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# Assessing changes in the genetic diversity of potato gene banks. 1. Effects of seed increase

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**Abstract** Effects of gene bank seed-increases on the genetic integrity of potato germ plasm is a major concern of gene bank managers. Thus the Association of Potato Inter-gene-bank Collaborators (APIC), a consortium of world potato gene bank leaders, initiated this joint research project using RAPD markers to determine genetic relationships between increased generations within accessions. *Solanum jamesii* ($2n = 2x = 24$) and *S. fendleri* ($2n = 4x = 48$), two wild potato species native to North America, were used as plant material. These species represented two major breeding systems found among *Solanum* species: outcrossing diploids and inbreeding disomic tetraploids, respectively. Comparisons were made between populations one generation apart and between sister populations generated from a common source. Fourteen such comparisons within *S. jamesii* accessions had an average similarity of 96.3%, and 21 such comparisons within *S. fendleri* accessions had an average similarity of 96.0%. No pairs of populations were significantly different, despite the fact that RAPD markers easily separated all of these very similar accessions within their respective species. Only one of six *S. jamesii* accessions analyzed showed a significant change in gene frequencies among generations. These findings indicate that there has been minimal loss or change of genetic diversity in *ex situ* germplasm using the gene bank techniques standard at NRSP-6 and other world potato gene banks.

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## Introduction

The cultivated potato, *Solanum tuberosum* L., is considered the most important tuber crop in the world and among the four most valuable crops world-wide. Germ plasm with adaptation to different climatic and cultural conditions has been essential to the development of improved varieties (Ross 1986).

Gene banks are recognized as one of the most important tools in the preservation, study, and utilization of valuable traits found in wild relatives, exotic species, and landraces of economic crops. The utility and value of potato gene banks has been documented in many areas of plant science such as breeding (Ross 1986; Hanneman 1989), conservation of genetic resources (Hawkes 1990; Huaman 1991), physiology (Smillie et al. 1983; Vega and Bamberg 1995), genetics (Gebhart et al. 1989; Peloquin et al. 1989; Bonierbale et al. 1993), and phylogeny and evolution (Hosaka and Spooner 1992; Spooner and Sytsma 1992). Therefore, effective and efficient management of the genetic resources in potato gene banks is of great importance to potato science and breeding.

The Association of Potato Inter-gene-bank Collaborators (APIC) was established to address problems of potato gene bank management from a global perspective (Bamberg et al. 1995). One of the primary APIC concerns has been to determine what has happened to the genetic diversity of germ plasm populations since they were originally deposited in gene banks. In nearly all cases, original germ plasm from populations growing in their natural habitats has been sampled as a small quantity of seeds or tubers. These are propagated by seed increase to facilitate evaluation and distribution to potato scientists. However, there has

been no empirical evidence demonstrating the effects of gene-bank seed-increase methods on the genetic integrity of collections. The potential of uncontrolled factors such as unintentional selection, differential fertility, and genetic drift for reducing genetic diversity of ex situ germ plasm has been pointed out (Widrlechner et al. 1989; Cross and Wallace 1994). Although it is plausible that these factors, and others, may have caused genetic attrition, it has been difficult to predict or quantify such losses because of the few appropriate genetic markers available. Now, however, empirical quantitative comparison of genetic diversity between serially increased populations can be conducted with sufficient precision to provide practical assessment of, and guidelines for, conservation methods in gene banks.

The development of DNA-based diagnostic markers has provided powerful tools for such measurements. Particularly, the PCR-based technique known as randomly amplified polymorphic DNA (RAPD) (Williams et al. 1990) is being used to tag genes of interest (Martin et al. 1991; Michelmore et al. 1991), to identify somatic hybrids (i.e., confirm parentage and recombination) (Helgeson et al. 1993), to characterize patterns of diversity in germ plasm organization (Newbury and Ford-Lloyd 1993; Nienhuis et al. 1994), and to quantify the genetic diversity in populations (Lynch and Milligan 1994; Link et al. 1995; Virk et al. 1995). The present study used RAPD markers to examine potential changes in the genetic diversity of model potato populations over several generations.

## Materials and methods

### Plant materials and coding

Fifty seven populations of two North-American wild potato species maintained at the Inter-Regional Potato Introduction Station (NRSP-6) at Sturgeon Bay were examined. Nineteen of these were derived from six accessions of *S. jamesii* ($2n = 2x = 24$); 38 were derived from 19 accessions of *S. fendleri* ($2n = 4x = 48$). In this context, the term *accession* signifies germ plasm collected from a specific wild location at a certain time and all of the populations (i.e. generations) derived from it. Twenty four plants of each population were grown in a green-house during the summer (June–August) of 1993 at NRSP-6.

Materials were sampled from populations generated by the gene bank for routine preservation and distribution. The protocol was to generate 20 plants per accession from original tubers or seeds. These were grown in the greenhouse and hand-intermated by bulking pollen from one flower from each plant, then pollinating all open flowers. This procedure was done for both self-compatible and self-incompatible species. Berries were harvested in bulk. After the first successful seed increase, original tuber genotypes were usually discarded, and original seed lots were often depleted. Thus subsequent seed-increases usually used a previous seed increase as a source of parents. Similar methods are used in other potato gene banks.

Accessions are designated by a PI number. Numbers beginning with "275" were collected by J. G. Hawkes in 1958. Those beginning with "458" were collected by D. Ugent and R. Ruhde in 1978 (see Bamberg and Martin 1993). Populations within accessions are differentiated by an extension to the accession number. Original samples from the wild, or their clonal derivatives, have the extension "ORIG". Populations which resulted from seed increase at the gene bank are designated by using the year of increase as the extension (see Table 1).

### DNA isolation

DNA was isolated from fresh leaves according to a procedure modified from that described by Williams and Ronald (1994) in which potassium ethyl xanthogenate (PEX) served to liberate DNA. Extracted DNA was dissolved and stored in TE buffer at $-20°C$ and quantified in a fluorometer (Hoefer Instruments Model TKO-100). Pooled DNA samples were produced from 15 to 20 individuals of each accession. The rationale for using pooled DNA was based on the procedure described by Virk et al. (1995), but DNA was diluted to the same concentration (10 ng/μL) and pooled to produce the final sample instead of pooling tissue. Hence, each individual within an accession was assured of equal representation in the pooled sample.

### RAPD markers and PCR amplification

Primers that represent random 10-nucleotide sequences obtained from Operon Technologies (Alameda, Calif.) were used in the RAPD assay. Amplification reactions (15-μl final volume) contained 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 0.1 mM EDTA, 5 mM DTT, 50% glycerol, 1.0% Triton X-100 as reaction buffer, 25 mM $MgCl_2$, 200 μM each of dATP, dCTP, dGTP and dTTP, 1 unit of *Taq* DNA Polymerase (Promega, Madison, Wis.), 25 ng of genomic DNA template and 0.2 μM of a random primer. PCR amplification was performed in a Perkin-Elmer Cetus DNA Thermal Cycler 9600. Amplification products were fractionated by electrophoresis in 1.5% agarose gels and visualized by ethidium bromide staining.

### Data analysis

#### *Analysis of pooled DNA samples*

Amplification products (bands) observed on the gel were scored as present (1) or absent (0). Pairwise comparisons of pooled populations, based on unique or shared polymorphic products, were used to generate genetic similarity coefficients. A similarity matrix was calculated based on the simple matching coefficient (Sneath and Sokal 1973):

$$S_{ij} = (a + d)/(a + b + c + d),$$

where $S_{ij}$ = the similarity coefficient; a = number of 1–1 matches, b = number of 1–0 matches, c = number of 0–1 matches and d = number of 0–0 matches. The similarity matrix, a cluster analysis, and graphic representations providing an agglomerate method of clustering populations (UPGMA) were developed using the NTSYS-pc program (Rohlf 1989). A chi-square analysis of genetic similarities was employed. The null hypothesis was an identical band status in paired populations representing the seed increase generations.

#### *Analysis of individuals within populations*

Changes in gene frequencies are also evidence of inadvertent selection or genetic bottlenecks imposed by ex situ seed increase. Therefore, gene diversity was assessed by the analysis of individuals within 18 populations of six *S. jamesii* accessions. Estimates of within- and

**Table 1** Identities and origins of *S. jamesii* and *S. fendleri* population used in this study

| Species[a] | PI number | Sample lot tested[b] | Source of tested sample |
|---|---|---|---|
| jam | 275169 | 1959 | HAW 1176 |
| jam | 275169 | 1966 | 1959 |
| jam | 275169 | 1971 | 1966 |
| jam | 275169 | 1985 | 1966 |
| jam | 275169 | 1986 | 1971 |
| jam | 458423 | 1978 | UGR 7-78 |
| jam | 458423 | 1980 | UGR 7-78 |
| jam | 458423 | 1981 | 1980 |
| jam | 458424 | 1978 | UGR 10-78 |
| jam | 458424 | 1980 | UGR 10-78 |
| jam | 458424 | 1981 | 1980 |
| jam | 458425 | 1978 | UGR 16-78 |
| jam | 458425 | 1980 | UGR 16-78 |
| jam | 458425 | 1981 | 1980 |
| jam | 458426 | 1978 | UGR 17-78 |
| jam | 458426 | 1980 | UGR 17-78 |
| jam | 458426 | 1981 | 1980 |
| jam | 458427 | 1980 | UGR 19-78 |
| jam | 458427 | 1981 | 1980 |
| fen | 275156 | 1959 | HAW 1156 |
| fen | 275156 | 1981 | 1959 |
| fen | 275157 | 1959 | HAW 1157 |
| fen | 275157 | 1981 | 1959 |
| fen | 275158 | 1959 | HAW 1158 |
| fen | 275158 | 1985 | 1959 |
| fen | 275158 | 1986 | 1959 |
| fen | 275161 | 1959 | HAW 1174 |
| fen | 275161 | 1985 | 1959 |
| fen | 275161 | 1986 | 1959 |
| fen | 458415 | 1980 | UGR 8-78 |
| fen | 458415 | 1981 | 1980 |
| fen | 275162 | 1959 | HAW 1177 |
| fen | 275162 | 1971 | 1959 |
| fen | 458420 | 1978 | UGR 14-78 |
| fen | 275163 | 1959 | HAW 1180 |
| fen | 275163 | 1973 | 1959 |
| fen | 275163 | 1975 | 1959 |
| fen | 458419 | 1978 | UGR 13-78 |
| fen | 458419 | 1981 | 1978 |
| fen | 275164 | 1959 | HAW 1204 |
| fen | 275164 | 1980 | 1959 |
| fen | 275164 | 1983 | 1959 |
| fen | 275166 | 1959 | HAW 1210 |
| fen | 275166 | 1975 | 1959 |
| fen | 458409 | UGR 1-78 | Wild |
| fen | 458409 | 1980 | UGR 1-78 |
| fen | 458411 | 1978 | UGR 3-78 |
| fen | 458411 | 1980 | UGR 3-78 |
| fen | 458413 | 1978 | UGR 5-78 |
| fen | 458413 | 1980 | UGR 5-78 |
| fen | 458417 | 1978 | UGR 11-78 |
| fen | 458417 | 1981 | 1978 |
| fen | 458421 | 1980 | UGR 15-78 |
| fen | 458421 | 1981 | 1980 |
| fen | 458422 | 1978 | UGR 18-78 |
| fen | 458422 | 1980 | UGR 18-78 |
| fen | 458422 | 1981 | 1978 |

[a] jam = *S. jamesii*, fen = *S. fendleri*

[b] Collector numbers represent material taken directly from the wild or clonal propagules thereof. These were tubers in all cases except UGR 1-78 which was botanical seeds. Original tuber clones were not maintained in the gene bank. *PI numbers* (*accessions*) *grouped by rows were collected from the same site*

between-population gene diversity by using RAPD markers were calculated using the analysis described by Lynch and Milligan (1994).

Populations were assumed to be in Hardy-Weinberg equilibrium. An unbiased estimator of $q$ was calculated from the proportion of sampled individuals in the population that did not exhibit the RAPD marker. Sampling variance of the frequency of null homozygotes and sampling variance of the allele frequency were also estimated (Lynch and Milligan 1994). From these estimates of allele frequencies, gene diversity within a population was obtained on the basis of the conventional measure of genetic diversity: the probability that a random pair of alleles will contain one marker and one null. Mean gene diversity was obtained by averaging all observed markers in a population.

Gene diversity between populations, defined as the probability that one allele randomly drawn from one population differs from one randomly drawn from another population, was also assessed via estimates of allelic frequencies. Mean gene diversity between populations was obtained by averaging over all observed markers.

Wright's measure of population differentiation (Fst) was calculated by using the procedure described by Lynch and Milligan (1994). In order to test the significance of the population differentiation, a computer simulation program which randomly takes allelic frequencies (1000 simulations) to reproduce simulated Fst coefficients was included in the analysis. The probability of obtaining the same or higher values of simulated Fst than those obtained from the actual data determines the significance of changes from one generation to the next.

## Results

### Analysis of RAPD markers

For the pooled DNA, a total of 74 primers were used to amplify the DNA of *S. jamesii*. Fifty one of these produced between one and six polymorphic bands per primer giving a total of 137 polymorphic bands for the genetic analysis. The remaining 23 primers produced only monomorphic bands and therefore were not considered in the analysis.

One hundred and seven primers were assayed for *S. fendleri* accessions and 76 of these produced 151 polymorphic bands. The number of polymorphic bands per primer ranged from one to five. The other 31 primers generated only monomorphic bands.

For the individual data analysis, 50 RAPD "loci" were detected and used to make estimates of gene

**Table 2** Statistical comparison of genetic similarities (GS) between populations within accessions

| Comparison[a] | 1-1 matches | | 0-0 matches | | $\chi^2$ [b] | *P* value | GS |
|---|---|---|---|---|---|---|---|
| | Obs | Exp | Obs | Exp | | | |
| *Solanum jamesii* | | | | | | | |
| 275169.1959 vs 275169.1966 F | 59 | 61 | 76 | 76 | 0.066 | 0.798 | 0.985 |
| 275169.1959 vs 275169.1971 | 59 | 61 | 76 | 76 | 0.066 | 0.798 | 0.985 |
| 275169.1959 vs 275169.1985 | 59 | 61 | 76 | 76 | 0.066 | 0.798 | 0.985 |
| 275169.1959 vs 275169.1986 F | 59 | 61 | 75 | 76 | 0.079 | 0.779 | 0.978 |
| 275169.1966 vs 275169.1971 F | 59 | 59 | 78 | 78 | 0.000 | 1.000 | 1.000 |
| 275169.1966 vs 275169.1985 F | 59 | 59 | 78 | 78 | 0.000 | 1.000 | 1.000 |
| 275169.1966 vs 275169.1986 | 59 | 59 | 77 | 78 | 0.013 | 0.910 | 0.993 |
| 275169.1971 vs 275169.1985 S | 59 | 59 | 78 | 78 | 0.000 | 1.000 | 1.000 |
| 275169.1971 vs 275169.1986 F | 59 | 59 | 77 | 78 | 0.013 | 0.910 | 0.993 |
| 275169.1985 vs 275169.1986 | 59 | 59 | 77 | 78 | 0.013 | 0.910 | 0.993 |
| 458423.1978 vs 458423.1980 S | 60 | 60 | 75 | 77 | 0.052 | 0.820 | 0.985 |
| 458423.1978 vs 458423.1981 | 56 | 60 | 73 | 77 | 0.474 | 0.491 | 0.942 |
| 458423.1980 vs 458423.1981 F | 57 | 62 | 73 | 75 | 0.457 | 0.499 | 0.942 |
| 458424.1978 vs 458424.1980 S | 73 | 74 | 60 | 63 | 0.156 | 0.693 | 0.971 |
| 458424.1978 vs 458424.1981 | 73 | 74 | 57 | 63 | 0.585 | 0.444 | 0.949 |
| 458424.1980 vs 458424.1981 F | 76 | 76 | 58 | 61 | 0.148 | 0.701 | 0.978 |
| 458425.1978 vs 458425.1980 S | 66 | 76 | 50 | 51 | 1.335 | 0.248 | 0.913 |
| 458425.1978 vs 458425.1981 | 68 | 84 | 50 | 53 | 3.217 | 0.073 | 0.861 |
| 458425.1980 vs 458425.1981 F | 63 | 69 | 53 | 57 | 0.802 | 0.370 | 0.921 |
| 458426.1978 vs 458426.1980 S | 74 | 80 | 53 | 56 | 0.611 | 0.435 | 0.934 |
| 458426.1978 vs 458426.1981 | 75 | 81 | 51 | 56 | 0.891 | 0.345 | 0.920 |
| 458426.1980 vs 458426.1981 F | 74 | 77 | 54 | 59 | 0.541 | 0.462 | 0.941 |
| 458427.1980 vs 458427.1981 F | 80 | 83 | 53 | 54 | 0.127 | 0.722 | 0.971 |
| Average genetic similarity | | | | | | | 0.963 |
| *Solanum fendleri* | | | | | | | |
| 275156.1959 vs 275156.1981 F | 70 | 72 | 72 | 79 | 0.676 | 0.411 | 0.940 |
| 275157.1959 vs 275157.1981 F | 72 | 73 | 78 | 78 | 0.014 | 0.907 | 0.993 |
| 275158.1985 vs 275158.1986 S | 67 | 69 | 82 | 82 | 0.058 | 0.810 | 0.987 |
| 275158.1959 vs 275158.1985 F | 69 | 70 | 81 | 81 | 0.014 | 0.905 | 0.993 |
| 275158.1959 vs 275158.1986 F | 67 | 70 | 81 | 81 | 0.129 | 0.720 | 0.980 |
| 275161.1959 vs 275161.1985 F | 67 | 71 | 76 | 80 | 0.425 | 0.514 | 0.947 |
| 275161.1959 vs 275161.1986 F | 66 | 71 | 76 | 80 | 0.552 | 0.457 | 0.940 |
| 275161.1985 vs 275161.1986 S | 67 | 71 | 77 | 80 | 0.338 | 0.561 | 0.954 |
| 275162.1959 vs 275162.1971 F | 86 | 87 | 61 | 64 | 0.152 | 0.697 | 0.974 |
| 275163.1959 vs 275163.1973 F | 65 | 66 | 84 | 85 | 0.027 | 0.870 | 0.987 |
| 275163.1959 vs 275163.1975 F | 66 | 66 | 79 | 85 | 0.424 | 0.515 | 0.960 |
| 275163.1973 vs 275163.1975 S | 66 | 66 | 79 | 85 | 0.424 | 0.515 | 0.960 |
| 275164.1959 vs 275164.1980 F | 68 | 70 | 72 | 81 | 1.057 | 0.304 | 0.927 |
| 275164.1959 vs 275164.1983 F | 69 | 70 | 73 | 81 | 0.804 | 0.370 | 0.927 |
| 275164.1980 vs 275164.1983 S | 75 | 77 | 72 | 74 | 0.106 | 0.745 | 0.974 |
| 275166.1959 vs 275166.1975 F | 67 | 69 | 76 | 82 | 0.497 | 0.481 | 0.947 |
| 458409.ORIG vs 458409.1980 | 66 | 66 | 79 | 85 | 0.424 | 0.515 | 0.960 |
| 458411.1978 vs 458411.1980 S | 77 | 80 | 70 | 71 | 0.127 | 0.722 | 0.974 |
| 458413.1978 vs 458413.1980 S | 59 | 67 | 79 | 84 | 1.253 | 0.263 | 0.914 |
| 458415.1980 vs 458415.1981 F | 71 | 72 | 78 | 79 | 0.027 | 0.871 | 0.987 |
| 458417.1978 vs 458417.1981 F | 69 | 76 | 70 | 75 | 0.978 | 0.323 | 0.921 |
| 458419.1978 vs 458419.1981 F | 68 | 69 | 78 | 82 | 0.210 | 0.647 | 0.967 |
| 458421.1980 vs 458421.1981 F | 77 | 80 | 71 | 71 | 0.113 | 0.737 | 0.980 |
| 458422.1978 vs 458422.1980 S | 63 | 67 | 83 | 84 | 0.251 | 0.617 | 0.967 |
| 458422.1978 vs 458422.1981 F | 60 | 67 | 80 | 83 | 0.840 | 0.359 | 0.933 |
| 458422.1980 vs 458422.1981 | 68 | 72 | 75 | 78 | 0.338 | 0.561 | 0.953 |
| Average genetic similarity | | | | | | | 0.959 |

[a] Suffix F indicates one filial generation between compared populations; S represents sister populations derived from a common source. Average inter-accession GS for *S. jamesii* from the same sites = 0.666; from different sites = 0.617. Average inter-accession GS for *S. fendleri* from the same sites = 0.903; from different sites = 0.647. For both species GS means for comparisons among accessions from the same site, and among accessions from different sites, are all highly significantly different ($P \leqslant 0.01$)

[b] $\chi^2$critical value of 3.84 for $P \leqslant 0.05$ and critical value of 6.63 for $P \leqslant 0.01$ for 1 *df*

diversity. It was assumed that RAPD bands of equal size were homologous and, when polymorphic within a species, represented a "locus" with two alleles (band/no band) (Riesenberg 1996).

## Analysis of *S. jamesii*

Based on pooled-DNA data analysis, a pairwise-comparison matrix among *S. jamesii* populations was generated using RAPD data. This matrix provided genetic similarity coefficients among populations (Table 2). Two types of similarities from Table 2 are of particular interest: filial populations separated by one generation, and sister populations derived from the same source. Populations derived from materials collected by Hawkes in 1958 (275169) provide five independent comparisons of populations separated by one generation (viz., 1959:1966, 1959:1986, 1966:1971, 1966:1985, 1971:1986). These similarities are 98.5%, 97.8%, 100%, 100% and 99.3%, respectively, with a mean of 99.1%. Sister populations derived from the same source for this accession (1971:1985) had a similarity of 100% (Table 2 and Fig. 1).

Populations derived from materials collected by Ugent and Ruhde in 1978 (458423, 458424, 458425, 458426, 458427) each allow one comparison of filial populations (viz., 1980:1981). The similarity of these are 94.2%, 97.8%, 92.1%, 94.1% and 97.1%, respectively, with a mean of 95.1%. For each of these accessions, with the exception of 458427, there is also one pair of sister populations derived from the same source (viz., 1978:1980). Their similarities are 98.5%, 97.1%, 91.3% and 93.4%, respectively, with a mean of 95.1% (Table 2 and Fig. 1).

## Analysis of individuals within populations

Estimates of gene diversity calculated from 50 RAPD markers in 18 populations of *S. jamesii* are shown in Table 3. The average gene diversity within populations within accessions was: 275169 (34.6%), 458423 (31.1%), 458424 (24.3%), 458425 (25.7%), 458426 (31.0%) and 458427 (30.1%), with an overall mean of 29.9%.

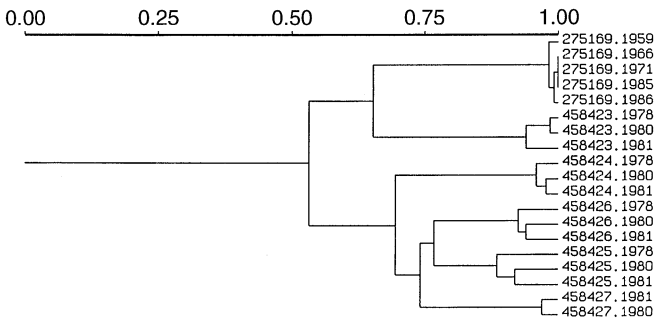


**Fig. 1** UPGMA phenogram of genetic relationships among *S. jamesii* populations based on RAPD marker analysis

**Table 3** Heterozygosity estimates of within- and between-population gene diversity (GD) and Wright's measure of population differentiation (Fst) using RAPD markers for 18 *S. jamesii* populations

| Population | GD within population[a] | GD between populations[b] | Fst | *P* value[c] |
|---|---|---|---|---|
| 275169.1959 | 0.353 | 0.022 | | |
| 275169.1966 | 0.335 | 0.025 | | |
| 275169.1971 | 0.368 | 0.020 | | |
| 275169.1985 | 0.354 | 0.020 | | |
| 275169.1986 | 0.321 | 0.035 | | |
| Accession mean | 0.346 | 0.024 | 0.0658 | 0.078 ns |
| 458423.1978 | 0.361 | 0.046 | | |
| 458423.1980 | 0.285 | 0.028 | | |
| 458423.1981 | 0.288 | 0.028 | | |
| Accession mean | 0.311 | 0.034 | 0.0986 | 0.004* |
| 458424.1978 | 0.240 | 0.017 | | |
| 458424.1980 | 0.232 | 0.018 | | |
| 458424.1981 | 0.258 | 0.012 | | |
| Accession mean | 0.243 | 0.016 | 0.0602 | 0.077 ns |
| 458425.1978 | 0.260 | 0.016 | | |
| 458425.1980 | 0.247 | 0.015 | | |
| 458425.1981 | 0.264 | 0.014 | | |
| Accession mean | 0.257 | 0.015 | 0.0553 | 0.074 ns |
| 458426.1978 | 0.314 | 0.011 | | |
| 458426.1981 | 0.306 | 0.011 | | |
| Accession mean | 0.310 | 0.011 | 0.0336 | 0.310 ns |
| 458427.1980 | 0.293 | 0.019 | | |
| 458427.1981 | 0.308 | 0.019 | | |
| Accession mean | 0.301 | 0.019 | 0.0581 | 0.084 ns |

[a] Averaged gene diversity estimate within populations for accession
[b] Averaged gene diversity estimate among populations for accession
[c] ns = not significant, * = significant

In contrast, average gene diversity observed among generations in each accession was low, with an overall mean of 2.0%. Wright's measure of genetic differentiation (Fst) indicated that only one accession had undergone significant change in gene frequency over generations: 458423 (Fst = 0.0986; $P = 0.004$) (Table 3). Of the 100 alleles assessed, no bands were lost in the distribution sample of all accessions tested, but in three cases bands appeared to have become fixed.

## Analysis of *S. fendleri*

Pooled DNA samples allowed the construction of a pairwise matrix of genetic similarity among *S. fendleri* populations (Table 2). In one case, an original wild population (458409.ORIG) could be compared with its first seed-increase (458409.1980). These populations were highly similar genetically (96%) (Fig. 2). In seven cases, the same seed lot was used to generate two sister populations in different years. These also had very close genetic similarities, with a mean of 96.1% (Table 2 and Fig. 2). Close resemblances were always found among

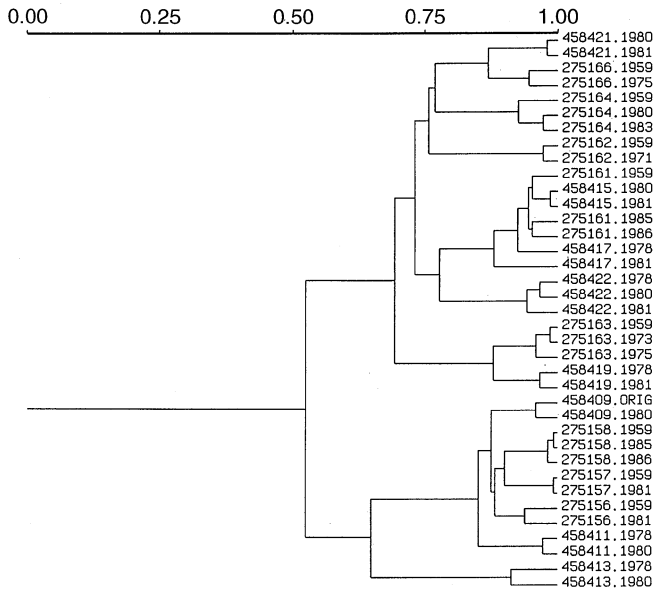


**Fig. 2** UPGMA phenogram of genetic relationships among *S. fendleri* populations based on RAPD marker analysis

populations separated by one generation. The average similarity of these filial generations was 95.9% (Table 2 and Fig. 2). Chi-square analysis indicated that, regardless of species, no pairs of populations within any given accession were significantly different.

## Discussion

Several studies have considered how methods affect the conservation of the genetic integrity of *ex situ* germ plasm (Ellis et al. 1985a, b; Breese 1989). For decades, much attention has focused on the genetic risks associated with small population size, particularly from inbreeding and genetic drift, but also from gene flow. Until now, a precise empirical assessment of how well diversity has been preserved over many years in gene banks has been unavailable (Ellstrand and Elam 1993).

### Appropriateness of the model species used

*S. jamesii* is a self-incompatible diploid, giving this species the potential for a high level of genetic variability within populations (Loveless and Hamrick 1984). The analysis of individuals revealed an average of 29.9% estimated mean gene diversity by population. The breeding system of *S. jamesii* (diploid outcrosser) is the most common of potato species (about 70%).

*S. fendleri* is a self-compatible allotetraploid with disomic inheritance that can reproduce by inbreeding or outcrossing (Hawkes 1966). Considering the typical spontaneous berry set of this species in greenhouse conditions, selfing can be assumed to be common in the wild. Thus, this species has a breeding system theoretically less vulnerable to the loss of genetic diversity since natural populations are assumed to be more homozygous and homogeneous (Jain 1975). However, outcrossing makes heterozygosity (and therefore vulnerability to genetic loss) possible. Alternatively, if wild "populations" exist as heterogeneous mixtures of several inbreds, genetic erosion in the gene bank could also occur. The breeding system of *S. fendleri* (disomic polyploid) is the second most common among accessions in potato collections (about 20%).

### RAPD marker analysis

Use of RAPD markers provided the power to detect small differences between these closely related populations within species. A preliminary analysis to discriminate these populations through the use of five morphological characters (which appeared to be polymorphic among populations) led to non-sensical, and presumably erroneous, population groupings (data not presented).

From the analysis of pooled DNA samples, no statistically significant differences among any generations within an accession were detected, despite the fact that all of these closely related accessions were clearly differentiated (Figs. 1 and 2). This leads to the conclusion that gene bank conservation methods have not significantly compromised the genetic integrity of either *S. jamesii* or *S. fendleri* populations.

The analysis of individuals for *S. jamesii* populations provides insights into changes in allele frequencies. The average estimated gene diversity within accessions ranged from 24.3 to 34.6%. This suggests that these populations have substantial heterozygosity vulnerable to loss. Interestingly, almost all cases revealed that small and non-significant variation in mean gene diversity occurred among generations within accessions, the mean gene diversity between generations within accessions being 2.0% (1.1–3.4%). Only in one accession, 458423, did significant loss of gene diversity occur: 1978 (36.1%): 1980 (28.5%). However, the same accession showed a very small change in the next generation of seed-increase, 1980 (28.5%): 1981 (28.8%), suggesting that allelic diversity was maintained. Wright's estimates of genetic differentiation among populations (Fst) also indicate almost no genetic differentiation over generations within accessions (see Table 3). These findings suggest that gene frequencies are generally stable over seed increase generations: exactly what is desired to maximize preservation of all alleles.

Efficient conservation of genetic diversity was not affected by the breeding system. The *S. jamesii* accessions' average similarity was 96.3% and that of *S. fendleri* was 95.9% (Table 2). No statistically significant

losses were detected between any filial generations or sister populations within accessions within either species. Even with nearly 100% effective seed increase methods, some small genetic change might be expected. These changes could be due to alleles at very low frequency, point mutations, genetic changes such as insertions, deletions, inversions, etc. (Williams et al. 1990), or sampling errors. However, even in accession 275169, where a population could be compared to its third serial generation, genetic similarity was still 97.8%.

In *S. fendleri*, a comparison could be made between original seed from the wild and its subsequent progeny (458409.ORIG vs 458409.1980). The close resemblance of 96% argues against the possibility that original samples from the wild were in some way "domesticated" at the first gene bank increase, causing substantial loss of diversity but making all subsequent generations stable.

## General conclusions

The results presented in this study confirm the usefulness of DNA-based markers as a powerful tool to address questions regarding the conservation of genetic resources. Loss of genetic diversity due to sexual propagation in the NRSP-6 gene bank was measured and found to be a very small part of the potential intra-accession variability. Given that these materials and methods are relevant to other potato species, and that similar methods are used for other potato gene banks, it appears that the genetic diversity of ex situ *Solanum* germ plasm is being adequately preserved.

The techniques used in this study could also be used to address other questions important to gene banks. For instance, one pair of *S. jamesii* accessions (275169 and 458423) were collected at the same location but by different collectors 20 years apart (Hawkes 1958 and Ugent and Ruhde 1978, respectively). These accessions from the same site resembled each other 66.6%, only slightly more than the average similarity of populations from different sites (61.7%). Likewise, three sets of *S. fendleri* accessions from identical locations also had a lower average inter-accession similarity (90.3%) than populations within accessions (95.9%). Were these sampled differently from the wild, or did the populations change that much over time, suggesting that periodic re-collections are worthwhile? Also, while the focus of this study was a comparison of intra-accession populations, it also demonstrated the power of RAPDs to clearly and quantitatively differentiate these closely related accessions of the same species. What geographic, climatic or reproductive variables best explain the partitioning of the diversity observed? Could such insights improve strategies for maximizing the efficiency of germ plasm collection and preservation? These questions will be the focus of future publications.

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