

Towards Strategic Genotype Selection for Allelic Mining: Genome Diversity Assessment in the Wild Potato, *Solanum bulbocastanum*

James M. Bradeen, Ryan L. Syverson, & Maria J. Sanchez

Department of Plant Pathology, University of Minnesota, 495 Borlaug Hall/1991 Upper Buford Circle, St. Paul, MN 55108 USA

Premise:

The wild potato *Solanum bulbocastanum* is a source of resistance to potato late blight (TAG 96:738-742; PNAS 100:9128-9133), nematodes (TAG 92:572-576), *Verticillium* wilt (unpublished), and aphids (D. Ragsdale, *personal communication*). This primitive species is distributed throughout central and southern Mexico and Guatemala (Fig 1) and is characterized by star-shaped flowers and simple leaves (Fig 2). We are characterizing allelic diversity at the *RB* late blight resistance locus using Long Range PCR (details at poster P165). Here we explore factors that may serve as predictors of genome and, ultimately, resistance allele diversity. We compare genetic distance measured via AFLPs with subspecies classification and geographic origin. We further compare within vs. between population diversity.



Fig 1. 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 Table 1.



Fig 2. *S. bulbocastanum* is a primitive species with distinctly star-shaped flowers (left) and simple leaves (right). The species is a source of potent disease resistance. The plant shown on the right is growing in *Verticillium* infested soil in Grand Rapids, MN.

Procedure:

41 *S. bulbocastanum* populations were obtained from the USDA Potato Genebank (Table 1). DNA was extracted from 2-5 single genotypes (individual plants) from each population. 31 polymorphic AFLPs from the primer combination E-AAC + M-CAC were scored for 151 genotypes; AFLP data were analyzed via UPGMA of the Jaccard's similarity coefficient using NTSYS-pc software (Fig 3). 27 populations were grown in a field at Becker, MN and herbarium specimens were prepared and deposited in the University of Minnesota Herbarium (Table 1). Subspecies classifications were determined numerically according to Hawkes (Smithsonian Press, 1990). Genetic similarity (Jaccard's) was correlated with geographic distance between populations via cophenetic correlation (normalized Mantel statistic Z) using NTSYS-pc.

Pop	Genotypes	USDA Potato Genebank #	USDA Classification	Our Classification	UM Herbarium #	Geographic Origin	Latitude	Longitude
1	a,b,c	24352	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	--		Moroles, Mexico	18,950 N	-99,680 W
2	a,b,c,d,e	24353	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	--		Moroles, Mexico	18,950 N	-99,680 W
3	a,b,c,d,e	25210	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	499871	Moroles, Mexico	18,950 N	-99,680 W
4	a,b,c	25518	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	499827	Jalisco, Mexico	20,983 N	-103,167 W
5	a,b,c,d	25518	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	499888	Jalisco, Mexico	20,983 N	-103,167 W
6	a,b,c	275185	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	499353	Federal District, Mexico	19,280 N	-98,540 W
7	a,b,c	275187	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	499825	Michoacan, Mexico	19,400 N	-100,350 W
8	a,b,c,d	275188	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>undetermined</i>	499834	Mexico, Mexico	19,500 N	-100,120 W
9	a,b,c,d	24345	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	492662	Federal District, Mexico	19,280 N	-98,540 W
10	a,b,c,d	275190	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	492592	Puebla, Mexico	19,040 N	-98,210 W
11	a,b,c	275191	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>undetermined</i>	492592	Puebla, Mexico	19,040 N	-98,210 W
12	a,b	275194	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>undetermined</i>	499832	Oaxaca, Mexico	17,033 N	-96,767 W
13	a,b,c,d	275195	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	499833	Oaxaca, Mexico	17,033 N	-96,767 W
14	a,b,c,d	275197	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	499833	Federal District, Mexico	19,280 N	-98,540 W
15	a,b,c,d,e	275198	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	499857	Mexico, Mexico	19,500 N	-100,120 W
16	a,b	275199	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	--		Mexico, Mexico	19,500 N	-100,120 W
17	a,b,c,d,e	243504	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	--		Federal District, Mexico	19,280 N	-98,540 W
18	a,b,c,d	275200	<i>S. bulbocastanum</i> ssp. <i>paritium</i>	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	499856	Huettenango, Guatemala	15,767 N	-91,565 W
19	a,b,c,d	282996	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	499829	Oaxaca, Mexico	17,033 N	-96,767 W
20	a,b,c,d	310960	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	499860	Puebla, Mexico	19,040 N	-98,210 W
21	a,b,c,d	347757	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	499829	Michoacan, Mexico	19,400 N	-100,350 W
22	a,b,c,d	347758	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	499829	Michoacan, Mexico	19,400 N	-100,350 W
23	a,b,c,d	365379	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	499830	Veracruz, Mexico	18,430 N	-96,180 W
24	a,b,c	498223	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	499828	Oaxaca, Mexico	17,033 N	-96,767 W
25	a,b,c	498224	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	492476	Michoacan, Mexico	19,400 N	-100,350 W
26	a,b	498225	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	492500	Michoacan, Mexico	19,417 N	-100,333 W
27	a,b,c,d	545711	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	--		Guerrero, Mexico	17,550 N	-99,500 W
28	a,b,c	545751	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	499842, 499867, 499869	Jalisco, Mexico	20,983 N	-103,167 W
29	a,b	545752	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	499863	Jalisco, Mexico	20,983 N	-103,167 W
30	a,b,c	558379	<i>S. bulbocastanum</i> ssp. <i>paritium</i>	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	497760	Chiapas, Mexico	16,187 N	-92,200 W
31	a,b	590303	<i>S. bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	492425	Jalisco, Mexico	20,017 N	-102,983 W
32	a,b	604051	<i>S. bulbocastanum</i>	--		Huettenango, Guatemala	15,767 N	-91,565 W
33	a,b,c,d	604065	<i>S. bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>paritium</i>	499840, 499862	Baja Verapaz, Guatemala	15,172 N	-90,295 W
34	a,b,c,d	604066	<i>S. bulbocastanum</i>	--		Baja Verapaz, Guatemala	15,172 N	-90,295 W
35	a,b,c,d	604073	<i>S. bulbocastanum</i>	--		Huettenango, Guatemala	15,313 N	-91,518 W
36	a,b,c,d	243506	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	499824	Federal District, Mexico	19,280 N	-98,540 W
37	a,b	604074	<i>S. bulbocastanum</i>	--		Huettenango, Guatemala	15,322 N	-91,548 W
38	a,b	243507	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	--		Federal District, Mexico	19,280 N	-98,540 W
39	a,b,c,d	243508	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>undetermined</i>	499866	Mexico, Mexico	19,500 N	-100,120 W
40	a,b,c,d	243509	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>dolichophyllum</i>	499811	Mexico, Mexico	19,500 N	-100,120 W
41	a,b,c,d	243510	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	<i>S. bulbocastanum</i> ssp. <i>bulbocastanum</i>	499826	Federal District, Mexico	19,280 N	-98,540 W

Results & Conclusions:

- Little genetic structure was detected and individuals from a common population were no more likely to cluster together than individuals from different populations. Maximal within population similarity was observed for population 11 (0.93); 12 inter-population similarities met or exceeded this value, including 5c/6b (1.00), 7c/10c (0.97), and 9d/17d (0.96).
- Genotypes do not associate based on subspecies classifications. Classifications are based on simply inherited or environmentally malleable morphologic traits (presence of trichomes, length vs. width of leaf) and may be artificial (D. Spooner, *personal communication*). Consistently, of 22 populations independently classified by the USDA (based primarily on materials collected in the native environment) and by our group (based on materials grown in Becker, MN), classification discrepancies were noted for 8 populations (36.4%).
- Geographic distance between two populations is poorly correlated ($r = -0.08128$) with genetic distance (Jaccard's similarity).

This study indicates that subspecies classifications and geographic origins do not predict genetic distance and that 2 individuals from a common population might be more genetically distant than 2 individuals from distinct populations. Currently we are completing additional AFLP analyses of a 62 genotype core collection. We are also pursuing allelic mining at the late blight resistance locus, *RB*, using long range PCR (see poster P165). We will examine each factor characterized here as a potential predictive factor for resistance allele diversity.

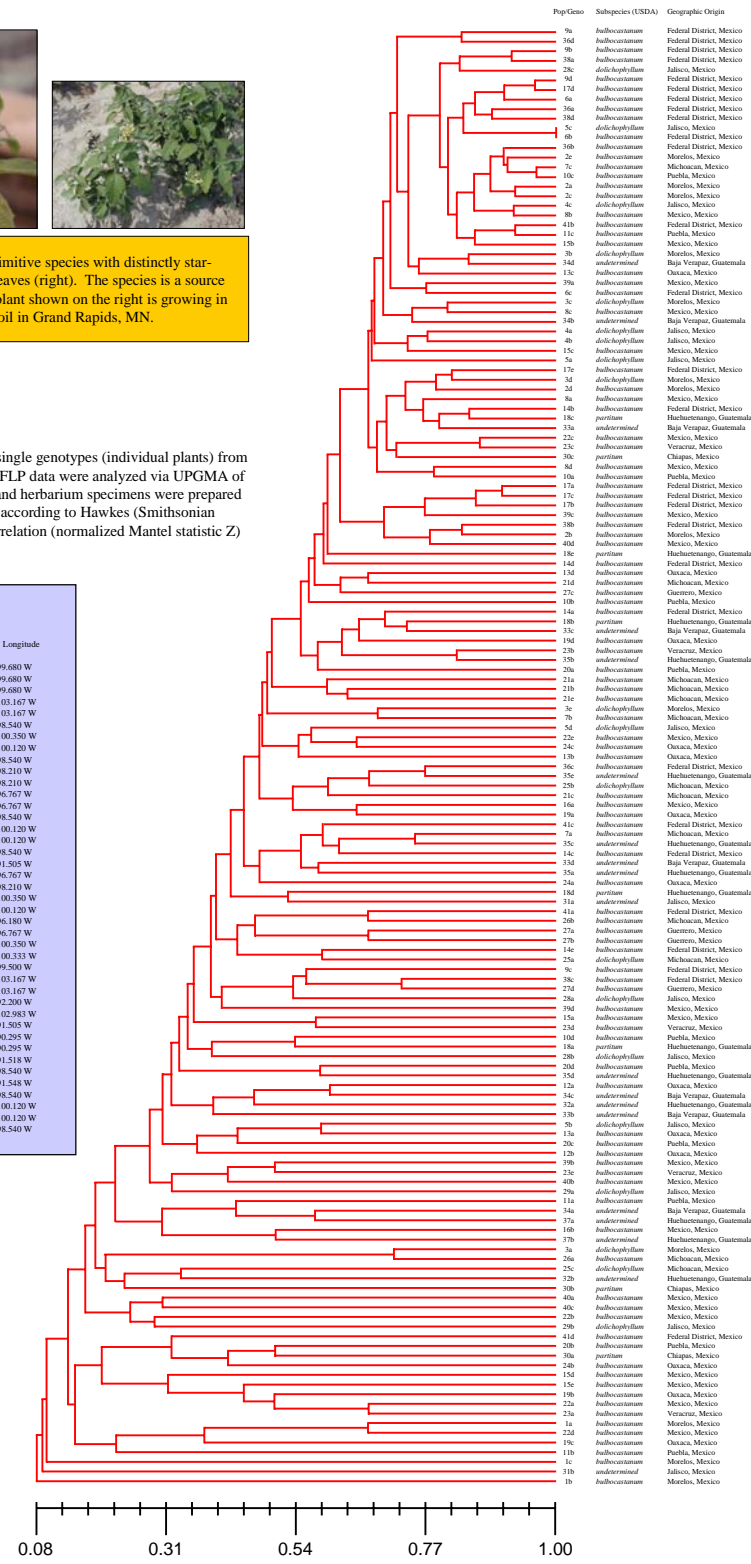


Fig 3. UPGMA dendrogram of 151 *S. bulbocastanum* genotypes generated from Jaccard's similarity coefficients calculated from 31 polymorphic AFLP fragments. Population and Genotype are listed in Table 1. Genotypes fail to cluster based on subspecies classifications and geographic origins. Individuals from a common population do not associate independent of individuals from different populations. Scale shows Jaccard's similarity coefficients.