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Ex Situ Conservation of Vegetatively Propagated Species: Development of a Seed-based Core Collection for *Malus sieversii*

Gayle M. Volk[1], Christopher M. Richards, Ann A. Reilley, and Adam D. Henk
National Center for Genetic Resources Preservation, U.S. Department of Agriculture, 1111 S. Mason Street, Fort Collins, CO 80521

Philip L. Forsline
Plant Genetic Resources Unit, U.S. Department of Agriculture, Geneva, NY 14456-0462

Herb S. Aldwinckle
Department of Plant Pathology, Cornell University, Geneva, NY 14456-0462

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Abstract. Seeds and scionwood of *Malus sieversii* Lebed. have been collected from wild populations of apple trees in Kazakhstan. Seedlings and grafted trees were planted in the orchards at the U.S. Dept. of Agriculture Plant Genetic Resources Unit in Geneva, N.Y. We developed core collections to capture the genetic and phenotypic diversity represented in the trees from each of two of the Kazakhstan collection sites. These core collections capture more than 90% of the genetic diversity of the original populations, as determined using seven unlinked simple sequence repeat markers and 19 quantitative traits. Since phenotypic evaluations of these materials have been completed, the 35 trees within each population will be used as parents in crosses so that the genetic diversity in the orchard populations can be captured as seed for long-term ex situ conservation. This strategy of storing seeds, rather than maintaining costly field collections, could be applied to other collections of wild plant materials in the National Plant Germplasm System.

Malus sieversii, the main progenitor of the domesticated apple (*Malus* ×*domestica* Borkh.), is endemic to the Republic of Kazakhstan (Harris et al., 2002; Hokanson et al., 1997; Luby et al., 2001). U.S. Dept. of Agriculture (USDA)-sponsored plant exploration teams have visited Kazakhstan to collect wild apple germplasm in 1989, 1993, 1995, and 1996. More than 130,000 seeds were collected from a total of 892 wild *M. sieversii* trees. Scionwood was also collected from 43 trees across 12 locations that displayed desirable horticultural characteristics (Luby et al., 2001).

Between 10 and 20 open-pollinated seeds of each tree from which seeds were collected were planted at the USDA Plant Genetic Resources Unit (PGRU) in Geneva, N.Y. Detailed phenotypic evaluations have been completed on the seedlings from two sampling sites (6 and 9) from the 1995 and 1996 trips (Fig. 1). Seedlings have been identified with resistance to apple scab (*Venturia inaequalis* Cooke), fire blight (*Erwinia amylovora* Burrill), and cedar apple rust (*Gymnosporangium juniperi-virginianae* Schwein), as well as characteristics such as large and flavorful fruit (Forsline and Aldwinckle, 2004).

Molecular methods were used to evaluate diversity within several *Malus* L. collections. Simple sequence repeats (SSRs) are an effective molecular technique for fingerprinting individual *Malus* ×*domestica* varieties. Many of the described SSR loci have numerous alleles in *M.* ×*domestica*, so allelic diversity can be described using fewer than 10 primer pairs (Hokanson et al., 1998, 2001). Representative core collections have been developed using molecular techniques for USDA National Plant Germplasm System (NPGS) collections of *Malus* and *Vitis* L. (Hokanson et al., 1998, 2001; Lamboy and Alpha, 1998).

It is both expensive and risky to maintain wild seedling populations in the field indefinitely. Many of the trees are susceptible to diseases such as apple scab and fire blight. Since phenotypic characterizations under field conditions have been completed, the *M. sieversii* collection can be stored as seeds at –20 °C for future use.

This paper describes the assembly of a genetically representative set of seedling trees from 43 half-sib families from two

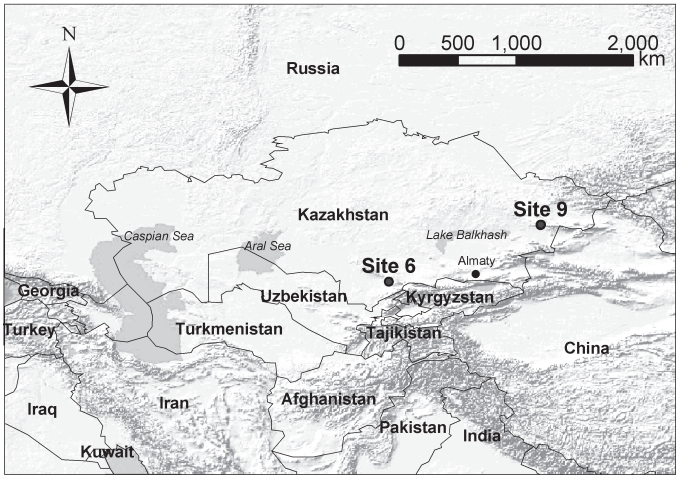

Fig. 1. Central Asia map shows the Kazakhstan site 6 and site 9 *M. sieversii* collection sites.

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[1]Corresponding author; e-mail: gvolk@lamar.colostate.edu

locations in Kazakhstan that were previously uncharacterized. Core collections representing these populations were identified using both phenotypic and genotypic data. These core collections represent more than 95% of the alleles present in the seedling populations and allow the curator to effectively capture the genetic diversity of these populations by performing directed crosses in the field.

Materials and Methods

Plant material. *Malus sieversii* plant materials were collected from 12 sites within Kazakhstan during exploration visits in 1989, 1993, 1995, and 1996. Seeds and scionwood from two of these sites, designated 6 and 9, were included in the present study (Fig. 1). Collectors at site 6 gathered seeds from trees along a stream (site 6.00; 600 m elevation) and in a xeric mixed scrub forest (site 6.01; 900 m elevation) in the Karatau Mountains (Fig. 2A). Overall, this dry region contained highly aromatic and firm fruit (Forsline et al., 2003, Luby et al., 2001). Seedlings from 14 half-sib families from site 6 were included in this study.

Seeds and scionwood were collected from 6 sub-sites (9.00–9.05) within the Semipalitinsk region of the Tarbagatai mountain range (Fig. 2B) in 1995 and 1996. Seedlings from 29 half-sib families and 12 grafted trees from scionwood from site 9 were included in this study. This dry continental forest is located 20 km north of Urdzhar and 3 to 5 km NE of Alexseyevka covering an area of 3.5 × 3 km. This region receives 400 mm of annual rainfall and has temperatures that range from +40 to –40 °C. The sub-sites within site 9 varied with respect to elevation (870 to 1120 m), environment (streamside vs. hillside), and tree density. Detailed site descriptions are presented by Dzhangaliev (2003) and Forsline et al. (2003).

Phenotypic characterization. Seedling trees produced from seeds collected from the 1995 and 1996 Kazakhstan expeditions were transplanted to an orchard setting (1997 for those collected in 1995, and 1998 for those collected in 1996) where disease resistance and fruit phenotypes were described for a collection of fruit-bearing seedling trees from each original half-sib family. Phenotypic characters included fire blight resistance, scab resistance, fruit flesh color, firmness, flavor, oxidation, juiciness, weight, shape texture, soluble solids percentage, fruit shape uniformity, fruit size uniformity, and harvest season. In addition, data were collected on fruit for ground color (base color), overcolor (color overlaying the base color), overcolor pattern and intensity, as well as russeting location, type, and coverage. All phenotypic characters were converted to categorical variables based on categories described in the Germplasm Resources Information Network (GRIN). Data were collected as described by Forsline and Aldwinckle (2004) and are available online (U.S. Dept. of Agriculture, 2004).

Molecular analysis. Genomic DNA from leaf tissue of 439 individual *M. sierversii* seedlings was extracted using DNeasy 96 plant kits (Qiagen, Valencia, Calif.). Two samples from each individual were collected and processed independently. *Malus* single sequence repeats (SSR) were amplified using unlinked primers (GD12, GD15, GD96, GD100, GD103, GD142, GD147, GD162) as described by Hokanson et al. (1998) and Hemmat et al. (2003). Forward primers, labeled with either IRD 700 or IRD 800, were obtained from MWG-Biotech (High Point, N.C.). Unlabeled reverse primers were purchased from IDT (Coralville, Iowa).

All polymerase chain reactions (PCR) were carried out in 15 μL total volume. For each reaction, 10 to 50 ng DNA template

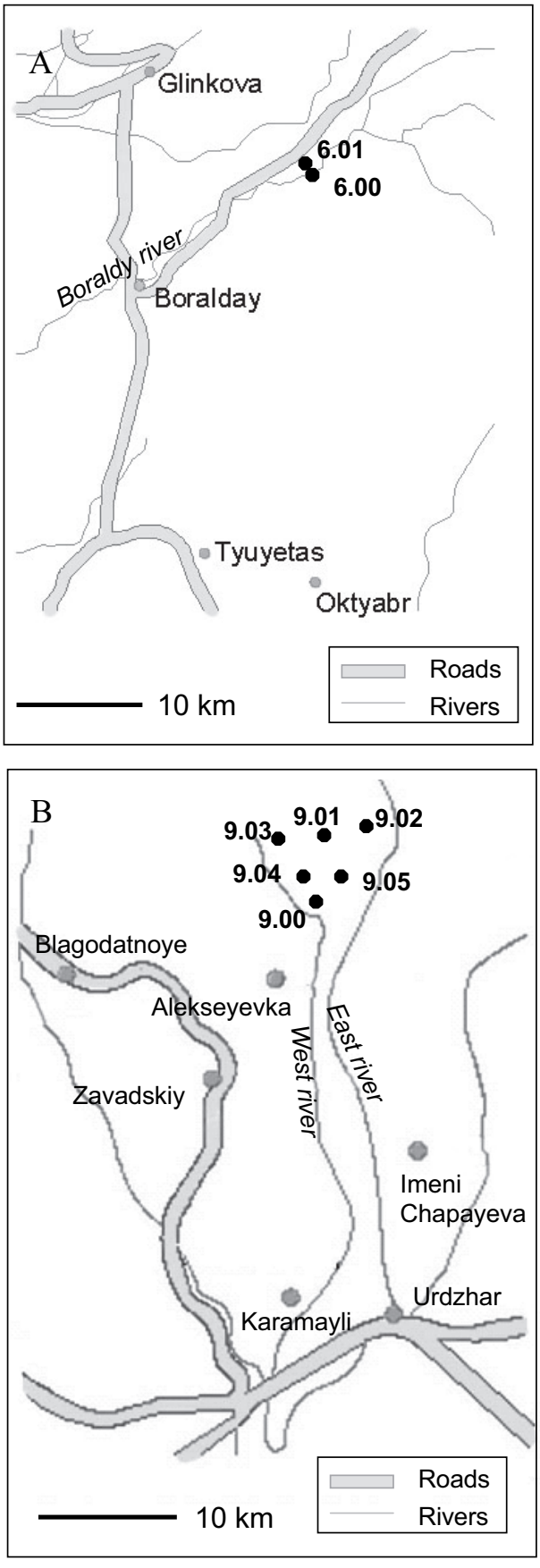

Fig. 2. *M. sieversii* accessions were collected from sites 6 (**A**) and 9 (**B**) in Kazakhstan.

and primers (0.3 to 0.7 pM) were combined with 1.5 units Taq Polymerase (Promega, Madison,Wis.), 1× Promega Magnesium free buffer (10 mM Tris-HCl, 50 mM KCl and 0.1% Triton X-100), 0.25 mM $MgCl_2$, and 0.25 mM dNTP.

PCR amplifications were carried out using MJ Research (Reno, Nev.) PTC 200 or Dyad Thermocyclers. A touch-down program reduced the annealing temperature 2° every other cycle starting at 63 °C and ending at 57 °C, followed by an annealing temperature of 55 °C for 20 additional cycles, and ended with a 2-min 72 °C extension. Completed PCR reactions were diluted 1:1 in 95% formamide, 50 mM EDTA, bromophenol blue loading dye, and denatured at 95 °C for 3 min. Gels (6.5% LI-COR KB Plus acrylamide; LI-COR, Lincoln, Nebr.) were run in 1× TBE (89 mM Tris, 89 mM boric acid, 20 mM EDTA) buffer for 1 h 45 min at 1500 V, 40 W, 40 mA, and 45 °C on a LI-COR 4200 DNA Sequencer. Digital images were collected from the sequencer using LI-COR Saga Generation2 software and were manually analyzed using the Saga software. Alleles from replicate samples were examined at each locus, and when alleles for replicates were not identical, data for that locus were entered as "missing" in subsequent analysis.

Genetic analysis and development of core collections. Genotypic data were initially analyzed for several descriptive measures of genetic diversity and divergence such as genetic differentiation (F_{st}), allelic richness, and gene diversity. Genetic analyses and significance testing were carried out using both GDA (Lewis and Zaykin, 2001) and FSTAT (Goudet, 1995).

Core collections were assembled for each sampling site using the maximization strategy outlined by Schoen and Brown (1995), and were implemented in the software package MSTRAT (Gouesnard et al., 2001). The MSTRAT algorithm maximizes the total number of alleles collected in a target core using equally weighted genotypic and phenotypic data. Genetic redundancy among individuals was examined using a feature of MSTRAT that plots samples of increasing sizes maximized for diversity against the amount of total diversity captured. If there is no redundancy and each individual contributes novel genetic diversity, an increase in core size is linearly related to an increase in core diversity. However, genetic redundancy is reflected by a curvilinear relationship where above some size class, further increases in sample sizes do not substantially increase the fraction of total diversity captured. These plots can be viewed as a saturation curve where the inflection point represents a minimum size of a core collection. Collections that are largely redundant saturate earlier (and require a small core size to capture most of the diversity) than collections that are not redundant. For each site, 10 alternative core collections with nearly equivalent maximal genetic diversity were assembled in MSTRAT by iteratively sampling individuals and evaluating their contribution to allelic diversity. A single core group was selected by choosing the core collection that represented the greatest number of half-sib populations and displayed the highest number of accessions with disease resistance characteristics.

Results

DNA was extracted from 174 *M. sieversii* seedlings from site 6, and 278 seedlings and trees grafted from scions from site 9 (Table 1). The original plantings included 591 individuals in 43 half-sib families, but some of these individuals were not alive when leaves were selected for DNA sampling in 2002.

As was reported previously, genetic variation was greater within each of the two sites than between the two sites (Lamboy et al., 1996). F_{st} values among collection sites 6 and 9 were modest but significant (F_{st} = 0.07, ±0.05 by bootstrap process, $P < 0.05$ by permutation), whereas the differentiation among half-sib families within a location was large (F_{st} = 0.19, ±0.04). Allelic richness (Petit et al., 1998) between the two regions was significantly different with each region having a set of private alleles. These findings support the creation of core collections for each sampling site.

For both site 6 and site 9, saturation analyses revealed that >95% of the possible variation could be captured with 35 member core collections (Fig. 3 A and B). While these estimates were robust using genetic data, some trees from which genotypes were taken in 2002 died or did not flower in 2004 (Table 1). We compared redundancy curves with all tree samples taken in 2002 (core-1), with all healthy, live tree samples in 2004 (core-2), and with all flowering tree samples in 2004 (core-3) to determine how mortality and phenology impacted the assembly of these core collections.

The site 6 core-1 collection included 35 accessions from the original 174 accessions. However, 14 of the original 174 accessions were not healthy and another 36 accessions were excluded from the original dataset because they were not flowering. Therefore, core-3 included 35 accessions selected from a population of 124 individuals. Similarly, the original dataset for site 9 had 278 accessions, but the core-3 collection was selected from only 170 of those accessions that were healthy and flowering in 2004. Despite a 29% or 39% reduction in the original population size, a 6% and 7 % reduction in diversity was noted between the core-1 and core-3 collections from sites 6 and 9, respectively (Fig. 3 A and B). One entire half-sib family was lost from sub-site 9.00.

For both sites, the frequency of alleles in the entire genotyped population was very highly correlated with allele frequency in the core-1, core-2, and core-3 collections (Fig. 4 A,B). Some alleles were lost as individuals were eliminated from the original populations. Site 6 had 64 alleles in the original population as determined in our SSR analyses. Core-1 included 63 of these alleles, while core-2 and core-3 had 61 and 60 alleles, respectively, represented. For site 9, the core-1, core-2, and core-3 collections included 62, 57, and 57 alleles of the original 64 alleles. In all, 72 of the total 76 alleles in the combined populations were captured in the core-3 collections from sites 6 and 9.

The core-3 collection for site 6 included representatives of each of the 14 half-sib families. All of the subsampling sites were represented in the site 9 core-3 collection. Eight of the 28 half-sib families for which DNA was available were not represented in the site 9 core-3 collection (Tables 1–3).

We further validated the core collections by comparing allelic and phenotypic frequencies between the original and core-3 collection populations. The core-3 collections represented individuals with disease resistance. Within the original populations at site 6 that were screened for disease resistance, there were 64% and 42% incidences of fire blight and scab resistance, respectively. The site 6 core-3 collection had 69% and 46% incidences of fire blight and scab resistance. In the original populations at site 9 screened for disease resistance, there were 51% and 37% incidence rates of fire blight and scab resistance, respectively. Sixty-three percent and 49% of the individuals in the core-3 collection exhibited fire blight and scab resistance, respectively. Thus, the selected core-3 collections provide valuable alleles for disease resistance from the original populations.

Table 1. *Malus sieversii* half-sib families from Kazakhstan collection sites 6 and 9.

Identification	Collection year	Site	Maternal ID	Trees sampled	Fruit sampled	Seeds collected	Seedlings in field	Sampled for DNA	Alive in 2004	Flowering in 2004
				#	#	#	#	#	#	#
GMAL 3975	1995	6.00		1	8	60	14	11	11	8
GMAL 3989	1995	6.00		1	8	--	14	14	14	14
GMAL 3999	1995	6.00		1	8	72	15	12	11	8
GMAL 4000	1995	6.00		1	8	43	14	11	7	5
GMAL 4002	1995	6.00		1	8	61	16	16	16	16
GMAL 3682	1995	6.01		1	150	933	12	11	11	6
GMAL 3683	1995	6.01		1	150	820	13	13	10	9
GMAL 3684	1995	6.01		1	70	325	15	13	10	8
GMAL 3685	1995	6.01		1	40	148	15	9	9	7
GMAL 3687	1995	6.01		1	150	645	14	9	9	4
GMAL 3688	1995	6.01		1	200	1225	15	13	12	6
GMAL 3689	1995	6.01		1	60	270	17	15	14	12
GMAL 3690	1995	6.01		1	150	760	15	14	14	11
GMAL 3691	1995	6.01		1	150	694	15	13	12	10
Total					1160	6056	204	174	160	124
GMAL 3607	1995	9.00	PI 613970	1	30	1020	15	5	3	0
GMAL 3762	1995	9.00		1	5	48	15	12	10	7
GMAL 3764	1995	9.00		1	5	45	14	14	12	7
GMAL 3775	1995	9.00		1	5	36	13	8	8	5
GMAL 3781	1995	9.00		1	5	33	13	12	10	9
GMAL 3784	1995	9.00		1	5	35	15	6	2	2
GMAL 3785	1995	9.00		1	5	32	12	3	3	3
GMAL 3616	1995	9.01	PI 613973	1	30	227	15	12	9	8
GMAL 3619	1995	9.01	PI 613974	1	30	250	13	8	6	6
GMAL 3626	1995	9.02		1	30	275	13	7	6	5
GMAL 3627	1995	9.02		1	60	326	13	13	12	12
GMAL 3629	1995	9.02		1	30	287	15	13	13	11
GMAL 3631	1995	9.02		1	30	229	13	3	2	1
GMAL 3608	1995	9.03	PI 613971	1	150	646	14	11	10	8
GMAL 3610	1995	9.03		1	30	124	12	10	9	9
GMAL 3614	1995	9.03	PI 613972	1	30	228	12	10	7	6
GMAL 3620	1995	9.04		1	15	55	13	11	10	9
GMAL 3622	1995	9.04		1	40	255	13	12	8	8
GMAL 3623	1995	9.04	PI 613975	1	30	218	13	6	6	5
GMAL 3625	1995	9.04	PI 613976	1	15	232	14	8	5	5
GMAL 3635	1995	9.05		1	30	256	13	4	3	3
GMAL 3636	1995	9.05	PI 613978	1	35	324	14	10	7	2
GMAL 3637	1995	9.05	PI 613979	1	50	323	15	11	5	4
GMAL 3638	1995	9.05		1	30	255	12	10	8	3
GMAL 3643	1995	9.05	PI 613977	1	150	1035	14	9	7	3
GMAL 4020	1996	9.05	PI 613954	1	4	32	12	10	10	5
GMAL 4024	1996	9.05	PI 613987	1	100	512	25	17	12	8
GMAL 4103	1996	9.05		60	8	72	5	4	2	1
GMAL 4155	1996	9.05		60	8	41	7	7	5	3
PI 613970	1996	9.00		1			1	1	1	1
PI 613973	1996	9.01		1			1	1	1	1
PI 613974	1996	9.01		1			1	1	1	1
PI 613971	1996	9.03		1			1	1	1	1
PI 613972	1996	9.03		1			1	1	1	1
PI 613975	1996	9.04		1			1	1	1	1
PI 613976	1996	9.04		1			1	1	1	1
PI 613978	1996	9.05		1			1	1	1	1
PI 613979	1996	9.05		1			1	1	1	1
PI 613977	1996	9.05		1			1	1	1	1
PI 613954	1996	9.05		1			1	1	1	1
PI 613987	1996	9.05		1			1	1	1	1
Total					995	7451	399	278	222	170

Discussion

Many research programs are utilizing wild *Malus* germplasm from Kazakhstan to increase disease resistance and stress tolerance for new apple cultivars (Forsline et al., 2003). However, few studies have looked at the molecular diversity of the wild materials. Lamboy et al. (1996) used 259 *M. sieversii* seedlings belonging to 31 half-sib families to determine allozyme diversity across four regions in Kazakhstan. Those plant materials were collected in 1989 and 1993. In those analyses, 85% of the enzyme variability was due to differences among half-sib families within locations, and only 15% was due to differences among regions. They concluded that thoroughly collecting from a few large populations effectively captured the diversity of this species.

Based on our SSR results, we also conclude that many alleles are shared between the *M. sieversii* seedlings from sites 6 and 9; however, allele frequencies do vary, as shown using F_{st} calculations and by comparing allelic richness estimates. The inherent

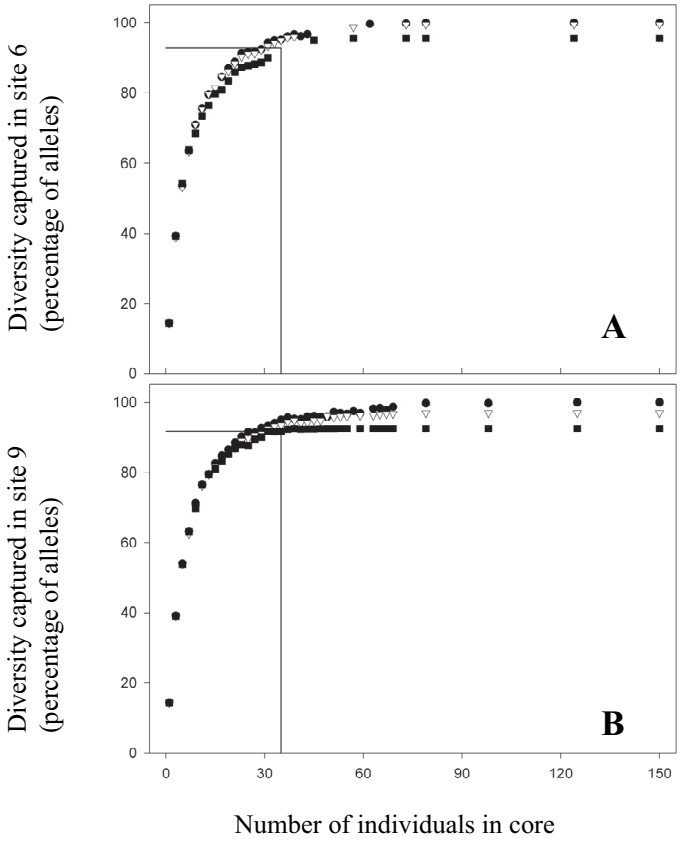

Fig. 3. Redundancy plots are shown for site 6 (**A**) and site 9 (**B**). Each plot shows the accumulation of allelic and phenotypic diversity with increasing core size. Diversity is represented as a percentage out of the total number of allelic and phenotypic states in the collection. Each point represents the mean of 20 runs using the maximization algorithm in MSTRAT software. The convex shape of the curve suggests that above a critical point adding more individuals to a core does not greatly increase diversity in the core collection. The intersection of the vertical and horizontal lines marks the size and diversity of the core collections proposed in this study. In each plot, the redundancy analyses are shown for the living seedlings in 2002 (circles), healthy seedlings in 2004 (triangles) and trees flowering in 2004 (squares) that were used to generate the core-1, core-2 and core-3 collections, respectively. In site 6 (**A**) the core-3 contains 35 individuals and captures 95.5% of the diversity and in site 9 (**B**) the core-3 contains 35 individuals and captures 92.5% of the diversity.

redundancy of alleles in the two populations allowed us to capture 90% of the alleles in the original population in the core-3 collections, despite the loss of 29% to 39% of the individuals from mortality, poor health, or "unwilling" phenology.

Core collections have been established for many plant collections to assist in collection maintenance and distribution. Often core collections are established based on sample geographical data and morphological descriptors (Clark et al., 1997; Holbrook et al., 1993; Skinner et al., 1999; Tohme et al., 1995). Neutral markers such as those available from isozymes, amplified fragment length polymorphisms, or SSR analyses also provide valuable data on allelic diversity and richness for the development of core collections (Clark et al., 1997). When core collections are designed based on genotypic characters, genetic representation is most complete (McKhann et al., 2004).

Most models use plant collections with thousands of accessions to base estimates for optimal core collection size. These models suggest that selection of 10% of the original accessions should retain roughly 70% of the alleles in the total collection (Schoen and Brown, 1995). Generally, this strategy preserves alleles that are not widespread, but may provide important sources of diversity (Bataillon et al., 1996; Schoen and Brown, 1995). A main objective of the study presented here was to identify individuals that effectively capture allelic and phenotypic diversity within two sites in Kazakhstan. The core collections we developed serve as sources for alleles, rather than sources of genotypes or clones. The maximization strategy used includes individuals in the core collection with high allelic richness and low redundancy. We determined that 35 accessions effectively capture greater than 95% of the alleles within the *M. sieversii* populations in sites 6 and 9. The designated core collections include more than 10% of the total accessions. For site 6, these 35 accessions represent 20% and 28% of the original and flowering populations, respectively. For site 9, these 35 accessions represent 13% and 21% of the original and flowering populations, respectively. Since the accessions from site 6, overall, have a greater allelic diversity per allele than the accessions from site 9, the core includes a larger percentage of individuals. The core size of 35 individuals per site falls within the range of 25 to 100 accessions per ecogeographical area suggested by Crossa et al. (1993).

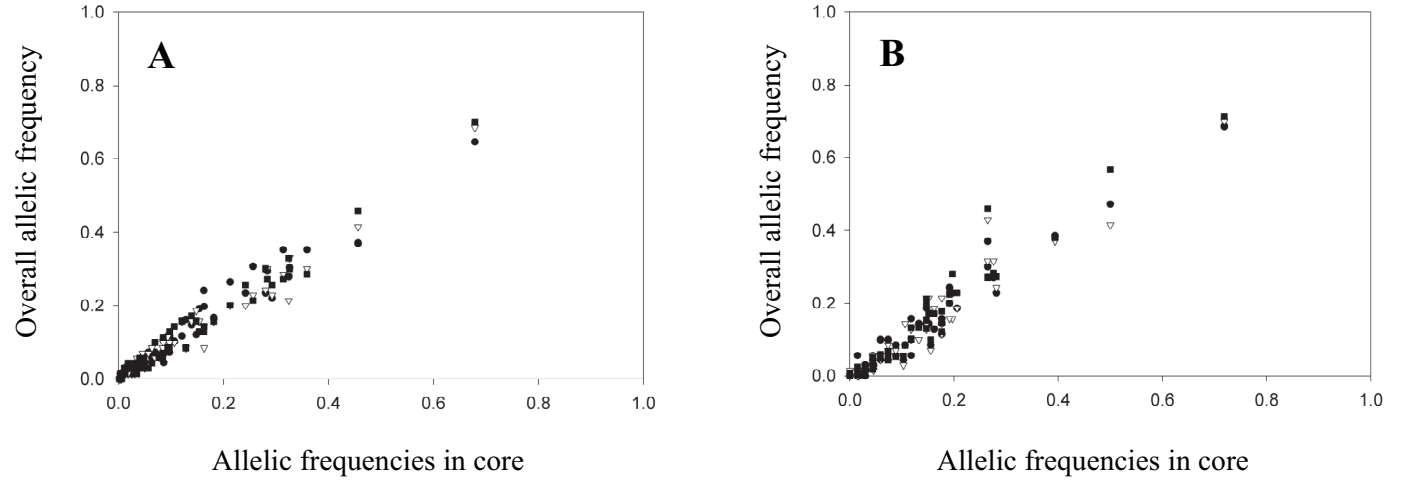

Fig. 4. Bi-plots show allelic frequencies in the core collection versus the complete collection of *M. sieversii* collected from Kazakhstan sites 6 (**A**) and 9 (**B**). These scatter plots show a strong positive correlation between allelic frequencies in core-1, core-2, and core-3 collections and their respective source collection (circles represent alleles from living seedlings in 2002, triangles represent alleles from healthy seedlings in 2004 and squares represent alleles from trees flowering in 2004). These data suggest that the core collections are genetically representative.

Table 2. *M. sieversii* phenotypic characterization data is provided for site 6 in Kazahstan.

Identification number	Subsite	Year collected	Characterization date	Fire blight resistance	Scab resistance	Flesh color	Flesh firmness	Flesh flavor	Flesh oxidation	Fruit ground color	Fruit juiciness	Weight (g)	Fruit overcolor	Overcolor on fruit %	Fruit overcolor pattern
GMAL 3682.e	6.01	1995	-	Resistant	Susceptible	-	-	-	-	-	-	-	-	-	-
GMAL 3682.f	6.01	1995	-	Resistant	Susceptible	-	-	-	-	-	-	-	-	-	-
GMAL 3682.k	6.01	1995	-	Resistant	Susceptible	-	-	-	-	-	-	-	-	-	-
GMAL 3683.d	6.01	1995	2001	Susceptible	Susceptible	Cream, green	Semifirm	Subacid	>10%	Lt green	Dry	25	Red	60	Striped
GMAL 3683.l	6.01	1995	2002	Resistant	Resistant	Cream, green	Firm	Astringent	>10%	Lt green	Dry	32	None	0	None
GMAL 3683.n	6.01	1995	2001	Susceptible	Susceptible	Cream, green	Firm	Acid	>10%	Green	Dry	32	Brown	10	Striped
GMAL 3684.a	6.01	1995	2003	Resistant	Resistant	Cream	Semifirm	Astringent	>10%	Green	Dry	35	Red	30	Striped
GMAL 3684.b	6.01	1995	2001	Susceptible	Susceptible	Cream, green	Semifirm	Subacid	>10%	Green	Dry	52	None	-	None
GMAL 3684.l	6.01	1995	2001	Susceptible	Resistant	Cream, green	Semifirm	Astringent	>10%	Green	Dry	35	Red	50	Blush
GMAL 3685.d	6.01	1995	2002	Susceptible	Resistant	Cream, green	Semifirm	Astringent	>10%	Green	Dry	34	Red	20	Striped
GMAL 3685.e	6.01	1995	2003	Resistant	Susceptible	Cream, green	Firm	Astringent	>10%	Green	Dry	23	Brown	10	Striped
GMAL 3685.f	6.01	1995	2002	Resistant	Susceptible	Cream, green	Semifirm	Astringent	>10%	Lt green	Dry	24	Pink	5	Blush
GMAL 3687.d	6.01	1995	-	Resistant	Susceptible	-	-	-	-	-	-	-	-	-	-
GMAL 3687.h	6.01	1995	2001	Susceptible	Susceptible	White	Soft	Sweet	5-10%	Green	Dry	47	None	0	None
GMAL 3688.n	6.01	1995	2003	Resistant	Resistant	Cream, green	Firm	Subacid	>10%	Lt green	Medium	33	None	-	None
GMAL 3689.c	6.01	1995	2001	Resistant	Resistant	Cream, green	Semifirm	Astringent	>10%	Lt green	Moderate	30	Red	1	Blush
GMAL 3689.n	6.01	1995	2003	Resistant	Resistant	Cream, green	Semifirm	Acid	>10%	Green	Medium	44	Pink	5	Striped
GMAL 3689.p	6.01	1995	2002	Resistant	Susceptible	Cream, green	Semifirm	Astringent	>10%	Lt green	Dry	28	-	-	-
GMAL 3690.d	6.01	1995	2001	Susceptible	Susceptible	Cream, green	Semifirm	Subacid	5-10%	Green	Dry	44	Pink	20	Striped
GMAL 3690.o	6.01	1995	2001	Resistant	Susceptible	Cream, green	Soft	Acid	1-4%	Lt green	Very dry	23	Brown	60	Striped
GMAL 3691.j	6.01	1995	-	Resistant	Susceptible	-	-	-	-	-	-	-	-	-	-
GMAL 3691.m	6.01	1995	2001	Susceptible	Susceptible	Cream, green	Semifirm	Subacid	>10%	Lt green	Very dry	38	Red	60	Blush
GMAL 3975.d	6.01	1995	2001	Resistant	Resistant	Cream, green	Firm	Subacid	>10%	Green	Medium	39	Red	10	Striped
GMAL 3975.g	6.01	1995	2001	Susceptible	Susceptible	Cream	Soft	Subacid	>10%	Green	Medium	26	None	0	None
GMAL 3975.k	6.01	1995	2001	Resistant	Resistant	Cream, green	Soft	Aromatic	0-1%	Yellow	Dry	39	None	0	None
GMAL 3975.l	6.01	1995	2000	Resistant	Resistant	Green	Hard	Subacid	5-10%	Green	Very juicy	47	Yellow	20	Blush
GMAL 3975.m	6.01	1995	2002	Resistant	Resistant	Cream, green	Semifirm	Subacid	>10%	Lt green	Dry	18	-	-	-
GMAL 3989.f	6.01	1995	2002	Resistant	Susceptible	Cream	Soft	Subacid	>10%	Lt green	Very dry	44	Pink	5	Striped
GMAL 3989.k	6.01	1995	2002	Resistant	Resistant	Cream, green	Soft	Subacid	>10%	Green	Medium	34	None	0	None
GMAL 3999.b	6.01	1995	2003	Resistant	Susceptible	White	Firm	Astringent	>10%	Green	Dry	19	Pink	1	Striped
GMAL 4000.b	6.01	1995	2003	Resistant	Resistant	Cream, green	Semifirm	Subacid	>10%	Green	Medium	16	Red	20	Striped
GMAL 4000.g	6.01	1995	2003	Susceptible	Susceptible	Cream, green	-	-	1-4%	Lt green	Dry	27	Pink	40	Striped
GMAL4002.d	6.01	1995	2002	Resistant	Resistant	Cream	Semifirm	Astringent	>10%	Lt green	Dry	21	Yellow	40	Blush
GMAL 4002.e	6.01	1995	2001	Resistant	Resistant	Cream, green	Semifirm	Astringent	5-10%	Lt green	Medium	29	None	0	None
GMAL 4002.h	6.01	1995	2002	Susceptible	Resistant	Cream, green	Soft	Subacid	>10%	Lt green	Very dry	34	-	-	Striped

Table 3. *M. sieversii* phenotypic characterization data is provided for site 9 in Kazakhstan.

Identification number	Subsite	Year collected	Characterization date	Fire blight resistance	Scab resistance	Flesh color	Flesh firmness	Flesh flavor	Flesh oxidation	Fruit ground color	Fruit juiciness	Weight (g)	Fruit overcolor	Overcolor on fruit %	Fruit overcolor pattern
GMAL 3764.e	9.00	1995	2002	Susceptible	Susceptible	Cream, yellow	Semifirm	Subacid	>10%	Lt green	Medium	47	Pink	5	Blush
GMAL 3764.l	9.00	1995	2002	Resistant	Susceptible	Cream, green	Semifirm	Subacid	>10%	Lt green	Very dry	29	Red	40	Striped
GMAL 3762.g	9.00	1995	2000	Resistant	Resistant	White	Soft	Subacid	0-1%	Green	Very dry	50	Yellow	60	Blush
GMAL 3762.n	9.00	1995	2003	Susceptible	Resistant	White, cream	-	-	1-4%	Lt green	Dry	29	None	0	None
GMAL 3781.b	9.00	1995	2003	Resistant	Susceptible	White	Soft	Subacid	>10%	Lt green	Dry	22	Yellow	90	Blush
GMAL 3781.c	9.00	1995	2003	Resistant	Susceptible	Pink	Firm	Astringent	>10%	Green	Dry	14	Red	90	Blush
GMAL 3781.h	9.00	1995	2002	Susceptible	Resistant	Cream	Soft	Subacid	>10%	Lt green	Moderate	11	None	-	-
GMAL 3781.n	9.00	1995	-	Resistant	Resistant	-	-	-	-	-	-	-	-	-	-
GMAL 3784.d	9.00	1995	2000	Susceptible	Resistant	Cream, green	Soft	Aromatic	>10%	Green	Medium	76	Yellow	20	Blush
GMAL 3785.b	9.00	1995	2003	Resistant	Susceptible	Cream	Semifirm	Astringent	>10%	Green	Medium	13	None	-	-
GMAL 3785.k	9.00	1995	2003	Susceptible	Resistant	Cream, yellow	Semifirm	Astringent	>10%	Lt green	Very dry	25	Yellow	95	Blush
GMAL 3616.d	9.01	1995	2001	Resistant	Susceptible	White, green	Soft	Subacid	>10%	Green	Dry	42	None	-	-
GMAL 3619.j	9.01	1995	2001	Resistant	Susceptible	Cream	Soft	Sweet	5-10%	Lt green	Medium	56	Orange	10	Blush
GMAL 3619.m	9.01	1995	2001	Resistant	Susceptible	Cream, green	Semifirm	Subacid	>10%	Lt green	Medium	50	Yellow	5	Striped
GMAL 4103.a	9.01	1996	2003	Resistant	Resistant	White	Soft	Subacid	1-4%	Lt yellow	Very dry	24	None	-	-
GMAL 3627.a	9.02	1995	2003	Resistant	Susceptible	Cream	Semifirm	Subacid	>10%	Lt green	Dry	34	None	-	-
GMAL 3627.l	9.02	1995	2002	Resistant	Resistant	Cream	Semifirm	Astringent	>10%	Lt yellow	Medium	27	Red	25	Striped
GMAL 3629.n	9.02	1995	2002	Susceptible	Resistant	Cream, green	Soft	Acid	>10%	Lt green	Dry	40	None	-	-
GMAL 4024.n	9.02	1996	2003	Susceptible	Resistant	Cream	Semifirm	Sweet	5-10%	Green	Dry	37	Red	75	Striped
GMAL 3608.b	9.03	1995	2002	Resistant	Susceptible	Cream	Soft	Aromatic	>10%	Lt green	Dry	38	Red	10	Striped
GMAL 3610.b	9.03	1995	2001	Resistant	Resistant	Cream, green	Semifirm	Astringent	>10%	Lt green	Medium	23	Pink	25	Blush
GMAL 3610.l	9.03	1995	2002	Resistant	Resistant	Cream, green	Semifirm	Astringent	>10%	Lt green	Medium	21	-	-	-
GMAL 3614.a	9.03	1995	2001	Susceptible	Resistant	Cream, green	Semifirm	Astringent	>10%	Lt green	Medium	33	Pink	30	Blush
GMAL 3614.g	9.03	1995	2001	Resistant	Resistant	Cream, green	Semifirm	Acid	>10%	Green	Dry	62	Brown	40	Blush
PI 613972.2	9.03	1995	2003	Susceptible	Susceptible	White	Semifirm	Subacid	1-4%	Yellow	Very dry	114	Pink	5	Blush
GMAL 3620.e	9.04	1995	-	Resistant	Susceptible	-	-	-	-	-	-	-	-	-	-
GMAL 3620.m	9.04	1995	2001	Resistant	Resistant	Cream, green	Semifirm	Astringent	>10%	Lt yellow	Dry	50	Red	5	Blush
GMAL 3622.m	9.04	1995	2001	Resistant	Susceptible	Cream, green	Semifirm	Astringent	>10%	Lt yellow	Very dry	28	Pink	10	Splashed
GMAL 3623.e	9.04	1995	2003	Susceptible	Susceptible	Cream	Hard	Acid	>10%	Green	Moderate	47	Red	65	Striped
GMAL 3623.f	9.04	1995	2002	Resistant	Susceptible	Cream, green	Semifirm	Subacid	>10%	Lt green	Dry	63	-	-	-
GMAL 3625.a	9.04	1995	2002	Susceptible	Resistant	Cream, green	Semifirm	Astringent	>10%	Lt green	Medium	43	-	-	-
PI 613975.1	9.04	1995	2003	Susceptible	Susceptible	Cream, green	Semifirm	Aromatic	>10%	Lt green	Medium	158	Pink	65	Striped
GMAL 3636.h	9.05	1995	-	Resistant	Resistant	-	-	-	-	-	-	-	-	-	-
GMAL 3638.b	9.05	1995	2003	Susceptible	Not tested	Cream, green	Semifirm	Subacid	5-10%	Lt green	Dry	65	Pink	10	Striped
GMAL 4020.l	9.05	1996	2002	Resistant	Susceptible	Cream, yellow	Semifirm	Subacid	>10%	Lt yellow	Medium	11	Red	80	Striped

Fruit surface with russet	Fruit russet	Fruit russet	Fruit shape	Fruit shape	Fruit size	Fruit texture	Harvest season	Soluble solids
%	location	type		uniformity	uniformity			%
	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
1	Pedicel	Extr. fine	Globose-conical	Variable	Variable	Coarse	Medium/late	13.8
0	None	None	Globose	Uniform	Uniform	Medium	-	11.8
5	Pedicel	Extr. fine	Globose	Variable	Variable	Coarse	Medium/late	12.6
2	Pedicel, calyx	Extr. fine	Flat-globose	Variable	Variable	Coarse	Medium	10.6
1	Pedicel	Extr. fine	Globose-conical	Uniform	Uniform	Medium	Early	11.0
10	Pedicel	Med. heavy	Globose-conical	Variable	Variable	Coarse	Medium/early	14.3
5	Entire fruit	Extr. fine	Flat-globose	Uniform	Uniform	Medium	-	12.4
10	Pedicel	Extr. fine	Globose	Uniform	Variable	Medium	Late	14.1
4	Entire fruit	Extr. fine	Globose	Uniform	Variable	Coarse	-	12.5
-	-	-	-	-	-	-	-	-
5	Pedicel	Extr. fine	Flat-globose	Variable	Uniform	Medium	Medium/late	13.5
3	Pedicel	Extr. fine	Flat-globose	Variable	Uniform	Medium	Early	9.9
1	Pedicel	Extr. fine	Globose	Uniform	Uniform	Coarse	Medium	13.7
2	Pedicel, calyx	Extr. fine	Flat-globose	Variable	Uniform	Medium	Late	11.2
1	Pedicel, calyx	Med. heavy	Globose-conical	Variable	Uniform	Medium	-	11.5
1	Pedicel	Extr. fine	Globose-conical	Uniform	-	Coarse	Extremely early	12.0
5	Pedicel	Extr. fine	Globose	Variable	Variable	Coarse	Early	12.0
-	-	-	-	-	-	-	-	-
1	Pedicel	Extr. fine	Globose	Uniform	Uniform	Medium	Medium	10.7
5	Pedicel	Extr. fine	Globose	Uniform	Uniform	Coarse	Medium/late	13.5
5	Pedicel	Extr. fine	Globose	Uniform	Uniform	Fine	Medium/late	11.5
1	Pedicel	Extr. fine	Globose-conical	Variable	Variable	Medium	Medium	12.1
-	-	-	Globose-conical	Uniform	Uniform	Fine	Early	11.5
5	Entire fruit	Extr. fine	Flat-globose	Uniform	Variable	Medium	-	11.6
1	Pedicel	Extr. fine	Flat-globose	Variable	Uniform	Fine	-	10.7
10	Entire fruit	Med. heavy	Globose	Variable	Variable	Medium	-	12.8
1	Pedicel	Extr. fine	Conical	Variable	Variable	Coarse	Medium/early	12.9
1	Calyx	Extr. fine	Globose-conical	Variable	Variable	Coarse	Early	11.0
1	Pedicel	Extr. fine	Globose	Uniform	Uniform	-	Very early	9.7
2	Calyx	Extr. fine	Flat-globose	Uniform	Variable	Medium	-	11.1
5	Pedicel	Extr. fine	Globose-conical	Variable	Variable	Medium	Medium	11.8
2	Pedicel, calyx	Med. heavy	Globose	Variable	Variable	Coarse	-	12.2

Fruit surface with russet	Fruit russet	Fruit russet	Fruit shape	Fruit shape	Fruit size	Fruit texture	Harvest season	Soluble solids
%	location	type		uniformity	uniformity			%
1	Pedicel	Extr. Fine	Globose	Variable	Variable	Medium	-	10.1
-	-	-	Flat-globose	Uniform	Uniform	Medium	-	11.0
-	-	-	Globose	Uniform	Uniform	Fine	Very early	10.7
1	Pedicel	Extr. Fine	Globose	Uniform	Uniform	-	Very early	10.1
1	Pedicel, calyx	Extr. Fine	Flat-globose	Variable	Variable	Medium	Early	8.0
1	Pedicel	Extr. Fine	Ellipsoid-conical	Uniform	Uniform	Coarse	Early	11.0
1	Calyx	Extr. Fine	Globose	Uniform	Uniform	Medium	Early	16.7
-	-	-	-	-	-	-	-	-
5	Pedicel	Extr. Fine	Globose-conical	Uniform	Uniform	-	Very early	12.2
1	Pedicel	Extr. Fine	Globose-conical	Variable	Variable	Medium	Medium early	11.4
1	Pedicel	Extr. Fine	Globose-conical	Variable	Variable	Medium	Early	12.6
10	Pedicel	Extr. Fine	Conical	Variable	Variable	Coarse	Early	13.3
1	Pedicel	Extr. Fine	Globose	Uniform	Uniform	Medium	Very early	11.8
5	Pedicel	Med. Heavy	Globose-conical	Variable	Variable	Coarse	Early	10.3
0	-	-	Flat-globose	Variable	Variable	Fine	Medium	10.8
5	Pedicel	Extr. Fine	Globose	Variable	Uniform	Coarse	Medium early	11.1
5	Pedicel	Extr. Fine	Globose	Variable	Variable	Coarse	-	11.4
2	Pedicel, calyx	Med. Heavy	Oblong	Variable	Variable	Medium	-	13.3
1	Pedicel	Extr. Fine	Globose	Variable	Variable	Medium	Early	11.4
2	Pedicel	Extr. Fine	Globose-conical	Uniform	Variable	Fine	-	12.0
1	Pedicel	Extr. Fine	Globose	Variable	Variable	Coarse	Very early	11.2
2	Pedicel	Extr. Fine	Globose-conical	Variable	Variable	Medium	-	11.0
0	None	None	Globose	Variable	Uniform	Coarse	Early	11.8
1	Pedicel	Extr. Fine	Flat-globose	Variable	Uniform	Medium	Very early	11.6
-	-	-	Globose	Uniform	Uniform	Medium	Very early	12.7
-	-	-	-	-	-	-	-	-
-	Pedicel	Extr. Fine	Globose-conical	Uniform	Uniform	Medium	Very early	11
5	Pedicel	Med. Heavy	Globose	Uniform	Uniform	Medium	Extremely early	9.7
1	Pedicel	Extr. Fine	Globose	Uniform	Variable	Coarse	Early	8.5
2	Pedicel	Extr. Fine	Globose	Variable	Variable	Coarse	-	11.3
20	Entire fruit	Med. Heavy	Flat	Variable	Variable	Coarse	-	13.1
15	Pedicel, calyx	Med. Heavy	Globose-conical	Variable	Variable	Medium	-	14.1
-	-	-	-	-	-	-	-	-
1	Pedicel	Extr. Fine	Conical	Variable	Variable	Medium	Early	11.9
<1	Pedicel	Extr. Fine	Globose	Variable	Uniform	Medium	-	12.5

This case example demonstrates the importance of having materials backed up at alternative field or storage conditions. Since the original 1995 and 1996 wild population orchards from Kazakhstan were planted in 1997, 15% and 30% of the individuals within the populations from sites 6 and 9, respectively, were lost before samples could be collected for DNA extraction in 2002. These trees were lost due to competition for resources from adjacent trees, disease infestation, and poor suitability to the PGRU field environment. Our core collections for site 6 and site 9 effectively captured the genetic diversity of the materials in the field from which DNA was collected. It was not possible to include 29% to 39% of the individuals for which DNA had been extracted, but 94% and 89% of the allelic diversity from sites 6 and 9 were captured in the respective core-3 collections. Efforts are now under way to back-up the *M. sieversii* field collection as seeds. While some trees will be kept as clonal representatives in the main *Malus* collection and as cryo-stored, dormant buds (Forsline et al., 1999; Towill et al., 2004), many of the *M. sieversii* trees will be removed from the orchard setting and their genetic resources distributed as seeds. This lowers the maintenance costs of the PGRU *Malus* collection as a whole and supplements the original seed collected from these sites in 1995 and 1996 for some of the accessions (Table 1).

The core-3 collections were validated to ensure that unique alleles contributed to the final core collection. The core-3 collections of 35 individuals from each of sites 6 and 9 captured most of the allelic diversity and also represented the allelic frequencies within each population. Despite the inclusion of 12 maternal parents (of the half-sib families) in the site 9 analyses, only two of those parents were selected for inclusion in the core-3 collection. This indicates that the maternal trees were not providing the bulk of the genetic diversity. Since *M. sieversii* is predominantly outcrossing, novel paternal genes were represented in the seedling accessions.

The site 6 and site 9 core-3 collections have been described and validated. Storing these populations as seeds when phenotypic evaluations are completed is less expensive than maintaining field tree collections. In order to minimize the effects of self-incompatibility (which is not characterized in these populations) and maximize the efficiency of the fieldwork, the following method for seed production has been proposed.

As stated previously, two sets of genetically representative seeds will be produced, one each for the site 6 and site 9 populations. Trees originating from different populations will not be crossed.

For the first site, four pools of bulked pollen (A, B, C, and D) will be collected from an equal number of flowers from unique sets of either nine, nine, nine, or eight of the 35 core-3 individuals. This eliminates some of the pollen competition that occurs if all the pollen is bulked, yet ensures that pollination will occur, even if some accessions share self-incompatibility alleles. Self-incompatibility has

not been characterized in this species but may impact the efficacy of this crossing design. By assembling four pollen pools, the practical logistics of implementing the field crosses is achieved while any variance in male reproductive success is partitioned. The effective population size of the resulting seeds can vary from ≈14 in the worst case scenario, where only one pollen parent per pool sires all the seeds, to 70, where each pollen parent sires equal numbers of seeds. Self-incompatibility characterization is needed in this species.

The 35 core-3 trees will also serve as females in the crossing design. All pollinated flowers will be emasculated, pollinated, and then bagged to prevent open pollination events. Twenty flowers from one branch of the first tree will be fertilized with pollen pool A. An additional 20 flowers will be fertilized with pollen pool B, and likewise for pollen pools C and D. Individual branches pollinated with different pollen pools will be labeled accordingly. The same procedure will be followed on each of the additional 34 trees. This process may need to be carried out in two successive years due to sparse flowering on some trees and/or poor fruit set after emasculation and pollination.

At harvest, the apples from each labeled branch of each tree will be kept separately from one another. Seeds will be removed from each of the 140 sets of apples (4 branches × 35 trees). Then, four seeds will be removed from each of the 140 sets of apple seeds and pooled, resulting in a final pool size of 560 apple seeds. These seeds represent one replicate of the entire population and will be stored as a group. Additional replicates are collected and each replicate will be stored separately from the other replicates. Assuming that at least four seeds form within each apple, we expect that at least 20 replicate sets of pooled seeds will be created. These replicates could be used for distribution purposes as well as long term storage at the PGRU and USDA's National Center for Genetic Resources Preservation in Ft. Collins, Colo. Properly dried apple seeds (adjusted to 25% relative humidity) should theoretically store for many years at –20 °C (Justice and Bass, 1978).

We have proposed a method to store field collections of vegetatively propagated plants as seeds. When field collections are fully characterized, it is more economical to preserve alleles present in these collections in seed form, rather than as field plants. Allelic diversity of field populations can be determined using molecular methods and neutral markers. Specific individuals that provide allelic richness in the population are selected for inclusion in core collections using a strategy that maximizes allelic richness. Once core collections are designated, seeds can be produced from the orchard collections and stored for distribution and future projects. This strategy could be applied to many other field collections of wild plant material in the NPGS.

Literature Cited

Bataillon, T.M., J.L. David, and D.J. Schoen. 1996. Neutral genetic markers and conservation genetics: Simulated germplasm collections. Genetics 144:409–417.

Clark, R.L., H.L. Shands, P.K. Bretting, and S.A. Eberhart. 1997. Managing large diverse germplasm collections. Crop Sci. 37:1–6.

Crossa, J., C.M. Hernandez, P. Bretting, S.A. Eberhart, and S. Taba. 1993. Statistical genetic considerations for maintaining germplasm collections. Theor. Appl. Genet. 86:673–678.

Dzhangaliev, A.D. 2003. The wild apple tree of Kazakhstan. Hort. Rev. 29:63–303.

Forsline, P.L. and H.S. Aldwinckle. 2004. Evaluation of *Malus sieversii* seedling populations for disease resistance and horticultural traits. EUCARPIA Symp. on Fruit Breeding and Genet.,1–5 Sept. 2003, Angers, France. Acta Hort. 663:529–534.

Forsline, P.L., H.S. Aldwinckle, E.E. Dickson, and S.C. Hokanson. 2003. Collection, maintenance, characterization, and utilization of wild apples from central Asia. Hort. Rev. 29:1–61.

Forsline, P.L., J.R. McFerson, W.F. Lamboy, and L.E. Towill. 1999. Development of base and active collections of *Malus* germplasm with cryopreserved dormant buds. Acta Hort. 484:75–78.

Goudet, J. 1995. FSTAT, a program for IBM PC compatibles to calculate Weir and Cockerham's (1984) estimators of F-statistics. J. Hered. 86:485–486.

Gouesnard, B., T.M. Bataillon, G. Decoux, C. Rozale, D.J. Schoen, and J.L. David. 2001. MSTRAT: An algorithm for building germ plasm core collections by maximizing allelic or phenotypic richness. J. Hered. 92:93–94.

Harris, S.A., J.P. Robinson, and B.E. Juniper. 2002. Genetic clues to the origin of the apple. Trends in Genet. 18:426–430.

Hemmat, M., N.F. Weeden, and S.K. Brown. 2003. Mapping and evaluation of *Malus* ×*domestica* microsatellites in apple and pear. J. Amer. Soc. Hort. Sci. 128:515–520.

Hokanson, S.C., W.F. Lamboy, A.K. Szewc-McFadden, and J.R. McFerson. 2001. Microsatellite (SSR) variation in a collection of *Malus* (apple) species and hybrids. Euphytica 118:281–294.

Hokanson, S.C., J.R. McFerson, P.L. Forsline, W.F. Lamboy, A.D. Djangaliev, and H.S. Aldwinckle. 1997. Collecting and managing wild *Malus* germplasm in its center of diversity. HortScience 32:173–176.

Hokanson, S.C., A.K. Szewc-McFadden, W.F. Lamboy, and J.R. McFersen. 1998. Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a *Malus* ×*domestica* borkh. core collection. Theor. Appl. Genet. 97:671–683.

Holbrook, C.C., W.F. Anderson, and R.N. Pittman. 1993. Selection of a core collection from the U.S. germplasm collection of peanut. Crop Sci. 33:859–861.

Justice, O.L. and L.N. Bass. 1978. Agriculture Handbook No. 506, Principles and practices of seed storage. U.S. Govt. Printing Office, Washington, D.C.

Lamboy, W.F. and C.G. Alpha. 1998. Using simple sequence repeats (SSRs) for DNA fingerprinting germplasm accessions of grape (*Vitis* L.) species. J. Amer. Soc. Hort. Sci. 123:182–188.

Lamboy, W.F., J. Yu, P.L. Forsline, and N.F. Weeden. 1996. Partitioning of allozyme diversity in wild populations of *Malus sieversii* L. and implications for germplasm collection. J. Amer. Soc. Hort. Sci. 121:982–987.

Lewis, P.O. and D. Zaykin. 2001. GDA user's manual. Dept. of Ecol. and Evolutionary Biol., Univ. of Connecticut. 27 July 2004. <http://lewis.eeb.uconn.edu.lewishome>.

Luby, J., P. Forsline, H. Aldwinckle, V. Bus, and M. Giebel. 2001. Silk road apples—Collection, evaluation, and utilization of *Malus sieversii* from Central Asia. HortScience 36:225–231.

McKhann, H.I., C. Camilleri, A. Berard, T. Bataillon, J.L. David, X. Reboud, V. Le Corre, C. Caloustian, I.G. Gut, and D. Brunel. 2004. Nested core collections maximizing genetic diversity in *Arabidopsis thaliana*. Plant J. 38:193–202.

Petit, R.J., A.E. Mousadik, and O. Pons. 1998. Identifying populations for conservation on the basis of genetic markers. Conservation Biol. 12:844–855.

Schoen, D.J. and A.H.D. Brown. 1995. Maximising genetic diversity in core collections of wild relatives of crop species, p. 55–76. In: T. Hodgkin, A.H.D. Brown, Th.J.L. van Hintum, and E.A.V. Morales (eds.). Core collection of plant genetic resources. IPGRI, Wiley Sayce, Rome.

Skinner, D.Z., G.R. Bauchan, G. Auricht, and S. Hughes. 1999. A method for the efficient management and utilization of large germplasm collections. Crop Sci. 39:1237–1242.

Tohme, J., P. Jones, S. Beebe, and M. Iwanaga. 1995. The combined use of agroecological and chracteriztaion data to establish the CIAT *Phaseolus vulgaris* core collection, p. 95–107. In: T. Hodgkin, A.H.D. Brown, Th.J.L. van Hintum, and E.A.V. Morales (eds.). Core collection of plant genetic resources. IPGRI, Wiley Sayce, Rome, Italy.

Towill, L.E., P.L. Forsline, C. Walters, J. Waddell, and J. Laufman. 2004. Cryopreservation of *Malus* germplasm using a winter vegetative bud method: Results from 1915 accessions. Cryoletters (in press).

U.S. Department of Agriculture. 2004. National genetic resources program. Germplasm resources information network (GRIN). USDA, ARS Natl. Germplasm Resources Laboratory, Beltsville, Md. 27 July 2004. <http://www.ars-grin.gov/cgi-bin/npgs/html/>.