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Genetic diversity of worldwide Jerusalem artichoke (*Helianthus tuberosus*) germplasm as revealed by RAPD markers

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ABSTRACT. Jerusalem artichoke (*Helianthus tuberosus*) is a wild relative of the cultivated sunflower (*H. annuus*); it is an old tuber crop that has recently received renewed interest. We used RAPD markers to characterize 147 Jerusalem artichoke accessions from nine countries. Thirty RAPD primers were screened; 13 of them detected 357 reproducible RAPD bands, of which 337 were polymorphic. Various diversity analyses revealed several different patterns of RAPD variation. More than 93% of the RAPD variation was found within accessions of a country. Weak genetic differentiation was observed between wild and cultivated accessions. Six groups were detected in this germplasm set. Four ancestral groups were found for the Canadian germplasm. The most genetically distinct accessions were identified. These findings provide useful diversity information for understanding the Jerusalem artichoke gene pool, for conserving

Jerusalem artichoke germplasm, and for choosing germplasm for genetic improvement.

Key words: *Helianthus tuberosus*; Diversity; Genetic structure; RAPD; Germplasm conservation

INTRODUCTION

The Jerusalem artichoke (*Helianthus tuberosus* L.) is a cold-hardy North American wild relative of the cultivated sunflower (*H. annuus* L.) and has been cultivated mainly for tubers since the 17th century (Kays and Nottingham, 2008). Its tubers are consumed as vegetable, and the inulin containing tubers can be used as raw material to produce various value-added products such as health food products, animal feed additive (Zaky, 1990) and bio-ethanol (Seiler, 2007). The crop, although largely abandoned after the Second World War (Serieys et al., 2010), has recently received renewed interest in genetic improvement for multiple purposes (Kays and Nottingham, 2008) and will play an increasing role in the improvement of economically important traits in sunflower such as oil characters and disease resistance (Sennoi et al., 2010; Breton et al., 2010). However, little attention has been paid to characterizing and conserving Jerusalem artichoke genetic resources, in contrast to sunflower germplasm (e.g., see Mandel et al., 2011).

Conserved Jerusalem artichoke genetic resources are relatively limited due to insufficient conservation efforts (van Soest et al., 1993; Volk and Richards, 2006; Kays and Nottingham, 2008). Currently, only several hundred Jerusalem artichoke accessions are maintained in plant germplasm collections worldwide. These include wild and weedy accessions, landraces, or traditional and obsolete cultivars, and advanced or improved cultivars. Some efforts have been made to characterize existing Jerusalem artichoke germplasm (Schittenhelm, 1989; Kays and Kultur, 2005; Serieys et al., 2010; Puttha et al., 2011). Little diversity research has been conducted on Jerusalem artichoke germplasm (Kays and Nottingham, 2008) and consequently, the Jerusalem artichoke gene pool is still poorly understood (Dozet et al., 1993, 1994; Wangsomnuk et al., 2006; El Gengaihi et al., 2009).

Characterization of plant germplasm using molecular techniques has played an increasingly important role in the management and utilization of plant genetic resources (Karp, 2002). It has also enhanced plant breeding in selection of diverse parents to widen the breeding gene pool (Fu, 2006). Random amplified polymorphism DNA (RAPD) was one of the first molecular markers developed. It contributed to early genetic diversity research thanks to its technical simplicity and feasibility (Williams et al., 1990). The RAPD marker requires no prior sequence information for the survey of plant genomes, but generally suffers from low resolution due to various issues associated with reproducibility, dominance and non-homologous DNA fragments, which are similar issues for other dominant markers (Koopman, 2005). However, successful RAPD applications assessing genetic diversity have been documented in many plants (Arif et al., 2010) such as sorghum (Iqbal et al., 2010), pomegranate (Ercisli et al., 2011) and sea buckthorn (Singh et al., 2006). Unfortunately, molecular markers have rarely been applied to assess genetic variation of Jerusalem artichoke (Dozet et al., 1993; Wangsomnuk et al., 2006; El Gengaihi et al., 2009).

This study was conducted using RAPD markers to characterize 147 diverse Jerusalem

artichoke accessions that originated from nine countries. The specific objective of this study was to assess their genetic diversity, structure, association and distinctiveness. It is our hope that this characterization effort can provide a set of baseline information for the conservation and utilization of Jerusalem artichoke germplasm.

MATERIAL AND METHODS

Plant material

A total of 147 Jerusalem artichoke accessions were used for this study (Table 1). The studied germplasm was obtained from three sources: 104 accessions from Plant Gene Resources of Canada (PGRC), Saskatoon, Canada; 25 accessions from the Gatersleben Gene Bank Department at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany; and 17 accessions from the North Central Regional Plant Introduction Station (NCRPIS), Iowa State University, USA. One accession of unknown origin, but cultivated in Thailand, was also included. The studied accessions of known origin represent germplasm from nine countries. Also, 21 accessions were collected from wild populations across the USA.

DNA extraction

Young leaf tissue was collected from at least three individual plants of one accession and bulked for DNA extraction following the modified method of Tai and Tanksley (1990) which was shown to be the best DNA extraction method for Jerusalem artichoke (Mornkham et al., 2011). The bulked tissue (30 mg) was ground with a homogenizer and 0.7 mL extraction buffer (100 mM Tris-HCl, pH 8, 50 mM EDTA, pH 8, 0.5 M NaCl, 1.25% SDS, 8.3 mM NaOH, 0.38% Na bisulfite) was added and mixed by vortexing. The sample was incubated at 65°C for 20 min and 0.22 mL 5 M potassium acetate added and mixed well. The tube was placed on ice for 40 min, followed by centrifugation for 3 min. The supernatant was transferred to a new tube. The DNA was precipitated by adding 0.7 volume of isopopanol, mixed well and centrifuged for 3 min. The supernatant was poured off and the pellet rinsed with 70% ethanol. The pellet was re-suspended in 300 µL TE (50 mM Tris-HCl, pH 8, 10 mM EDTA) by briefly vortexing, and incubated at 65°C for 5 min, followed by vortexing again. Ammonium acetate (150 µL 7.4 M) was added and mixed well before centrifugation for 3 min and removal of the supernatant to the new tube. The DNA was precipitated by mixing with 330 µL isopropanol and centrifuged for 3 min. The pellet was rinsed with 70% ethanol and re-suspended in 100 µL TE, incubated at 65°C for 5 min, and then vortexed. The DNA was re-suspended in 150 µL TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). The purity and quality of genomic DNA were assessed after digestion with RNaseA (Sigma), and quantified on 1% agarose gel against a known concentration of 100 bp DNA ladder plus (Vivantis). The extracted genomic DNA was stored at -20°C until further use.

RAPD analysis

Thirty decamer primers (Operon Technologies, Alameda, CA) were initially screened using bulked DNA from 38 accessions of Jerusalem artichoke (PI547241, PI613241, AMES2714, AMES2722, AMES2723, AMES2730, AMES2736, AMES2746, AMES8380,

Table 1. List of 147 Jerusalem artichoke accessions with country origin, average dissimilarity and the cluster inferred from the STRUCTURE program.

Acc	Orig/Sour	AD	StC	Acc	Orig/Sour	AD	StC	Acc	Orig/Sour	AD	StC
KKU001	UNK/U	0.360	2	JA55	USA/P	0.333	3	JA131	CAN/P	0.345	6
CN52867	RUS/P	0.363	2	JA58	RUS/P	0.344	3	JA132	CAN/P	0.343	6
JA1	CAN/P	0.338	1	JA59	RUS/P	0.313	3	JA133	CAN/P	0.344	6
JA2	CAN/P	0.339	1	JA60	RUS/P	0.339	3	JA134	CAN/P	0.331	6
JA3	CAN/P	0.347	1	JA61	RUS/P	0.319	3	JA135	CAN/P	0.344	6
JA4	CAN/P	0.329	1	JA66	USA/P	0.335	3	AMES2714*	USA/N	0.384	1
JA5	CAN/P	0.345	1	JA67	USA/P	0.352	2	AMES2722*	USA/N	0.377	1
JA6	CAN/P	0.335	1	JA69*	USA/P	0.310	3	AMES2723*	USA/N	0.355	1
JA7	CAN/P	0.338	1	JA70*	USA/P	0.313	3	AMES2729*	USA/N	0.369	2
JA8	CAN/P	0.335	1	JA71*	USA/P	0.316	3	AMES2730*	USA/N	0.363	1
JA9	CAN/P	0.328	1	JA72*	USA/P	0.354	3	AMES2736*	USA/N	0.370	1
JA10	CAN/P	0.339	1	JA73*	USA/P	0.326	3	AMES2746*	USA/N	0.356	1
JA11	CAN/P	0.349	1	JA74*	USA/P	0.337	3	AMES2747*	USA/N	0.356	1
JA12	CAN/P	0.341	4	JA75	CAN/P	0.347	3	AMES8380	USA/N	0.382	1
JA13	CAN/P	0.322	4	JA78	FRA/P	0.323	3	AMES22229	CAN/N	0.358	1
JA14	CAN/P	0.338	4	JA81	FRA/P	0.354	2	PI451980*	USA/N	0.382	1
JA15	CAN/P	0.364	4	JA86	FRA/P	0.356	6	PI503262*	USA/N	0.369	1
JA16	CAN/P	0.336	4	JA87	FRA/P	0.341	6	PI547230*	USA/N	0.359	1
JA17	CAN/P	0.335	4	JA88	RUS/P	0.343	6	PI547232*	USA/N	0.367	1
JA18	CAN/P	0.344	4	JA89	FRA/P	0.359	2	PI547233*	USA/N	0.384	1
JA19	CAN/P	0.363	4	JA91	RUS/P	0.329	6	PI547237*	USA/N	0.369	1
JA20	CAN/P	0.348	4	JA92	RUS/P	0.334	6	PI547241*	USA/N	0.402	1
JA21	CAN/P	0.353	4	JA93	RUS/P	0.317	6	HEL53	DEU/I	0.357	2
JA22	CAN/P	0.352	4	JA95	RUS/P	0.329	6	HEL61	RUS/I	0.340	2
JA23	CAN/P	0.336	4	JA97	FRA/P	0.332	6	HEL62	RUS/I	0.350	2
JA24	CAN/P	0.351	4	JA98	FRA/P	0.332	6	HEL65	RUS/I	0.383	2
JA25	CAN/P	0.343	4	JA100	FRA/P	0.322	6	HEL66	O-U/I	0.356	2
JA26	CAN/P	0.341	4	JA102	DEU/P	0.367	2	HEL68	UNK/I	0.364	2
JA27	CAN/P	0.340	4	JA105	RUS/P	0.309	6	HEL69	UNK/I	0.334	2
JA28	CAN/P	0.314	5	JA106	CAN/P	0.325	6	HEL231	DEU/I	0.325	2
JA29	CAN/P	0.325	5	JA107	CAN/P	0.313	6	HEL243	DEU/I	0.350	2
JA30	CAN/P	0.336	5	JA108	CAN/P	0.318	6	HEL246	UNK/I	0.357	2
JA31	CAN/P	0.334	5	JA109	CAN/P	0.313	6	HEL248	DEU/I	0.360	2
JA32	CAN/P	0.318	5	JA110	CAN/P	0.324	6	HEL250	FRA/I	0.339	2
JA33	CAN/P	0.318	5	JA111	CAN/P	0.323	6	HEL253	UNK/I	0.350	2
JA34	CAN/P	0.326	5	JA112	CAN/P	0.329	6	HEL256	UNK/I	0.326	2
JA35	CAN/P	0.331	5	JA113	CAN/P	0.330	6	HEL257	UNK/I	0.346	2
JA36	CAN/P	0.324	5	JA114	CAN/P	0.330	6	HEL265	O-H/I	0.350	2
JA37	CAN/P	0.352	2	JA116	CAN/P	0.332	6	HEL267	O-Y/I	0.325	2
JA38	CAN/P	0.352	2	JA117	CAN/P	0.334	6	HEL272	FRA/I	0.336	2
JA42	CAN/P	0.321	5	JA118	CAN/P	0.326	6	HEL278	UNK/I	0.343	1
JA43	CAN/P	0.337	5	JA119	CAN/P	0.332	6	HEL280	UNK/I	0.346	1
JA44	CAN/P	0.323	5	JA120	CAN/P	0.332	6	HEL293	O-P/I	0.371	1
JA45	CAN/P	0.329	5	JA122	CAN/P	0.336	6	HEL308	UNK/I	0.362	1
JA46	CAN/P	0.324	5	JA123	CAN/P	0.324	6	HEL324	UNK/I	0.347	2
JA47	CAN/P	0.326	5	JA125	CAN/P	0.336	6	HEL327	UNK/I	0.349	6
JA48	CAN/P	0.322	5	JA126	CAN/P	0.334	6	HEL335	UNK/I	0.363	2
JA49	CAN/P	0.331	3	JA127	CAN/P	0.340	6				
JA50	CAN/P	0.331	3	JA128	CAN/P	0.333	6				
JA54	USA/P	0.347	3	JA130	CAN/P	0.336	6				

Acc = accession label described in Kays and Nottingham (2008), * = an accession collected from a wild population in the USA; Orig/Sour = country origin and germplasm source, country code following ISO 3166-1 alpha-3 country code, RUS = the former Union of Soviet Socialist Republics (USSR), O-H = other-Hungry, O-Y = other-the former Yugoslavia, O-P = other-Poland, O-U = other-Ukraine, UNK = unknown origin, Source I = The Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany, P = Plant Gene Resources of Canada (PGRC), N = The North Central Regional Plant Introduction Station (NPRPIS), USA, U = unknown; AD = average dissimilarity; StC = clusters obtained from the STRUCTURE program.

AMES22229, JA1, JA2, JA3, JA4, JA5, JA6, JA7, JA8, JA9, JA10, JA11, JA12, JA13, JA14, JA15, JA16, JA17, JA18, JA19, JA20, JA21, JA22, JA23, JA24, JA25, JA26, JA27 and JA28) to determine the suitability of each primer for the study. The informative primers were then selected for further analysis based on their ability to detect distinct, clearly resolved, and reproducible amplified products.

The polymerase chain reaction (PCR) was run in a final volume of 25 ng DNA template, 0.4 U Taq DNA polymerase (Vivantis), 1.0 µL 10X buffer (750 mM $NH_4(SO_2)_4$, 0.1% Tween 20, Fermentas), 1.5 mM $MgCl_2$ (Fermentas), 0.2 mM dNTPs (Vivantis), 1.0 µM RAPD primer in a 0.20 ml PCR tube. The amplification was performed in a "CG1-96" thermocycler (Corbett Research, Germany). The amplification regime consisted of 95°C for 2 min; then 45 cycles at 94°C for 30 s, annealing temperature (Ta) °C for 30 s, and 72°C for 90 s; and a final extension at 72°C for 5 min.

The RAPD amplification products were analyzed by electrophoresis on 1.2% agarose gels, run in 1X TBE, visualized under UV transilluminator and photographed. The PCR were done three times independently. Only repeatable amplified DNA fragments were manually scored as 1 or 0 for presence or absence, respectively, for each sample.

Data analysis

The RAPD data were analyzed for the levels of polymorphism with respect to primer and sample by counting the number of polymorphic bands and generating summary statistics of band frequencies. Shannon's entropy was calculated following Russell et al. (1993) to estimate the diversity content per locus, as this estimate does not require strict genetic assumptions such as marker inheritance and sample ploidy. The entropy-based diversity content provides a measure of the effective number of alleles per marker locus (Reyes-Valdes and Williams, 2005). These analyses were performed by using a SAS program written in SAS IML (SAS Institute Inc., 2008).

To assess the genetic differentiation among the Jerusalem artichoke accessions, an analysis of molecular variance (AMOVA; Excoffier et al., 1992) that was based on the dissimilarity matrix of pairwise accessions was also performed using Arlequin version 3.1 (Excoffier and Lischer, 2010). This analysis permitted the partition of the total RAPD variation into within- and among-group variation components, and provided measures of inter-group genetic distance as the proportion of the total RAPD variation residing between any two groups (Excoffier et al., 1992). Two models of genetic structuring were examined based on the country origin and germplasm status (wild versus cultivated) of an accession. Significance of resulting variance components and inter-group genetic distances was tested with 10,000 random permutations.

The model-based Bayesian method available in the program STRUCTURE version 2.2.3 (Pritchard et al., 2000; Falush et al., 2003, 2007) was used to detect population structure and to assign accessions to subpopulations. The STRUCTURE program was run 30 times for each subpopulation (K) value, ranging from 2-10, using the admixture model with 10,000 replicates for burn-in and 10,000 replicates during analysis. The final population subgroups were determined based on 1) the likelihood plot of these models, 2) the change in the second derivative (ΔK) of the relationship between K and the log-likelihood (Evanno et al., 2005), and 3) the stability of grouping patterns across 30 runs. For a given K with 30 runs, the run

with the highest likelihood value was selected to assign the posterior membership coefficients to each accession. A graphical bar plot was then generated with the posterior membership coefficients.

The genetic associations of the Jerusalem artichoke accessions were assessed using two approaches. A principal component analysis of 147 accessions was performed using NTSYS-PC 2.01 (Rohlf, 1997) based on the similarity matrix of 337 RAPD bands, and plots of the first three resulting principal components were made to assess the accession associations and to identify genetically distinct accessions. A neighbor-joining analysis of 147 accessions was also made using PAUP* (Swofford, 1998) and a radiation tree was displayed using MEGA 3.01 (Kumar et al., 2004) to confirm the genetic association of individual accessions and to identify any genetic clustering without restriction to known characteristics.

To assess the genetic distinctiveness of the Jerusalem artichoke accessions, the similarities of each accession with the remaining accessions assayed were calculated using the simple matching coefficient (Sokal and Michener, 1958): $S_{ij} = (a+d)/(a+b+c+d)$, where S_{ij} is the RAPD similarity between the accession i (i = 1 to n) and the other accession j [j = 1 to (n-1)], a is the number of bands (from all RAPD loci) shared in both i and j, b is the number of bands present in i but not shared in j, c is the number of bands present in j but not shared in i, and d is the number of bands absent from both i and j. The RAPD dissimilarity for each pair of accessions can be defined as 1- S_{ij}. The average RAPD dissimilarity for the accession i can be obtained by averaging all of the n-1 RAPD dissimilarities that the accession was associated with. This average dissimilarity measures the overall genetic difference between the accession (i) of interest and the remaining accessions assayed. A higher average dissimilarity obtained from unlinked markers means that the accession has a more distinct genetic background than the other accessions (Fu, 2006). This assessment was done using a specific SAS program written in SAS IML.

RESULTS AND DISCUSSION

RAPD variation

A total of 30 RAPD primers were screened on 38 selected accessions and only 13 of them were found to be informative with reproducible patterns of DNA fragments over three replications (Table 2). The 13 primers detected a total of 357 DNA bands with fragment sizes ranging from 210 to 2600 bp, of which 337 (94.4%) were polymorphic. The number of DNA bands detected by a primer ranged from 20 (the primers OPE9 and OPS1) to 33 (the primer OPS2) and averaged 27.5. Similarly, the high percentages of polymorphic bands were observed for these primers, ranging from 90% (the primer OPE9) to 100% (the primers OPS1 and OPS6). The most informative primer was the primer OPS2 with the highest Shannon's entropy of 9.55, followed by the primer OPE2 with Shannon's entropy of 8.56. Relatively, the least informative primer was the primer OPE9 with Shannon's entropy of 4.06. The average Shannon's entropy for these primers was 6.74.

The observed occurrence frequencies of the 337 bands ranged from 0.007 to 0.993 with an average of 0.48. Interestingly, an average of 16.9 bands was observed for each 0.05 interval of occurrence frequency ranging from 0 to 1. For example, 10 bands had a frequency of 0.05 or less in the 147 accessions and 20 bands displayed a frequency of 0.95 or higher.

Table 2. List of 13 RAPD markers used and polymorphism detected in the 147 Jerusalem artichoke accessions.

	Sequence	No. of bands	Size range (bp)	Polymorphic bands (%)	Entropy-based diversity content
OPA02	CTTCTGACTG	29	400-2,600	96.6	6.819
OPA10	GTGATCGCAG	31	210-1,500	90.3	7.599
OPA20	GTTGCGATCC	27	290-1,260	92.6	6.367
OPE1	CCCAAGGTCC	29	260-2,500	93.1	6.289
OPE2	GGTGCGGGAA	32	250-2,000	93.8	8.564
OPE8	TCACCACGGT	23	340-1,640	95.7	5.497
OPE9	CTTCACCCGA	20	300-1,760	90.0	4.062
OPS1	CTACTGCGCT	20	230-1,430	100.0	4.711
OPS2	CCTCTGACTG	33	220-2,450	97.0	9.551
OPS4	CACCCCCTTG	32	330-2,000	93.8	7.006
OPS6	GATACCTCGG	26	280-1,580	100.0	7.214
OPS12	CTGGGTGAGT	32	320-2,500	90.6	7.603
OPS15	CAGTTCACGG	23	410-2,150	95.7	6.298
Total or mean		357		94.5	6.737

These RAPD variations were consistent with some earlier reports (Doetz et al., 1993, 1994; El Gengaihi et al., 2009), and also were compatible with those RAPD results reported in sunflower (e.g., see Lawson et al., 1994) and those detected by other markers (e.g., see Quagliaro et al., 2001; Wangsomnuk et al., 2006). Such large RAPD variation is expected (Hamrick and Godt, 1998), as Jerusalem artichoke is an outcrossing, hexaploidy (2n = 6x = 102) plant (Swanton et al., 1992).

Genetic differentiation

Partitioning RAPD variation into groups by AMOVA revealed weak genetic differentiations with respect to germplasm status (wild versus cultivated) and country origin. Only 4.4% RAPD variation resided between the Jerusalem artichoke accessions collected from wild populations and breeding programs. It seemed that the wild accessions displayed a little more RAPD variation than the cultivated materials measured by the group-specific F_{ST} values (0.04423 and 0.04441, respectively). Note that a higher F_{ST} value would mean higher inbreeding and consequently imply less genetic diversity. Similarly, only 6.7% RAPD variation harbored among the Jerusalem artichoke accessions originated from nine countries, and more than 93% RAPD variation was present within the accessions of a given country. Note that all the AMOVA components reported here were statistically significant at $P < 0.0001$.

The genetic differentiations at the country level were relatively larger, ranging in the pairwise country F_{ST} value from 0.001 to 0.183 (Table 3). The large pairwise country F_{ST} value was obtained between accessions from Germany and the former Union of Soviet Socialist Republics (USSR) followed by those accessions from Germany and Canada. These results indicate the accessions from Germany were more differentiated from those from the USSR and Canada. However, based on the country-specific F_{ST} values, the largest RAPD variation was observed within the accessions originated from the USA (0.0621), followed by Canada (0.0659), and the least RAPD variation was within the accessions from Germany (0.0871) (Table 3). These results could be biased by variable sample sizes, but should be useful for acquiring diverse germplasm from other countries.

Table 3. Proportional RAPD variations (F_{ST}) within and among the Jerusalem artichoke accessions originated from different countries, estimated from the analysis of molecular variance of 337 RAPD markers.

Country	No. of samples	Group-specific F_{ST}	Pairwise group F_{ST}					
			Canada	USA	USSR	German	France	Others
Canada	75	0.0659						
USA	26	0.0621	0.0557					
USSR	14	0.0704	0.0383	0.0581				
Germany	5	0.0871	0.1797	0.1569	0.1827			
France	10	0.0694	0.0294	0.0434	**0.0073**	0.1252		
Others	4	0.0790	0.0945	0.0649	0.0860	**0.0375**	**0.0390**	
Unknown	13	0.0690	0.0898	0.0561	0.0952	0.0495	0.0497	**0.0001**

USSR = the former Union of Soviet Socialist Republics; Others = Hungry, Poland, Ukraine or the former Yugoslavia. All pairwise group F_{ST} values were statistically significant at $P < 0.05$, except for non-significant ones highlighted in bold.

Genetic structure

The model-based inference of genetic structure within the 147 Jerusalem artichoke accessions by STRUCTURE considered K = 2 to 10 clusters and revealed six optimal clusters with the highest log-likelihoods (Figure 1). The log-likelihoods increased slowly from K = 2 to 6 and started to decrease after K = 6 (Figure 1B). The optimal cluster with K = 6 was also supported from the rate of change in the second derivative of the log-likelihoods over various Ks analyzed (Figure 1C; Evanno et al., 2005), in which the dramatic change in the derivative was found at K = 6.

The average distance between individual accessions in the same cluster for six clusters was 0.346, 0.285, 0.248, 0.277, 0.220 and 0.274, respectively, for clusters 1 to 6. The mean value of cluster-specific F_{ST} was 0.094, 0.259, 0.344, 0.270, 0.410 and 0.253, respectively, for clusters 1 to 6. The overall proportions of membership of the sample in each of the six clusters were 0.205, 0.190, 0.113, 0.129, 0.109 and 0.254, respectively, for clusters 1 to 6. The detailed memberships of the 147 accessions in each cluster are given in Table 1, which was based on the highest level of inferred ancestry for one cluster from one STRUCTURE run with the highest log-likelihood of data (-22364.3). At the inferred ancestry level of 0.80 or lower, 52 accessions had multiple memberships for various clusters; at 0.90 or lower, 85 accessions displayed multiple memberships for different clusters (Figure 1A).

These optimal clusters were further confirmed with additional genetic structure analysis done with the BAPS software (Corander et al., 2004). The BAPS application revealed the same optimal number of clusters with more than 90% memberships matched with those inferred from the STRUCTURE software (results not shown).

The optimal clusters detected here may truly reflect the current Jerusalem artichoke gene pool, as several clusters reflected either the wild populations sampled or the consequence of long term Jerusalem artichoke breeding, particularly in Canada. They could also be confounded by the bias of sampling Jerusalem artichoke germplasm worldwide. Adding other representative samples to such structural analysis would help to verify and correct the sampling bias. However, these findings are significant and could be used as rough guides for further germplasm research such as the development of core sets for germplasm conservation and association mapping of genes for genetic improvement of Jerusalem artichoke.

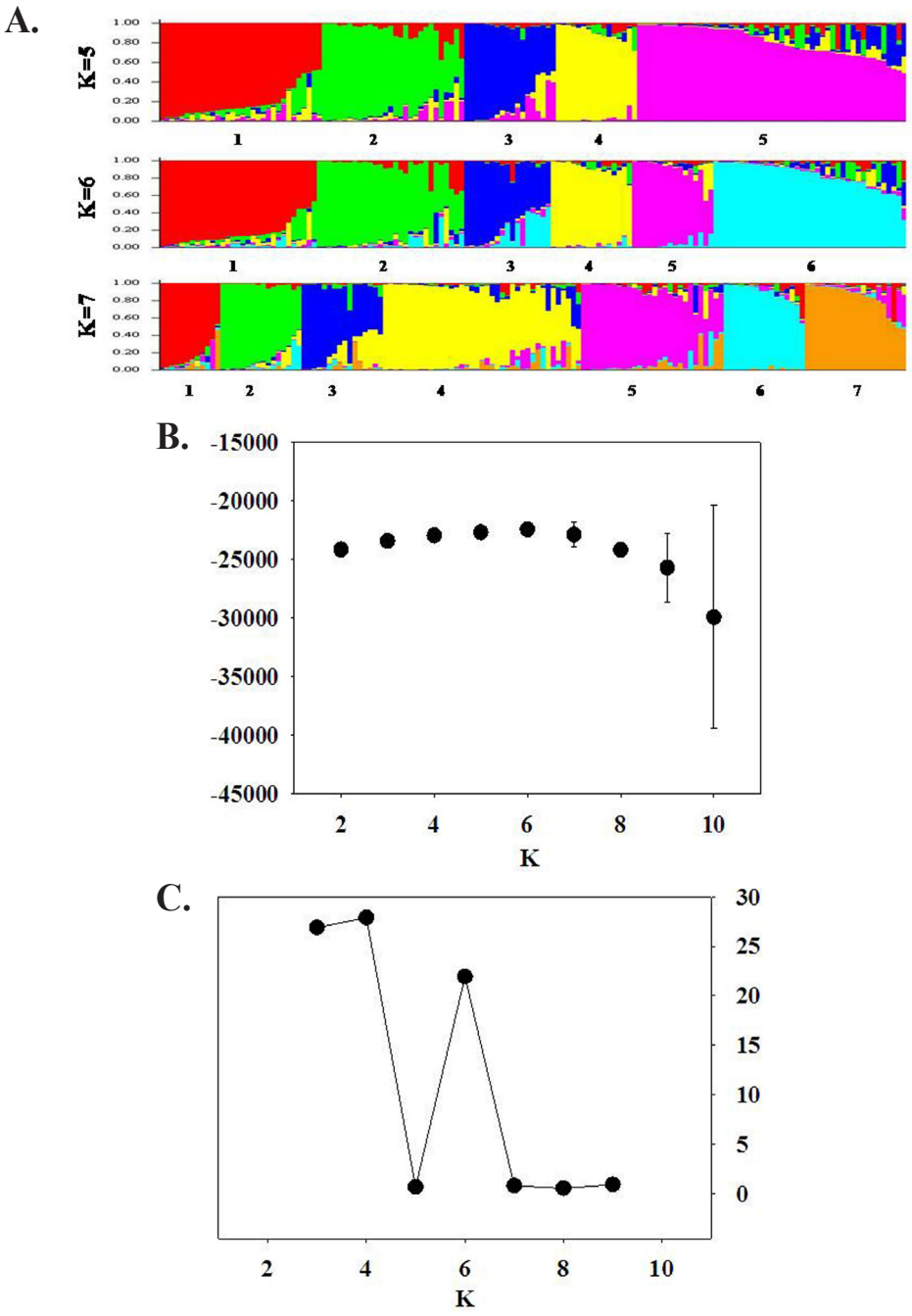

Figure 1. The genetic structure of the 147 Jerusalem artichoke accessions inferred by STRUCTURE and the sensitivity assessment of inference by STRUCTURE. **A.** genetic structure inferred by STRUCTURE with clusters of K = 5, 6, 7. **B.** The log-likelihood profiles for models with K = 2 to 10. **C.** The rates of change in log-likelihood for models with K = 2 to 10.

Genetic association

Clustering of the 147 Jerusalem artichoke accessions by neighbor-joining (NJ) analysis revealed several variation patterns (Figure 2). First, up to eight major clusters were detected, but they were not well separated due to relatively low RAPD resolution. Second, the accessions from the USA appeared to display in three separate groups; the accessions from Canada could form up to four groups; the accessions from the former Soviet Union also were well dispersed among several clusters; the accessions from Germany were located only in one large group. Third, the accessions collected from the wild populations in the USA were separated into two groups; the six accessions from Texas (JA69 to JA74) were located together in one cluster, while the other 15 accessions collected from other parts of the USA were grouped together. This may suggest the presence of geographical variation within this species. The last two patterns of genetic association were consistent with those inferred from the principal component analysis (PCA) of RAPD markers (Figure 3). For example, the accessions from the USA were allocated into multiple groups, so were the wild accessions. However, PCA clustering could identify only up to five major clusters with some level of separation. This difference could reflect the weakness of a PCA analysis to group individuals or the low resolution of RAPD markers.

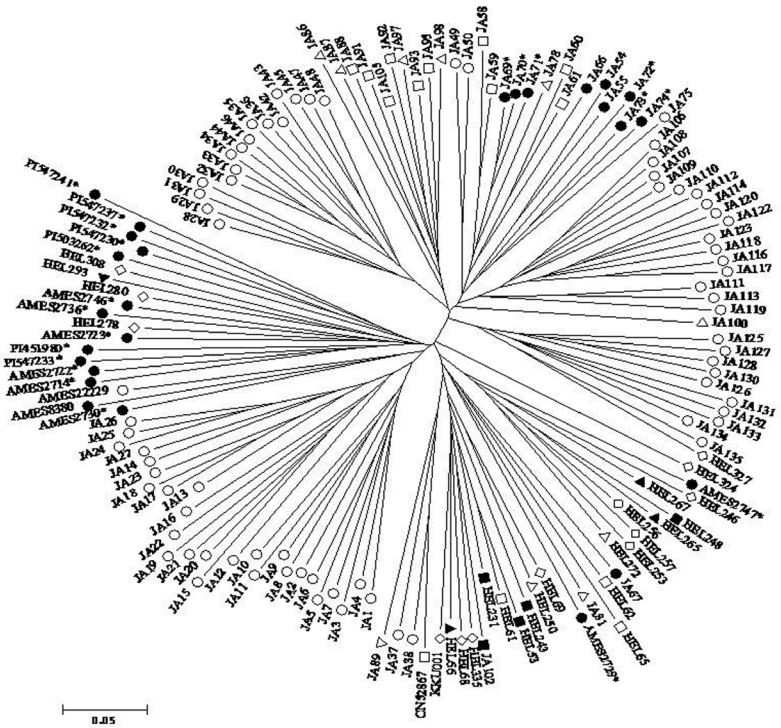

Figure 2. The neighbor-joining (NJ) tree displaying the genetic associations of the 147 Jerusalem artichoke accessions representing nine countries. Each accession is labeled with its country origin: open circle for Canada; filled circle for the USA; open square for the former Union of Soviet Socialist Republics (USSR); filled square for Germany; open triangle for France; filled triangle for other four countries (Hungry, Poland, Ukraine or the former Yugoslavia); and open diamond for unknown origin. The accession with a star was collected from a wild population in the USA.

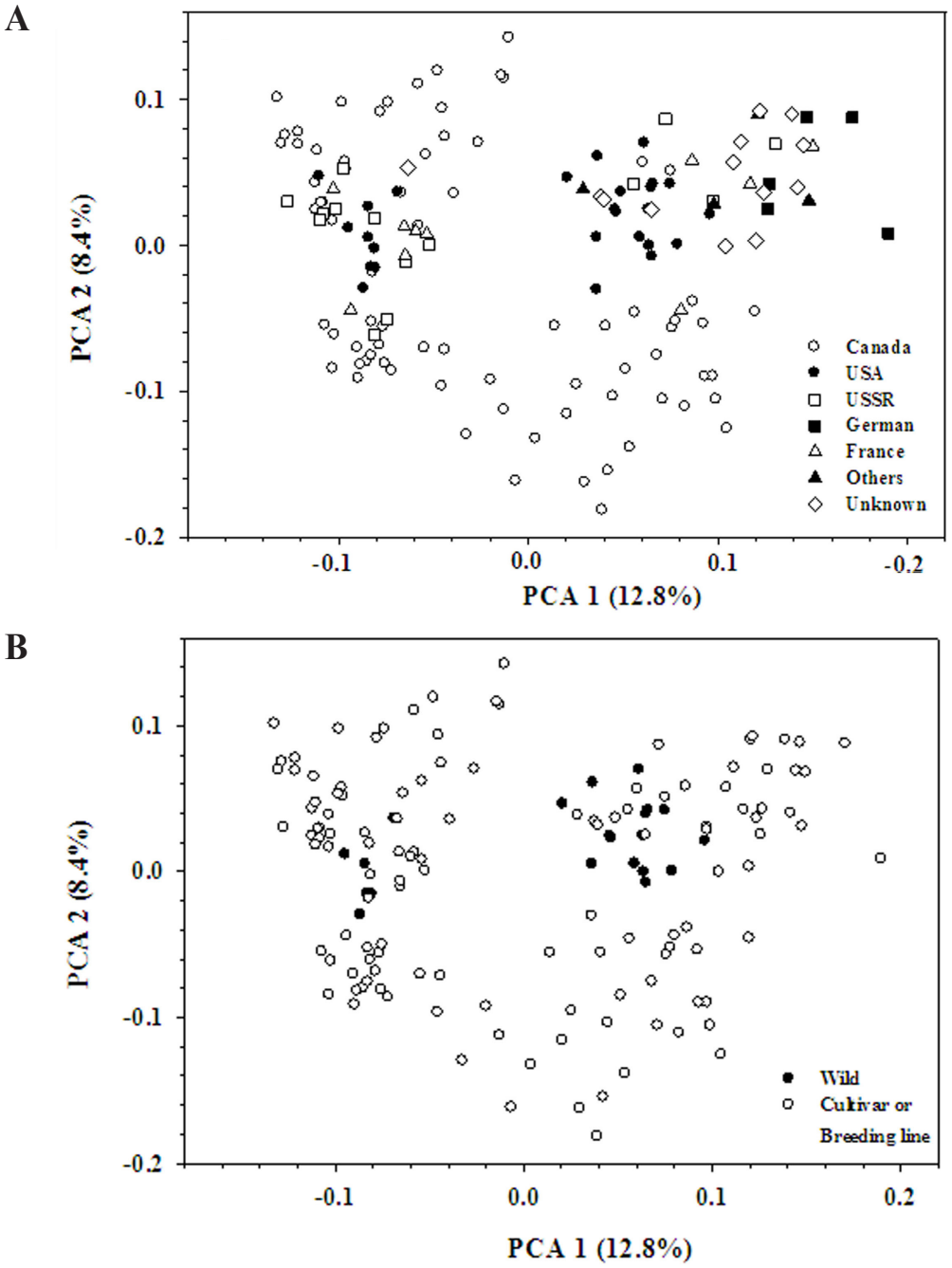

Figure 3. The genetic associations of 147 Jerusalem artichoke accessions inferred from the principal component analysis of 337 RAPD markers. Two sub-figures are the same, but labeled differently for various accession features. **A.** accessions are identified with country origin. **B.** accessions collected from wild populations in the USA are distinguished from cultivators or breeding lines.

The patterns of clustering revealed here are consistent with the six optimal clusters inferred as above from model-based STRUCTURE analysis. Although the detailed parentages of the breeding materials are unknown, the estimated genetic relationships could serve as a useful guide for parental selections in Jerusalem artichoke breeding, as they are more informative than parental selection and traditional pedigree analysis (Fu et al., 2009). The clustering by either NJ or PCA appeared to reveal the Canadian germplasm had four ancestral lines (Figure 2). These lines were quite distinguished from the germplasm from other countries. Also, the relationships revealed in Figure 2 and 3 also are useful to identify the origin of some germplasm. For example, the three accessions of unknown origin (HEL308, HEL280 and HEL278) were probably acquired from the USA collection, as they were closely related to those wild collections. Moreover, the accessions acquired from IPK, Germany, were originated from many countries, but they still were largely grouped together. This finding suggests a relatively narrow base in this set of germplasm, except for the three mentioned introductions from the USA wild collections. This may reflect the cultivated gene pool present in Europe, which are different from those in Canada.

Genetic distinctiveness

The genetic distinctiveness of a Jerusalem artichoke accession was measured by the average dissimilarity (AD) of the accession against the remaining accessions assayed. The higher the AD, the greater is the distinctiveness of the genetic background. The AD of the accessions ranged from 0.309 for JA105 to 0.402 for PI547241 with a mean of 0.341 (Table 1). The nine most distinctive accessions with AD of 0.37 or higher were PI547241, PI547233, AMES2714, HEL65, PI451980, AMES8380, AMES2722, HEL293 and AMES2736. These distinct accessions are mainly those wild collections in the USA. The nine less distinctive accessions with AD smaller than 0.318 were JA105, JA69, JA59, JA70, JA107, JA109, JA28, JA71 and JA93. These accessions are largely from breeding materials from the USA, Canada and the former Soviet Union.

The ADs shown in Table 1 are limited to only the 147 accessions assayed. The AD values would change if more accessions were assessed. This measure can recognize the distinctiveness, but not necessarily the relatedness, of accessions (Fu, 2006). For example, two closely related cultivars that were quite distinct from the remaining cultivars could have similar higher levels of AD than the others and both cultivars would have been identified as genetically distinct. It is important to recognize these limitations when the relative measure of genetic distinctiveness reported here is used as a guide for selecting specific germplasm with distinct genetic background in Jerusalem artichoke breeding.

CONCLUSIONS

The RAPD analysis reported here, although expected with low resolution, generated several interesting patterns of genetic variation in the 147 Jerusalem artichoke accessions. More than 93% RAPD variation resided within accessions of a country. Weak genetic differentiation was observed between wild and cultivated accessions. Six optimal groups were detected in this germplasm set. Four ancestral groups were found for the Canadian germplasm. Most genetically distinct accessions were identified. These findings provided the first set of

useful diversity information for understanding the Jerusalem artichoke gene pool, conserving Jerusalem artichoke germplasm, and utilizing distinctive germplasm for genetic improvement.

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