INTRODUCTION
Genetic identity of apple rootstocks and the relationship among them and with other Malus germplasm can now be investigated by means of recently developed multi-allelic mapped microsatellite markers (synonymous for Simple Sequence Repeats or SSR markers). There are currently ~250 of such markers available publicly from laboratories around the world. This investigation 1) provides a benchmark to test the genetic identity of unknown rootstocks 2) it provides information on the relationship among rootstocks and answer questions about parentage, similarity and breeding potential, and 3) with more genetic information about important rootstock traits such as disease resistance (fireblight, replant disease etc.) dwarfing and precocity becoming available, this investigation will provide information about new sources of genes and rootstocks.

OBJECTIVES
1. Genotype commercial and experimental rootstocks that have been developed by several breeding programs around the world (including Geneva rootstocks) with a subset of 50-80 microsatellite markers that are well distributed among the 17 linkage groups of apple.
2. Test fingerprinting on unknown or questionable samples.
3. Study the relationship of these rootstocks with recently acquired wild accessions from Kazakhstan and China of Malus orientalis and Malus sieversii as well as other wild species to identify potential germplasm for breeding.
4. Compile a public database of the allelic composition of such rootstocks and make it available on the internet.

All objectives were accomplished except for the publication of data over the internet.

SIGNIFICANT FINDINGS
1. All rootstocks (except for clones of M.9), wild species and scion variety checks were uniquely identified with a subset of microsatellite DNA markers.
2. The DNA fingerprints were used on rootstocks in a Wenatchee (WA) experimental orchard to cull trees of Geneva 3041 that had been mis-labeled and traced the problem back to an admixture in a Geneva stoolbed.
3. Relationships among rootstocks were established using the data collected:
   a. M.9 and related rootstocks grouped together with the cultivated apple scion checks (Malus X domestica Borkh.) – confirming the origin of M.9 as a chance French seedling selection.
   b. Most commercial rootstocks share many genetic regions with M.9. Exceptions are some Japanese rootstocks and most of the Geneva rootstocks.
   c. The new AR series of rootstocks clustered together with the Budagovsky series.
   d. The genetic diversity in some wild apple species that are more closely related to cultivated apple (Malus orientalis, Malus sieversii) has not been tapped yet for rootstock development.

METHODLOGY
Leaf and apical meristem tissue for DNA extraction was harvested from bud-wood and rootstock liners of a collection of 96 commercial rootstock varieties, wild species and reference scion varieties (Table 1). The tissue was introduced in a unique well position of a 2 ml polypropylene microtiter
plate (Fisher Scientific, Pittsburgh, PA) and then lyophilized in preparation of DNA extraction using the Aquapure genomic DNA extraction kit (Bio-Rad Laboratories, Hercules, CA) methodology. Apple DNA primers corresponding to 86 mapped microsatellite (SSR) loci well distributed throughout all 17 chromosomes of the apple genome (Table 2) were synthesized by IDT (Integrated DNA Technologies Inc. Coralville, IA). Three fluorescent labels (6-FAM-blue, HEX-green, and NED-yellow) corresponding to filter set D of the ABI-PRISM 3100 DNA fragment analyzer were chosen for multiplexing markers into the same PCR reaction. Forward primers labeled with 6-FAM and HEX were synthesized by MWG Biotech Inc. (High Point, NC) and NED labeled primers were synthesized by Applied Biosystems (Foster City, CA). DNA of M.9 and *Malus robusta* CV. Robusta 5 were used in annealing temperature gradient PCR (ATG-PCR) to evaluate DNA profiles and optimum annealing temperatures for all available SSR markers. PCR reactions for SSR markers were performed in 15 µl volumes of a uniform reaction mixture [3 mM MgCl, 0.2 mM dNTPs, 15-20 ng of DNA, 0.4 µM of each primer, Taq polymerase, and commercial buffer (Promega, Madison, WI)] incorporating 10 µl of light weight mineral oil overlay (Fisher Scientific, Pittsburgh, PA). The PCR reactions were resolved in 2 % agarose (Bio-Rad Laboratories, Hercules, CA) by staining with ethidium bromide (0.5 µg/ml). Gels were visualized with the Dark Reader™ trans illuminator (Clare Chemical Inc., Denver, CO) and CCD camera system. The same reaction products were then resolved with a 36 cm capillary on a ABI-PRISM 3100 DNA fragment analysis system. Over 3500 electropherograms (Figure 2) were analyzed with the Genescan and Genotyper software (ABI-PRISM) and allele size information was manually checked for accuracy and entered into a database for analysis. A genetic similarity analysis was conducted with NTSYS 2.02 software for phylogenetic analysis (Exeter Software, Setauket, NY). Jaccard’s coefficient of similarity was calculated and a similarity tree was built using the SAHN clustering UPGMA method (Unweighted Pair-Group Method, Arithmetic average) within NTSYS. We are in the process of organizing the data for publication in peer reviewed journals and on the internet.

**Results and Discussion**

**SSR primer synthesis and optimization**

The adoption of a new technology (SSR markers) in a new laboratory always requires some degree of adaptation and optimization of methods. Although microsatellite markers amplify a specific locus through specific PCR primers (18-25 bp long), subtle variation in sequences surrounding the repeat region used for primer design may impart instability of such markers due to subtle variations of PCR conditions (e.g. annealing temperature, salt concentration etc.) (Fazio et al., 2002). We used annealing temperature gradient PCR (ATG-PCR) to test the performance of SSR primer pairs on two diverse rootstock parents (M.9 and Robusta 5) at annealing temperatures ranging from 45°C to 65°C. We are utilizing the ATG profiles generated in this experiment to increase the reproducibility of results in our laboratory and to discover polymorphisms (Figure 1).

![Figure 1. ATG-PCR with three SSR primer pairs. Each set is the result of PCR with M.9, Robusta 5 DNA (alternating) at 8 annealing temperatures ranging 45°C (leftmost in each set) to 65°C (rightmost in each set).](image-url)
**Genotyping**

All SSR primer pairs amplified detectable products. These products were visualized in agarose gel electrophoresis to check for proper amplification of the target DNA fragments prior to the more expensive process of allele sizing and fingerprinting in a sequencing apparatus (ABI PRISM 3100). Allele sizing produced more than 3,500 electropherograms (figure 2) that were translated into more than 20,000 genotypic data points. Allele size data for the entries listed in table 1 has been entered into a database. This data, which cannot be included in this report because of size constraints, is available upon request to WTFRC members and collaborators.

**Analysis of genetic diversity**

Several interesting findings were obtained from the analysis of this genetic data. Please refer to figure 3 which graphically synthesizes the results of this study. The apple rootstock M.9, which was an East Malling selection (1912) from a French open pollinated seedling population called Jaune de Metz, has been classified a crabapple, however it shared many genetic similarities with the domesticated apple samples that were included in this study (Golden Delicious, Reinette, and Northern Spy). Among its wild apple relatives *Malus orientalis* and *Malus sieversii* (with a Jaccard’s similarity coefficient of 0.29 and 0.26 respectively) were more closely related than all other species examined. These two species originating from Kazakhstan and the Caucasus region have been the object of intense exploration and represent some of the best material for breeding, discovery of useful traits, and potentially direct use as rootstock material. We could noticeably identify a central cluster in the tree diagram of figure 3 which represented most of the dwarfing derivatives of M.9 (M.27, Mark, G.30, P.14 etc.). The Budagovsky series was clustered together with the new AR series of rootstocks, J TE C, Pillnitz AU 51-4 and at times with M.26 (data not shown). Most of the elite Geneva rootstocks grouped together in a different cluster from M.9, this probably due to their common parentage and to the intense disease screening and selection process that may have skewed the DNA toward the fireblight resistant parent Robusta 5. The Japanese rootstocks Naga, JM.4, Marubakaido and the Geneva rootstocks Novole and G6589 all derived from crosses with *M. prunifolia* clustered together with the only *M. prunifolia* accession in this study.

Currently, we are undertaking several genetic mapping projects involving horticultural traits (dwarfing, precocity) and resistance to biotic stresses (replant disease, fire blight) with the ultimate goal of cloning the factors involved in these critical traits. The data developed in this project will have a synergic effect in the identification of these critical genes or genetic regions and the development of novel genotypes for the apple industry.

Although this is a final report for the Washington Tree Fruit Research Commission we regard it more of an interim report since the invaluable genetic data gathered in this study continues to be the basis for breeding decisions and for discovery of useful genes in apple.
Practical use of this study
During a visit to the Geneva rootstock test orchard in Wenatchee WA in September 2002 and 2003 we became aware of the possibility that one of the entries (several trees of Geneva 3041) in that particular trial had originated from a stoolbed suspected to have a mixture of genotypes. After DNA testing of the stoolbed and of suckers from one of the trees in question we established that the genotypes of those trees were not what they were labeled as and that the mislabeled trees had derived form the questionable stoolbed. The data gathered in this study will continue to be a benchmark for identity testing where questions arise.

Figure 3. Tree diagram of genetic similarity among entries in table 1.
<table>
<thead>
<tr>
<th>Rootstock Entry</th>
<th>Pedigree or PI Number</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Commercial and Experimental rootstocks from other programs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AR-295-6</td>
<td>Unknown</td>
<td>East Malling- Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>AR-486-1</td>
<td>Unknown</td>
<td>East Malling- Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>AR-628-2</td>
<td>Unknown</td>
<td>East Malling- Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>M.26</td>
<td>M.16, M.9</td>
<td>Acquired from Willow Drive (Geneva stoolbed)</td>
</tr>
<tr>
<td>M.27</td>
<td>M.13, M.9</td>
<td>Acquired from TRECO (Geneva stoolbed)</td>
</tr>
<tr>
<td>M.7</td>
<td>English Selection</td>
<td>Acquired from Willow Drive (Geneva stoolbed)</td>
</tr>
<tr>
<td>M.9 EMLA</td>
<td>French Selection</td>
<td>USDA-PGRU stoolbed</td>
</tr>
<tr>
<td>M.9 Nic29</td>
<td>French Selection</td>
<td>Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>M.9 Nic8</td>
<td>French Selection</td>
<td>Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>M.9 Pajam1</td>
<td>French Selection</td>
<td>Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>M.9 Pajam2</td>
<td>French Selection</td>
<td>Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>M.9 T337</td>
<td>French Selection</td>
<td>Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>MM.106</td>
<td>N. Spy, M.1</td>
<td>Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>MM.111</td>
<td>N. Spy, Merton 793</td>
<td>Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>Bud.118</td>
<td>Moscow pear, M.8, M.9</td>
<td>Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>Bud.491</td>
<td>Unknown</td>
<td>Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>Bud.9</td>
<td>M.8, Red Standard</td>
<td>Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>J.9</td>
<td>M.9, OP</td>
<td>Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>J-TE-C</td>
<td>Unknown</td>
<td>Czech Rep.- Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>J-TE-G</td>
<td>Unknown</td>
<td>Czech Rep.- Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>JM.1</td>
<td>M. prunifolia, M.9</td>
<td>Japan-Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>JM.4</td>
<td>M. prunifolia, M.9</td>
<td>Japan-Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>JM.7</td>
<td>M. prunifolia, M.9</td>
<td>Japan-Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>MARK</td>
<td>M.9, OP</td>
<td>M9 open pollinated (Geneva stoolbed)</td>
</tr>
<tr>
<td>Marubakaido</td>
<td>M. prunifolia</td>
<td>Japan (Geneva stoolbed)</td>
</tr>
<tr>
<td>Naga</td>
<td>M. prunifolia</td>
<td>Japan (Geneva stoolbed)</td>
</tr>
<tr>
<td>P.1</td>
<td>M.4, Antonovka</td>
<td>Polish Series-Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>P.14</td>
<td>M.9, OP</td>
<td>Polish Series-Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>P.18</td>
<td>M.4, Antonovka</td>
<td>Polish Series-Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>P.22</td>
<td>M.9, Antonovka</td>
<td>Polish Series-Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>Pi-AU-51-11</td>
<td>M.4 OP</td>
<td>Pillnitzer Series-Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>Pi-AU-51-4</td>
<td>M.4 OP</td>
<td>Pillnitzer Series-Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>Pi-AU-56-83</td>
<td>M.11 OP</td>
<td>Pillnitzer Series -Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>Supporter 4</td>
<td>M.4, M.9</td>
<td>Pillnitzer Series -Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>V.1</td>
<td>Kerr, M.9 OP</td>
<td>Vineland, Canada-Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td>V.2</td>
<td>Kerr, M.9 OP</td>
<td>Vineland, Canada-Acquired from Meadow Lake (Geneva stoolbed)</td>
</tr>
<tr>
<td><strong>Geneva Commercial and Elite Rootstocks</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5030</td>
<td>B.491, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>Novole</td>
<td>M. Prunifolia</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>G.11</td>
<td>M.26, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>G.65</td>
<td>M.27, Beauty Crab</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>3041</td>
<td>M.27, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>4202</td>
<td>M.27, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>6143</td>
<td>M.27, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>5046</td>
<td>Novole, B.146</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>6589</td>
<td>Novole, B.9</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>G.16</td>
<td>O.3, M. floribunda</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>4013</td>
<td>O.3, Novole</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>3007</td>
<td>O.3, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>4210</td>
<td>O.3, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>4213</td>
<td>O.3, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>4214</td>
<td>O.3, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>4814</td>
<td>O.3, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>5012</td>
<td>O.3, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>5087</td>
<td>O.3, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>5179</td>
<td>O.3, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
</tbody>
</table>
### Rootstock Entry Table

<table>
<thead>
<tr>
<th>Rootstock Entry</th>
<th>Pedigree or PI Number</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>5257</td>
<td>O.3, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>5757</td>
<td>O.3, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>5890</td>
<td>O.3, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>5935</td>
<td>O.3, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>6210</td>
<td>O.3, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>6253</td>
<td>O.3, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>6874</td>
<td>O.3, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>6969</td>
<td>O.3, Robusta5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>6006</td>
<td>PK-14 (Bud), Robusta 5</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>6879</td>
<td>Robusta5, M.9</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>7707</td>
<td>Robusta5, M.9</td>
<td>(Geneva stoolbed)</td>
</tr>
<tr>
<td>G.30</td>
<td>Robusta5, M.9</td>
<td>(Geneva stoolbed)</td>
</tr>
</tbody>
</table>

### Wild Species and Reference Accessions

<table>
<thead>
<tr>
<th>Species</th>
<th>Accession</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malus angustifolia</td>
<td>589763</td>
<td>United States (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus asiatica</td>
<td>594099</td>
<td>China (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus coronaria</td>
<td>589976</td>
<td>Canada (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus doumeri</td>
<td>589882</td>
<td>United States (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus fusca</td>
<td>589975</td>
<td>United States (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus fusca</td>
<td>589941</td>
<td>United States (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus kansuensis</td>
<td>594097</td>
<td>China (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus orientalis</td>
<td>594095</td>
<td>Russia (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus orientalis</td>
<td>594101</td>
<td>Russia (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus prunifolia</td>
<td>594103</td>
<td>Japan (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus sieversii</td>
<td>596283</td>
<td>Kazakhstan (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus siversii</td>
<td>596282</td>
<td>Kazakhstan (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus siversii</td>
<td>596280</td>
<td>Kazakhstan (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Eriolobus trilobata</td>
<td>589397</td>
<td>United Kingdom (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus toringo</td>
<td>594094</td>
<td>United States (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus toringoides</td>
<td>589393</td>
<td>United Kingdom (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus transitoria</td>
<td>589384</td>
<td>United Kingdom (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus transitoria</td>
<td>589422</td>
<td>United Kingdom (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus tschonoskii</td>
<td>589395</td>
<td>United Kingdom (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Malus iensis</td>
<td>590015</td>
<td>United States (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Crimson Beauty</td>
<td></td>
<td>Malus. X domestica (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Esopus Spitzen</td>
<td></td>
<td>Malus. X domestica (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Nertchinsk</td>
<td></td>
<td>Malus. X domestica (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Northern Spy</td>
<td></td>
<td>Malus. X domestica (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Reinette Sim</td>
<td></td>
<td>Malus. X domestica (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Wijcik Mac</td>
<td></td>
<td>Malus. X domestica (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Yellow Trans</td>
<td></td>
<td>Malus. X domestica (Plant Genetic Resource Unit Orchard)</td>
</tr>
<tr>
<td>Golden Delicious</td>
<td></td>
<td>Malus. X domestica (Plant Genetic Resource Unit Orchard)</td>
</tr>
</tbody>
</table>

### Literature review

Since their initial implementation (Litt and Luty, 1989), PCR markers based on microsatellites have become the marker of choice for many genetic studies in higher eukaryotes. Microsatellites are synonymous with simple sequence repeats (SSRs). The development of microsatellite markers requires the characterization of sequences flanking the repeat motif followed by the design of flanking PCR primers. Many higher eukaryote genomes contain numerous, randomly dispersed tandem repeat sequences (Condit and Hubbel, 1991; Cregan, 1992; Roder et al., 1995; Tautz and Renz, 1984). Microsatellites are tandem repeats with a basic motif of less than six base pairs and are synonymous to simple sequence repeats (Staub et al., 1996c). These repeat sequences are highly variable in length depending on the number of repeat units giving rise to length polymorphisms (different alleles). Microsatellite markers offer several advantages over other types of markers (RAPD, AFLP, RFLP):
• They are highly polymorphic. Since these sequences are found generally in non-coding regions of DNA (including introns), little natural selection pressure has been applied to these sequences allowing mutations to be transmitted and conserved in a population in high frequencies.
• They are inherited in a codominant Mendelian manner, and thus can be highly informative in mapping and marker assisted selection.
• They represent multiple alleles at a locus. Due to their high variability several alleles (fragment sizes) may be observed at a specific locus. This attribute (i.e., greater allelic variation) allows the estimation of genetic distance within a species and for variety identification (Staub and Meglic, 1993; Staub et al., 1996b).

Microsatellite markers have been used in apple for scion cultivar identification (Guilford et al., 1997), as markers linked to columnar growth habit in apple (Hemmat et al., 1997), for map merging (Gianfranceschi et al., 1998; Maliepaard et al., 1998), for germplasm management and identification (Hokanson et al., 1998; Hokanson et al., 2001; Vornam et al., 2000), for the identification of homozygous lines after anther culture (Kenis et al., 2000) and finally to identify breeding parents in a scion breeding program (Kenis et al., 2001). One hundred and forty new microsatellite markers have been recently developed and used to build a framework map of apple that can be aligned with previously published maps (Liebhard et al., 2002). Very little is known about the phylogeny of commercial apple rootstocks and their relationship to wild species of apple. One study that used RAPD markers identified the relationship of SJM series of rootstocks to standards like M.9 and M.26 and developed a DNA fingerprinting system based on 13 RAPD markers, however these markers are non-specific and error prone (Staub et al., 1996a). This work represents the beginning of a new more comprehensive study needed for apple rootstocks and will provide useful information for genetic improvement and a benchmark for genetic fingerprinting.

**Budget:**

<table>
<thead>
<tr>
<th>Item</th>
<th>Year 1 (2003)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salaries</td>
<td>0</td>
</tr>
<tr>
<td>Benefits (38.31%)</td>
<td>0</td>
</tr>
<tr>
<td>Wages¹</td>
<td>4,000</td>
</tr>
<tr>
<td>Benefits (38.31%)</td>
<td>1,532</td>
</tr>
<tr>
<td>Equipment</td>
<td>0</td>
</tr>
<tr>
<td>Supplies²</td>
<td>4468</td>
</tr>
<tr>
<td>Travel</td>
<td>0</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>10,000</strong></td>
</tr>
</tbody>
</table>

¹Temporary laboratory technician hired for 3 months.
²Supplies include primer synthesis, laboratory chemicals (enzymes, buffers, etc.).

We are grateful to the Washington Fruit Tree Research Commission for the funds that were made available for this project. This budget covered partially the expenses incurred in this project. We are also grateful to the International Dwarf Fruit Tree Association for their contributions to this project.
REFERENCES


