Theor Appl Genet (1997) 95: 199–204

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Assessing changes in the genetic diversity of potato gene banks. 2. In situ vs ex situ

Received: 28 October 1996 / Accepted: 7 March 1997

Abstract An important question in the conservation of potato germ plasm is whether germ plasm in the gene bank, although stable, still represents the in situ populations from which it was collected, sometimes many decades ago. The answer would direct objective decisions regarding the value of re-collections and in situ preservation. The present study was undertaken as a project of the Association of Potato Inter-gene-bank Collaborators (APIC). It measured genetic differentiation between potato germ plasm maintained in the US gene bank for many years and current in situ populations re-collected from the same original sites in the wild. *Solanum jamesii* and *Solanum fendleri* from the United States were used as representatives of potato germ plasm. Re-collections were carried out in 1992 at the same locations at which gene bank-conserved accessions had been collected in 1958 and 1978. RAPD markers revealed significant genetic differences between gene bank-conserved and re-collected in situ populations for all seven comparisons of *S. jamesii* (diploid outcrosser), and 12 of 16 comparisons within *S. fendleri* (tetraploid inbreeder). The average genetic similarities were 65.2% for *S. jamesii* and 80.4% for *S. fendleri*. Possible explanations and consequences of these unexpectedly large differences are discussed.

Key words RAPD markers · Genetic diversity · Potato gene banks · *Solanum jamesii* · *S. fendleri* · APIC · Wild potato populations · Ex situ conservation

Communicated by B. S. Gill

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Introduction

Exotic potato species are a valuable source of traits of interest to breeders (Jansky et al. 1990). There are more than 200 recognized wild and cultivated tuber-bearing *Solanum* species, growing primarily in the mountain chains from the southwestern United States through Mexico and Central America to central Chile and across northern Argentina to the Atlantic coast of South America (Correll 1962; Hawkes 1990).

Potato gene banks are essential for conserving, evaluating and using this genetic diversity for breeding; (Gebhart et al. 1989; Peloquin et al. 1989; Hanneman 1989; Spooner and Sytsma 1992; Bonierbale et al. 1993). A major concern defined by APIC, the Association of Potato Intergenebank Collaborators, is the lack of empirical scientific information on potential factors affecting methods for preserving gene bank diversity (Bamberg et al. 1995). In del Rio et al. (1997), comparisons of populations (generations) within accessions increased at the NRSP-6 gene bank were considered. Differences in such populations were small and none were statistically significant. However, some of the accessions analyzed were collections from exactly the same geographical sites but collected at different times (i.e., re-collections). These were significantly different, raising the question of whether germ plasms in the gene bank, although stable, still represent the current in situ populations from which they were collected, sometimes several decades ago. The answer has a major impact on gene bank decisions regarding the value of re-collections, the handling of existing samples, and in situ preservation.

The PCR-based technique of random amplified polymorphic DNA (RAPD) has been shown to be

useful for the analysis of germ plasm organization (Nienhuis et al. 1994) and the resolution of intraspecific differences (Hu and Quiros 1991; del Rio et al. 1997), and was used in the present study.

Materials and methods

Plant material

Twenty two populations of *Solanum jamesii* Torrey (Table 1), a diploid (2n = 2x = 24), and thirty eight of *Solanum fendleri* A. Gray (Table 2), a tetraploid (2n = 4x = 48), both originating in the southwestern United States (Correll 1962), were analyzed. Although most *Solanum* species occur in Latin America, use of these North-American species facilitated rapid access to the places of collection and the immediate availability of collected material because quarantine was not required. About 90% of all potato species are either outcrossing diploids, like *S. jamesii*, or inbreeding polyploids, like *S. fendleri*, so these two species were considered appropriate models to represent potato germ plasm.

Comparisons were made between accessions from exactly the same wild collection site. Each site was represented by up to three accession samples: HAW (J. Hawkes) collections made in 1958, UGR (D. Ugent and R. Ruhde) made in 1978, and SBV (A. Salas, J. Bamberg and S. Vega) made in 1992 (see Bamberg and Martin 1993). Populations from the HAW and UGR accessions included the first true-seed produced from originally collected tubers and subsequent generations made by seed increase. Data from multiple generations of the same accession were pooled for comparisons to other accessions. SBV collections were represented by clonally propagated samples of plants taken directly from the wild.

Data generation and analysis

Up to 24 plants from each population were grown for DNA sampling in a screen-house during the summer (June–August) of 1993 at NRSP-6. Procedures of DNA extraction, RAPD methods and data analysis were as described in del Rio et al. (1997). Pooled DNA samples from each population were employed. A genetic similarity matrix was produced and an agglomerative cluster analysis (UPGMA) based on similarity coefficients was made for each particular comparison. These were developed using the NTSYS-pc program (Rohlf 1989). Chi-square tests were employed to measure the significance of the genetic similarities.

Plants from two *S. jamesii* accessions were also analyzed individually to assess gene-diversity parameters. These estimates of within- and between-population gene diversity, as well as Wright's measure of population differentiation (Fst), quantified changes in population gene frequencies. They were obtained according to the procedure described by Lynch and Milligan (1994).

Table 1 Identities and characteristics of each gene bank-conserved and corresponding re-collected *S. jamesii* accession

Original collection site near	PI number	Sample lot tested[a]	Source of tested sample[b]
Silver City, New Mexico	275169	1959	Wild (HAW 1176)
	275169	1966	1959
	275169	1971	1966
	275169	1985	1966
	275169	1986	1971
	458423	1978	Wild (UGR 7-78)
	458423	1980	Wild (UGR 7-78)
	458423	1981	1980
	564048	SBV 17 (orig)	Wild
Nelson Reservoir, Arizona	458426	1978	Wild (UGR 17-78)
	458426	1980	Wild (UGR 18-78)
	458426	1981	1980
	564051	SBV 24 (orig)	Wild
Eagar, Arizona	458425	1978	Wild (UGR 16-78)
	458425	1980	Wild (UGR 16-78)
	458425	1981	1980
	564054	SBV 28 (orig)	Wild
Quemado, New Mexico	458427	1980	Wild (UGR 19-78)
	458427	1981	1980
	564055	SBV 29 (orig)	Wild
Socorro, New Mexico	458428	1978	Wild (UGR 20-78)
	564056	SBV 31 (orig)	Wild

[a] Seed families identified by their year of generation from the indicated source, except for SBV collections, which were clonal replicates of plants or tubers taken directly from the wild

[b] UGR and HAW collections listed were clonal replicates of materials taken directly from the wild, used for seed increase and discarded. Other sources are seed families identified by their year of generation

Table 2 Identities and characteristic of each gene bank-conserved and corresponding recollected *S. fendleri* accession

Original collection site near	Pl number	Sample lot tested[a]	Source of tested sample[b]
Tucson, Arizona	458421	1980	Wild (UGR 15-78)
	458421	1981	1980
	564024	SBV 1 (orig)	Wild
Sierra Vista, Arizona	275165	1959	Wild (HAW 1209)
	275166	1959	Wild (HAW 1210)
	275166	1975	1959
	564025	SBV 3 (orig)	Wild
Wilcox, Arizona	275162	1959	Wild (HAW 1177)
	275162	1971	1959
	458420	1978	Wild (UGR 14-78)
	564027	SBV 5 (orig)	Wild
	275163	1959	Wild (HAW 1180)
	275163	1973	1959
	275163	1975	1973
	458419	1978	Wild (UGR 13-78)
	458419	1981	1978
	564028	SBV 6 (orig)	Wild
Cloudcroft, New Mexico	458409	UGR 1-78 (orig)	Wild (UGR 1-78)
	458409	1980	Wild (UGR 1-78)
	564030	SBV 8 (orig)	Wild
	275158	1959	Wild (HAW 1158)
	275158	1985	1959
	275158	1986	1959
	564031	SBV 9 (orig)	Wild
	275156	1959	Wild (HAW 1156)
	275156	1981	1959
	564032	SBV 10 (orig)	Wild
	275157	1959	Wild (HAW 1157)
	275157	1981	1959
	564033	SBV 11 (orig)	Wild
	458411	1978	Wild (UGR 3-78)
	458411	1980	Wild (UGR 3-78)
	564034	SBV 12 (orig)	Wild
Reserve, New Mexico	458417	1978	Wild (UGR 11-78)
	458417	1981	1978
	564041	SBV 20 (orig)	Wild
Alpine, Arizona	458418	1981	1978
	564042	SBV 23 (orig)	Wild

[a] Seed families identified by their year of generation from the indicated source. SBV collections were clonal replicates of plants or tubers taken directly from the wild. UGR 1-78 (orig) were original seeds (collected from the wild)

[b] UGR and HAW collections listed were clonal replicates of materials taken directly from the wild, used for seed increase and discarded, except for wild (UGR 1-78) which was collected as seeds. Other sources are seed families identified by their year of generation

Results

RAPD markers produced

The 22 accessions of *S. jamesii* were analyzed using 51 random primers which gave at least one clear and reproducible polymorphic band. These primers each produced an average of 2.7 polymorphic and 12.3 monomorphic bands. Primer OP-AT 07 generated the most polymorphic bands (six), while ten others produced only one polymorphic band. Another 23 primers generated only monomorphic bands in all the *S. jamesii* populations and were not used in the analysis. Of the 596 bands generated using the 51 primers, a total of 137 (23%) were polymorphic in *S. jamesii*.

When 107 random primers were used on *S. fendleri*, 76 generated a total of 151 polymorphic bands with 31 primers producing only monomorphic bands. The average number of polymorphic and monomorphic

bands produced per primer was 2.0 and 9.5, respectively. Thus, 20.9% of bands were polymorphic in *S. fendleri*. Primers OP-D 20 and OP-AA 14 were found to be the most polymorphic in *S. fendleri* with five bands each. Twenty others generated only one band.

Within populations of *S. jamesii*, 50 segregating RAPD bands were produced and analyzed for estimates of within- and between-population gene diversity.

Analysis of *S. jamesii*

In all cases accessions re-collected in different years at the same site were highly significantly different. Similarity ranged from 50.5% to 73.7%, with an average of 65.2% (Table 3 a). As noted in del Rio et al. (1997), the mean similarity was 61.6% for accessions from different sites, and 96.3% for generations within accessions.

Analysis of *S. fendleri*

For this species, 12 of 16 re-collections were significantly different (Table 3 b). Average similarity of the seven comparisons of HAW (1958): SBV (1992) was 77.8%. Average similarity of the seven comparisons of UGR (1978): SBV (1992) was 81.8%. Thus, samples re-collected in 1978 were closer to 1992 re-collections than samples originally collected in 1958. The average similarity of the two comparisons of HAW (1958): UGR (1978) accessions was 85.0%. As noted in del Rio et al. (1997), the mean similarity for accessions from different sites was 64.7%. and 95.9% for generations within accessions.

The nature of genetic differentiation

The analysis of *S. jamesii* individuals provided information on the nature of change between SBV (1992)

Table 3 Chi-square analysis of genetic similarity (GS) of gene bank-conserved and re-collected populations

Comparison[a]	GS	1-1 Matches		0-0 Matches		χ^2	*P* value[b]
		Obs	Exp	Obs	Exp		
a) *Solanum jamesii*							
275169 (0.991) vs 564048	0.505	27	59	42	77	33.829	0.000**
458423 (0.956) vs 564048	0.538	27	61	47	76	30.213	0.000**
275169 vs 458423	0.654	37	60	52	77	16.517	0.000**
458425 (0.896) vs 564054	0.680	49	70	40	61	13.400	0.000**
458426 (0.932) vs 564051	0.737	54	78	45	58	9.648	0.002**
458427 (0.971) vs 564055	0.726	68	83	32	54	11.858	0.001**
458428 vs 564056	0.723	55	72	44	65	10.799	0.001**
Mean[c] (0.963)	0.652						
b) *Solanum fendleri*							
458421 (0.980) vs 564024	0.843	63	77	63	73	3.790	0.052 ns
275165 vs 564025	0.755	60	74	54	77	9.519	0.002**
275166 (0.947) vs 564025	0.729	58	72	54	79	10.977	0.001**
458420 vs 564027	0.755	58	84	56	67	9.854	0.002**
275162 (0.974) vs 564027	0.695	52	88	53	63	16.315	0.000**
275162 vs. 458420	0.821	72	88	52	63	4.830	0.028*
275163 (0.969) vs 564028	0.782	49	70	69	81	8.001	0.005**
458419 (0.967) vs 564028	0.792	51	72	69	80	7.555	0.006**
275163 vs. 458419	0.880	61	70	72	81	2.186	0.139 ns
458409 (0.960) vs 564030	0.841	63	69	64	82	4.473	0.034*
275158 (0.987) vs 564031	0.806	58	69	63	83	6.178	0.013*
275156 (0.940) vs 564032	0.838	65	75	62	77	4.153	0.042*
275157 (0.993) vs 564033	0.838	58	73	69	79	4.174	0.041*
458411 (0.974) vs 564034	0.858	66	77	64	74	3.061	0.080 ns
458417 (0.921) vs 564041	0.782	51	71	67	81	7.658	0.006**
458418 vs 564042	0.854	59	72	70	79	3.373	0.066 ns
Mean[c] (0.959)	0.804						

[a] Accession codes represent germ plasm from a particular original collection site at a particular time. Numbers starting with "275" collected by Hawkes in 1958, "458" by Ugent and Ruhde in 1978, "564" by Salas, Bamberg and Vega in 1992. Data from multiple generations of the same accession were pooled for comparisons to other accessions

[b] * $\geqslant$ critical χ^2 value = 3.84 for $P = 0.05$, ** $\geqslant$ critical χ^2 value = 6.63 for $P = 0.01$ for 1 *df*; ns = not significant at $P \leqslant 0.05$

[c] Values given in parentheses are the weighted mean intra-accession population similarities (for comparison). Mean similarity among accessions from different sites for *S. jamesii* = 0.617, for *S. fendleri* = 0.647. From the same sites, *S. jamesii* 1958 vs 1978 = 0.654, 1978 vs 1992 = 0.680, 1958 vs 1992 = 0.505; *S. fendleri* 1958 vs 1978 = 0.850, 1978 vs 1992 = 0.818, 1958 vs 1992 = 0.778

Table 4 Estimates of within- and between-population gene diversity (GD) and Wright's measure of population differentiation (Fst) of gene bank-conserved and re-collected populations for two *S. jamesii* accessions

Population	GD within populations	GD between populations	Fst
458426.1978	0.314	0.011	
458426.1981	0.306	0.011	
564051.1992	0.140	0.185	
458426 vs 564051			0.3335**
458427.1980	0.293	0.019	
458427.1981	0.308	0.019	
564055.1992	0.109	0.194	
458427 vs 564055			0.3638**

** = significant at $P \leqslant 0.01$ level

re-collections and those collected previously from the same sites. Two such comparisons from the 1992 and 1978 collections (564051 vs 458426; 564055 vs 458427) were made. SBV collections from 1992 were found to have less within-population gene diversity (less heterogeneity) than their corresponding 1978 collections (Table 4).

Estimated gene diversity between populations collected at different times was much greater than that between generations within accessions. Gene diversity of 1992:1978 collections averaged 18.9%, but only 1.5% for generations within accessions (see Table 4). Thus, Wright's estimates of genetic differentiation (Fst) revealed that gene frequencies of accessions re-collected in 1992 have significantly diverged from those collected in 1978 and subsequently maintained at the gene bank.

Comparing SBV 1992 re-collections as a group with all previous materials in the gene bank from corresponding sites (HAW 1958 and UGR 1978 collections), re-collections of *S. jamesii* gained 11 new bands and lost 16, with an overlap of 110 bands (80%). For *S. fendleri*, re-collections gained three new bands and lost nine, with an overlap of 139 bands (92%).

Discussion

Observed differences between genebank and re-collected samples were surprisingly large. For *S. jamesii*, differences among collections were almost as great as differences among sites. Although all differences among *S. jamesii* accessions were highly significant at $P \leqslant 0.01$, only some of those among *S. fendleri* were significant at the same level. Four such comparisons revealed no significant differences. The different breeding systems of these species might explain why re-collections of *S. jamesii* appear to have diverged more than those of *S. fendleri*. Diploidy and self-incompatibility in *S. jamesii* presumably generate high levels of heterozygosity and, therefore, greater vulnerability to loss of diversity (Loveless and Hamrick 1984). The species cannot be forced to self (J. Backlund, personal communication), and does not set spontaneous fruit in the absence of pollinators (authors' greenhouse observations). Under this breeding system, effects of genetic drift, gene flow, and effective population size are more pronounced. Self-incompatible plants in small populations may also suffer from lack of a mate or pollinator. Byers and Meagher (1992) pointed out that small populations do not maintain a large diversity of *S* alleles. Thus, rare compatible individuals could rapidly change population structure in the wild if sexual reproduction become favored. Similarly, forced sexual reproduction in the gene bank might ampilify alleles of rare compatible individuals, magnifying sampling differences by collectors. In contrast, a self-compatible disomic tetraploid like *S. fendleri* is expected to produce more homozygous and homogeneous populations (Loveless and Hamrick 1984), making it less subject to change. It can be assumed that selfing predominates in nature, since all but two of these *S. fendleri* populations readily produce berries with many seeds in the absence of pollinators (authors' greenhouse observations).

Wright (1965) predicted that every finite population will experience genetic drift, but that the effects of such drift become more pronounced as population size decreases. Populations with continually small effective population size will be especially susceptible to the loss and the re-organization of variation by genetic drift (Frankel and Soulé 1981; Barrett and Kohn 1991). Any population that undergoes occasional fluctuations to a small size may also lose genetic variation by chance (Ellstrand and Elam 1993). Such fluctuations comprise population-bottleneck and founder/colonization events (Barrett and Kohn 1991). The species used in this study were commonly found as small localized colonies of less than 100 individuals. This is also generally true of other potato species in the wild (J. G. Hawkes, C. M. Ochoa, D. M. Spooner, personal communication).

No empirical data is available on the activity of potato pollinators, other agents that might move seeds or tubers, or habitat changes over time which have influenced the effective population size and number. Gene flow homogenizes population structure and counteracts the effects of drift and diversifying selection. Nevertheless, gene flow can also be detrimental to small populations because, under certain conditions, it can reduce local variation, prevent local adaptive differentiation, and reduce fitness. Thus, populations can undergo genetic differentiation from one generation to another (Allendorf 1983; Slatkin 1987).

The data in Table 3 suggest that the SBV re-collections of 1992 are, on average, slightly more diverged from the HAW collections of 1958 (34 years apart) than

the UGR collections of 1978 (14 years apart). This trend would be expected if the genetic differences noted in accessions are due to evolutionary processes (Slatkin 1987).

Apparent differentiation may be explained, however, even if populations in nature are generally stable. There may have been differences in the sampling protocols of the three expeditions, either due to different methods of the collectors or because different particular individuals of the population were favored as having more showy or collectable propagules in different years. The possibility of different collecting methods cannot be tested because, in most cases, original collected genotypes were not preserved. The possibility that natural populations consist of different genotypes in different years is being investigated by comparing SBV 1992 collections to re-collections within a period assumed to be too short for significant genetic divergence to occur (1–3 years).

Re-collections may appear to be different if accessions go through a domesticating bottleneck when they enter gene banks, after which they are stable. In one comparison of originally collected and genebank increased populations (del Rio et al. 1997), *S. fendleri* 458409. ORIG and 458409. 1980 had a 96% similarity, suggesting that no great loss of diversity had occurred. Comparisons are planned between SBV 1992 collections and their seed increases to further examine this possibility.

Findings in the analysis of *S. jamesii* individuals also indicate that plants from the 1992 SBV re-collections are more homogeneous (Table 4), with less gene diversity compared to their previous collections from the same sites. Thus, the population structure of germ plasm distributed from the gene bank does not necessarily represent that found in the source population in the wild. This is also being investigated by comparing SBV 1992 original genotypes with their seed-increase progeny and will be reported in future publications. Such comparisons may provide valuable insights into the relative prevalence of seed and tuber reproduction in the wild.

Comparing SBV 1992 re-collections as a group with all previous materials in the gene bank from corresponding sites (HAW 1958 and UGR 1978 collections), re-collecting both gained and lost bands. Thus, in situ preservation may be important for the back-up of diversity already present in gene banks and for the preservation of new diversity which can be accessed in future re-collections.

Acknowledgements The authors thank the University of Wisconsin Peninsular Agricultural Research Station program and staff as well as USDA/OICD for financial support. We also thank Dr. S. Peloquin, Ms. J. M. Cale, Dr. W. Lamboy, Dr. P. Bretting, Dr. J. Bacher, Mr. Peter Hoff and Dr. J. Nienhuis for their scientific and technical assistance.

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