011	*S. bulbocastanum ssp. bulbocastanum*
30	031-0037	A,C,D,E	498223	*S. bulbocastanum ssp. bulbocastanum*
31	031-0038	A,B,C,D,E,	498224	*S. bulbocastanum ssp. dolichophyllum*
32	031-0039	A,B,C,E	498225	*S. bulbocastanum ssp. bulbocastanum*
33	031-0040	A,B,C,D	545711	*S. bulbocastanum ssp. bulbocastanum*
34	031-0041	A,B,C,D,E	545751	*S. bulbocastanum ssp. dolichophyllum*
35	031-0042	A,B,C,D	545752	*S. bulbocastanum ssp. dolichophyllum*
36	031-0044	A,B,C,D,E	558379	*S. bulbocastanum ssp. partitum*
37	031-0045	B,D,E	590930	*S. bulbocastanum*
38	031-0046	A,B,C	604051	*S. bulbocastanum*
39	031-0047	A,B,C,D,E	604065	*S. bulbocastanum*
40	031-0048	A,B,C,D,E	604066	*S. bulbocastanum*
41	031-0049	A,B,C,D,E	604073	*S. bulbocastanum*
42	031-0050	A,B,C,D,E	604074	*S. bulbocastanum*

Results:

1. AFLP data do not support subspecies classification: subspecies do not cluster independently.
   - We prepared 38 herbarium specimens from Minnesota field grown *S. bulbocastanum* accessions. Specimens were deposited in the UM Herbarium. We determined subspecies classification for these specimens using the criteria of Hawkes (1990). Classifications are based on the presence or absence of trichomes on flower parts and the width to length ratio of fully expanded leaves. These are simple traits, which may be conditioned by few genes and may be environmentally malleable.
   - In agreement with conclusions reached via our molecular data, 19 of 38 of our herbarium classifications disagreed with USDA Potato Genebank classifications based on herbarium specimens collected in a natural environment (i.e. Mexico or Guatemala). Consistently, Dr. David Spooner (USDA ARS, University of Wisconsin), a leading expert on potato taxonomy, no longer recognizes as valid *S. bulbocastanum* subspecies classifications (personal communication).

2. Geographic distance between populations was poorly correlated with genetic distance.
   - Matrices of geographic distance between populations and genetic similarity between genotypes were compared, resulting in a correlation of -0.08325.
   - These same comparisons were executed for the secondary data set and the results are listed in Table 2.

3. Substantial diversity exists at both the Inter-population and Intra-population level.
   - Individual genotypes did not cluster into specific population (PI) groups (Fig 3). This topology suggests that there exists considerable diversity within populations, as well as between populations.

Table 2:

	Correlation	Geographic Origin	EAAC/MCAC	EAAG/MCAA	EAAG/MCAT
EAAC/MCAC	r-value	-0.29794	0		
EAAG/MCAA	r-value	-0.17034	0.0879	0	
EAAG/MCAT	r-value	-0.31275	0.38026	0.08851	0

Conclusions:

We found that previously described subspecies classifications were not supported by our data and geographic distance between populations did not correlate well with observed genetic distance. We also identified substantial genetic diversity at both the inter- and intra-population levels. Hence, a stratified sampling methodology based on subspecies classification, geographic origin, or inter- vs. intra-population variation may be of limited use to researchers attempting to characterize genebank holdings of *S. bulbocastanum* for desirable traits while maximizing genetic diversity.

Like most *Solanum* species, *S. bulbocastanum* is an obligate outcrossing species with severe inbreeding depression. We expect our finding may extend to other obligate outcrossing species, including both potato and non-potato species, but may be of limited applicability to self-pollinating species.

References:

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Helgeson JP, Pohlman JD, Austin S, Haberlach GT, Wielgus SM, Ronis D, Zambolim L, Tooley P, McGrath JM, James RV (1998) Somatic hybrids between *Solanum bulbocastanum* and potato: a new source of resistance to late blight. Theor Appl Genet 96:738-742

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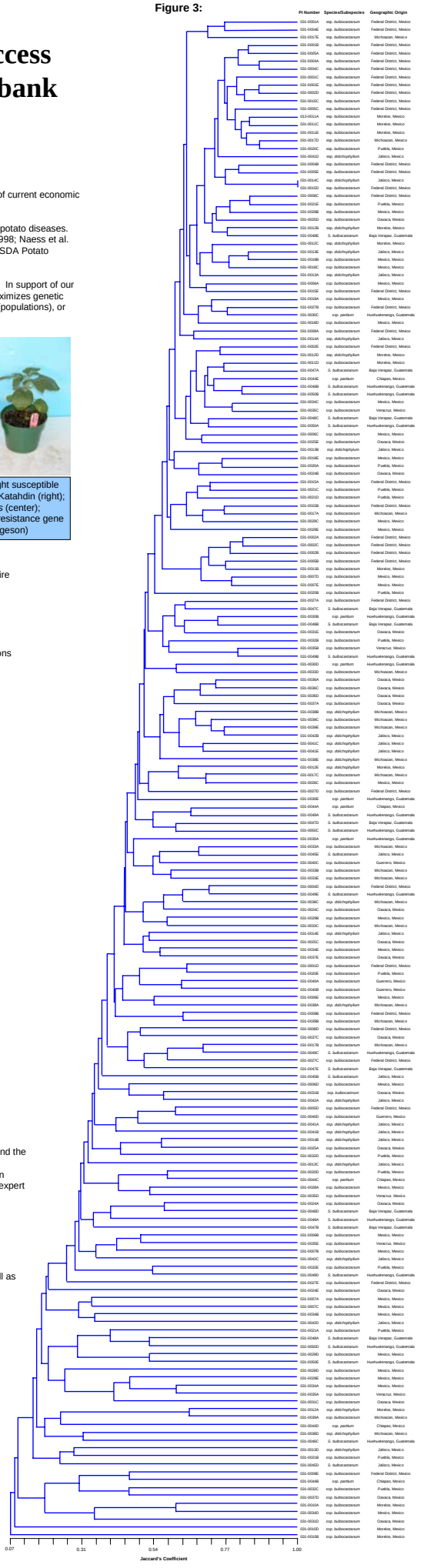
Figure 3:



Dendrogram based on 31 polymorphic AFLP fragments and 192 genotypes from 42 populations.