

AN ABSTRACT OF THE THESIS OF

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Title: SIMPLE SEQUENCE REPEAT MARKER DEVELOPMENT AND FINGERPRINTING IN CUPHEA LANCEOLATA AIT. AND C. VISCOSISSIMA JACQ.

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Steven J. Knapp

Microsatellites or simple sequence repeats (SSRs) are high-throughput sequence based DNA markers that have been exploited as tools for measuring genetic distance and diversity in evolutionary studies, protection of intellectual property, genetic resource conservation management, cultivar identification and molecular breeding of crop plants. We isolated and sequenced 1095 clones from genomic libraries enriched for CA, GA and GAA repeats. Unique SSRs were identified in 425 clones and primers were designed for 271 SSR sequences. Of the latter, 184 primer pairs produced clean SSR markers. Twenty nine functional SSR markers were used for genetic diversity study on 94 accessions of *Cuphea*. The *Cuphea* genotypes in the study panel included 20 accessions of *C. lanceolata* and 74 accessions of *C. viscosissima*. The mean number of alleles for *C. lanceolata* and *C. viscosissima* were 10.97 and 4.76 respectively. Similarly, markers amplifying *C. lanceolata* had a higher mean polymorphic information content (PIC) score (0.74) as compared to *C.*

viscosissima (0.42). In *C. lanceolata*, a positive correlation was observed between maximum repeat count (MRC) and number of alleles ($r=0.40$) as well as MRC and standard deviation of molecular weight (SDMw) ($r=0.72$). *C. viscosissima* demonstrated a similar trend, albeit with a greater magnitude of correlation, between MRC and number of alleles ($r=0.70$) and MRC and SDMw ($r=0.78$). The SSR markers described herein are powerful tools for efficiently and accurately discriminating between *Cuphea* genotypes.

Simple Sequence Repeat Marker Development And Fingerprinting In
Cuphea Lanceolata Ait. And *C. Viscosissima* Jacq.

by

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Simple Sequence Repeat Marker Development And Fingerprinting In *Cuphea lanceolata* Ait. And *C. Viscosissima* Jacq.

Introduction

The genus *Cuphea*, with 260 species, is the largest numerically in the Lytheraceae. Most are perennial or facultative annual herbs with zygomorphic, intensely colored flowers. *Cuphea*, a new world genus, is distinguished from a closely related genus, *Lythrum* by a unique seed dispersal mechanism.

Cuphea disperses seed through a dorsal abscission layer along the corolla tube. The placenta of the wildtype rotates upward 100 to 120° from its origin within the corolla tube. The fully exposed seeds mature and dehisce after the placenta separates from the corolla tube, the parchment thin fruit carpels remaining within the corolla tube. The non-shattering phenotypes discovered in the inter-specific hybrids disrupt this process. Placentas of the fruits of non-shattering genotypes fail to rotate, so the seeds mature and dehisce within the corolla tube. The dorsal abscission layer arises within these phenotypes, but most of the seed is retained within the corolla tube. The placenta dries over the developing seed, further decreasing shattering. The carpels are torn irregularly by the expanding and maturing seed (Knapp and Crane, 2000c).

The two species we investigated, *Cuphea viscosissima* and *Cuphea lanceolata*, are found in the southeastern United States and on the central

plateau of Mexico respectively. In the latter, we studied *C. lanceolata* f. *silenoides* and *C. lanceolata* f. *typica*. The inter-fertility of autogamous *C. viscosissima* and allogamous *C. lanceolata* species has facilitated domestication and breeding efforts in the genus. These species share characteristics that make them useful experimental organisms for genetic studies. They are diploid with $x = 6$ chromosomes, and have relatively small genomes (unpublished data). *C. lanceolata*, with the most northeastern distribution of any *Cuphea* in Mexico, is the only species whose range approaches that of *C. viscosissima*, which is restricted to the eastern United States.

The seeds of *Cuphea* are oil rich, with concentrations between 16 - 42% (Earle *et al.*, 1960; Miller *et al.*, 1964). In the Lytheraceae, this genus has unique seed oil chemistry. *Cuphea* has an unmatched profile of fatty acid synthesis rendering it a model system for studying medium chain fatty acid (MCFA) biosynthesis. Unlike most angiosperm lipids that contain primarily linoleic acid (30-59%), *Cuphea* seed oils contain remarkably high quantities of MCFA (38-85%) (Graham *et al.*, 1981; Wolf *et al.*, 1983; Thompson, 1984; Hirsinger 1985). The oils of wild populations of *C.*

lanceolata and *C. viscosissima* average 83.2% and 70.1% capric acid (10:0) respectively (Graham, 1989; Knapp and Tagliani, 1991). In addition, *C. viscosissima* lines have been developed with as much as 28.4% caprylic acid (8:0), 14.0% lauric acid (12:0) and 29.4% myristic acid (Knapp and Tagliani, 1991). A correlation between fatty acid patterns and chromosome number, pollen morphology and other taxonomic characters that are useful in systematics has been observed (Graham *et al.*, 1988).

Caprylic and capric acid – rich medium chain triglycerides have been used to treat disorders of lipid metabolism, epilepsy, gallstones and other diseases, and are a source of rapidly absorbed energy for critically ill patients (Bach and Babayan, 1982). In addition, caprylic and capric acids may have nutritional benefits not yet exploited for general public (Babayan, 1987).

Presently, the primary commercial application of MCFAs is the use of lauric acid and myristic acids in manufacture of soap and detergents (Knaut and Richter, 1985). The industrialized western world is heavily dependant upon imported coconut (*Cocos nucifera* L.) and palm kernel (*Elaeis guineensis* Jacq.) oils as the primary source of MCFAs (Thompson, 1984). Imported supplies are subject to periodic fluctuations in price and supply, creating repercussions that are directly felt by the chemical

industry. Hence, it is deemed desirable to develop renewable sources of MCFA s in the temperate region. Moreover, every MCFA except lauric acid is more concentrated in seed oils of *C. lanceolata* and *C. viscosissima* than in oils of coconut and palm kernel. The higher concentration of individual fatty acids would reduce the processing needed for their purification. In addition because *Cuphea* is adapted to temperate climates, the production sources of MCFA s would be diversified and help ensure long term supplies and stable prices (Webb, 1992). This potential economic value of *Cuphea* has triggered domestication of this "very perplexing" (Nevling, 1958) weed and breeding of promising accessions. *Cuphea*, as an economically viable crop, promises a large domestic market. Several temperate species of *Cuphea* have been proposed as candidates for domestication. However, seed shattering and seed dormancy are the major barriers within the genus.

Plant and animal domestication is the most important development in the past 13,000 years of human history (Diamond, 2002). The history of agriculture is a classic case of taming wild plant species by selecting non-shattering and non-dormant populations. Natural selection has facilitated the survival of *Cuphea* in the wild by maximizing seed dispersal. The domestication of *Cuphea* calls for reversing the evolution of perfect seed dispersal. The difficult task of developing commercially

desirable non-shattering and non-dormant cultivars of *Cuphea* has been underway at Oregon State University for nearly two decades.

Genetic diversity is the basis of crop breeding and improvement (Allard, 1960). Furthermore, examining the genetic variability within a gene pool of exotic and elite breeding material could make crop improvement more efficient by directed accumulation of favorable alleles. Genetic diversity for domesticating *Cuphea* has been supplied by two interfertile taxa, *C. viscosissima* and *C. lanceolata* f. *silenooides* (Brandt and Knapp 1993; Ali and Knapp 1996a). Oddly, *C. lanceolata* f. *typica* x *C. lanceolata* f. *silenooides* and *C. lanceolata* f. *typica* x *C. viscosissima* are sterile, whereas *C. lanceolata* f. *silenooides* x *C. viscosissima* are fertile. *C. viscosissima* and *C. lanceolata* are geographically isolated and seem to have diverged as species during evolution of this genus. Moreover, the formae of *C. lanceolata* are allogamous and morphologically much more similar to each other than either are to *C. viscosissima*, an autogamous species (Graham 1988; Knapp and Tagliani, 1991). The early analyses of intersubspecific and interspecific hybrids were performed before any of the taxa had been collected on a significant scale from the wild. Brandt and Knapp (1993) and Ali and Knapp (1996b) only had one wild population each of *C. viscosissima* and *C. lanceolata* f. *typica* and a synthetic population of *C. lanceolata* f. *silenooides* (LN43) produced from germplasm

accessions collected from botanical gardens (Crane et al. 1995a).

Subsequently, 74 wild populations of *C. viscosissima* and 20 wild populations of *C. lanceolata* were collected and are the basis for analyses of SSR diversity presented here.

Several key wild characteristics of *Cuphea* have been modified through classical breeding, e.g., seed dormancy and seed shattering have been reduced (Crane et al. 1995a,b; Knapp and Crane 2000a,b,c). Near-isogenic lines and other novel breeding stocks have been developed for domestication traits; however, the tools needed for mapping phenotypic and quantitative trait loci (QTL) have not been fully developed.

The genetic diversity of germplasm collections can be established from pedigree records, morphological traits, isozyme and DNA markers (Smith et al., 1990; Mumm and Dudley, 1994). The development of DNA marker technologies has re-defined plant breeding. Molecular marker technologies can be broadly categorized as hybridization and polymerase chain reaction (PCR)-based methods. Restriction fragment length polymorphism methods, RFLP (Botstein et al., 1980) have found restricted applications since the development of PCR (Mullis et al., 1986). PCR-based marker systems include random amplified polymorphic DNAs, RAPDs (Williams et al., 1990; Welsh and Mc Clelland, 1990), amplified fragment length polymorphism, AFLP (Vos et al., 1995) and

simple sequence repeats, SSRs (Morgante and Olivier, 1993). Selection of a DNA marker system for plant breeding depends on project objectives, population structure, genomic diversity of species under investigation, time required for analysis and cost per unit of information obtained (Staub *et al.*, 1996). The utility of the DNA markers is also governed by their abundance, reproducibility, multiplex ratio, information content and convenience (Powell *et al.*, 1996a, 1996b; Russell *et al.*, 1997; Pejic *et al.*, 1998).

Ideal DNA markers for plant breeding are co-dominant, time and cost effective, abundant, and highly polymorphic. SSR markers are sequence based DNA markers that utilize differences in the number of DNA repeat units at a given locus and are a valuable source of genetic markers. SSRs have been extensively used for genomic diversity and evolutionary studies in many species e.g. human (Bowcock *et al.*, 1994), mouse (Dietrich *et al.*, 1996), *Drosophila* (Goldstein and Clark., 1995), *Arabidopsis* (Innan *et al.*, 1997), rice (Yang *et al.*, 1994) and sunflower (Yu *et al.*, 2001; Tang *et al.*, 2001). SSRs are one of the most abundant and polymorphic classes of DNA markers in plant genomes (Akkaya *et al.* 1992; Morgante and Olivieri 1993; Wang *et al.* 1994; Röder *et al.* 1995; Powell *et al.* 1996a,b). Large numbers of SSR markers can be efficiently developed by mass sequencing clones isolated from SSR-enriched genomic DNA libraries (Karagyozov *et al.* 1993; Edwards *et al.* 1996) and genotyped using high-throughput

methods (Carrano et al. 1989; Reed et al. 1994; Schwengel et al. 1994; Diwan and Cregan, 1997). Both have been key factors behind the rapid development of dense second generation genetic linkage maps for several plant species (Cregan et al. 2000; Ramsay et al. 2000; Temnykh et al. 2000; Tang et al. 2002).

We presently have a limited understanding of the nature and distribution of genetic diversity of *Cuphea*. On the basis of allozyme polymorphisms in a synthetic population of LN43, *C. lanceolata* seemed to be a highly polymorphic species (Knapp and Tagliani 1989). Webb et al. (1992), in an analysis of RFLPs, found significant polymorphisms among a random sample of *C. lanceolata* f. *silenoides* inbred lines. There are no reports of molecular genetic diversity in *C. viscosissima*. Several years ago, our laboratory screened 12 allozyme loci for polymorphisms among 24 wild *C. viscosissima* populations originally collected from half of the natural range of the species (unpublished data). We observed a null allele for one locus in one population; otherwise the 12 loci were completely monomorphic. Genetic diversity, as measured by allozyme polymorphisms, seems to be exceptionally sparse in *C. viscosissima*.

To date, there has been no report on the development of SSR markers in *Cuphea*. The present study was undertaken to develop SSR

markers for *C. viscosissima* and *C. lanceolata* in numbers sufficient for constructing a 'complete' genetic linkage map, routinely mapping phenotypic and quantitative trait loci, and performing marker-assisted selection.

Webb et al. (1992) developed a sparse genetic linkage map for *C. lanceolata* using 5 allozyme and 32 RFLP markers. Because the number of loci on the allozyme-RFLP map was low, the six linkage groups they reported may not correspond to the six chromosome pairs of the two species. Slabaugh et al. (1997) developed and mapped sequence-based DNA markers for 22 fatty acid synthesis genes. Since then, no additional DNA markers have been developed and the density of the genetic linkage map has not been increased. Numerous SSR and other sequence-based DNA markers are needed for molecular breeding and the development of a dense genetic linkage map for *Cuphea*.

The goals of the present study, aside from the development of a large collection of SSR markers, were to reassess genetic diversity in *C. viscosissima* and *C. lanceolata* by surveying SSR polymorphisms among wild populations collected over the last two decades. Thus the objectives of the present investigation were:

1. Develop and characterize SSR markers for *Cuphea*
2. Estimate genetic distances among various *Cuphea* accessions
3. Assess the patterns of genetic diversity and relationships among
Cuphea accessions
4. Conduct a principal component analysis of the *Cuphea*
accessions

Material and Methods

Construction of genomic libraries enriched for CA, GA and GAA repeats

Genomic DNA was isolated from an inter-specific *Cuphea* line, PSR 23, as per Porebski *et al.*, (1997) with minor modifications. The isolated DNA was sent to Genetic Identification Services (GIS, Chatsworth, CA) for construction of SSR enriched libraries. The CA, GA and GAA repeat libraries were constructed according to procedures described by Karagyzov *et al.* (1993) and Edwards *et al.* (1996). To ensure isolation of microsatellites from throughout the genome, genomic DNA was digested with blunt end restriction enzymes. The genomic libraries were constructed from fragments with size ranging from 300 – 800 bp. The inserts were ligated to adaptors harboring Hind III cloning sites. The constructs were amplified with primers complementary to one adaptor sequence. The amplified fragments were trapped with magnetic beads coated with (CA)₁₅, (GA)₁₅ or (GAA)₁₅ to capture the amplified fragments. The captured fragments were released and amplified by PCR, followed by a second round of enrichment. Fragments were released from magnetic beads by eluting into solution and ligated to Hind III cloning sites in pUC 19 plasmids. The plasmids were multiplied in *E. coli* strain DH5α. The yield of recombinant cells was approximately 220 colonies/μl.

Colony PCR and DNA sequencing

The genomic libraries were plated on LB-agar medium with 75 µg/ml ampicillin. The recombinant clones were selected with the IPTG and X-Gal method involving screening blue-white colonies. The white colonies were transferred to LB-agar medium in rectangular plates in a pattern corresponding to the 96-well format and incubated overnight at 37°C. Bacterial colonies were picked and used as templates for colony PCR. The PCR reaction was executed with universal forward primer (5'- CGCCAGGGTTTCCCAGTCACGAC-3') and reverse primer (5'- TCACACAGGAAACAGGCTATGAC-3') in a MJ PTC 200 thermocycler manufactured by MJ Research Inc., Watertown, MA. The reaction volume of 35 µl contained 1x buffer, 2.5 mM MgCl₂, 0.2 mM of each dNTPs, 1% Tween-20, 1.5 mM cresol red, 3% sucrose, 0.2 µM of each primer, 0.75 unit Taq polymerase (Qiagen, Valencia, CA, USA) and colony picks as template DNA. An initial start up denature at 95°C for 5 minutes was followed by 35 cycles of 94°C for 15 seconds, 55°C for 30 seconds, 72°C for 30 seconds and a final extension of 72°C for 5 minutes. Amplicons were tested on 1.5% agarose gel to visualize successful PCR and determine product size. The colonies that produced bands between 300 – 1000 bp were selected for sequencing. The selected PCR products were purified using QIA Quick PCR purification kits (Qiagen, Valencia, CA,

USA). Approximately 25-50 ng PCR product was sequenced on an ABI 3730 DNA Analyzer at the Genomics Center, University of Nevada-Reno, NV. The sequencing reaction utilized ABI Big Dye Terminator Cycle Sequencing Ready Reaction Kit v3.1 chemistry. M13 forward primer (1.5-3.0 pmoles) was used for sequencing reactions.

Sequence analysis and primer design

The sequences were analyzed for the presence of SSRs using SSRIT Software (<http://brie2.cshl.org:8082/gramene/searches/ssrtool>). The sequences harboring SSRs were further analyzed with the SEQLAB function of Genetics Computing Group (GCG) software version 1.1 developed by the Department of Genetics, University of Wisconsin, Madison. Plasmid sequences flanking the genomic inserts were trimmed and redundant sequences were identified using the PILEUP feature of GCG Software version 1.1. Unique sequences harboring ≥ 5 repeats were selected for primer design. The primer length ranged from 20-27 bp (average 22 bp), GC content was between 45-60% (average 50%) and Tm was between 59 and 63°C (average 61°C). Other features of primer pairs include Tm variance of 2 °C, maximum 3' stability of 9.0, maximum self complementarity of 6.0 and maximum 3' self complementarity of 2.0. The expected allele length produced by each primer pair ranged from 100-500 bp. The forward primers were fluorescently tagged with FAM, HEX or NED.

Plant material

Ninety four *Cuphea* accessions were obtained from United States Department of Agriculture National Plant Germplasm System (<http://www.ars-grin.usda.gov>). Seventy four accessions of *C. viscosissima* and 20 accessions of *C. lanceolata* were included in the genetic diversity study (Appendix 2). Based on the floral characters, most of the plants in the *C. lanceolata* panel could be classified as *C. lanceolata* f. *silenoides* and *C. lanceolata* f. *typica*. Four accessions had characters intermediate to forma *silenoides* and forma *typica* and yet remain unclassified.

Cuphea seeds were germinated at room temperature on moist blotter paper in covered 11 x 11 x 3 cm plastic boxes. Seedlings were transplanted into pots containing potting soil (pumice:peat moss:sandy loam) in trays for further growth. A single plant from each accession was transplanted to 1 gallon plastic pots and grown in a greenhouse with 16/8 day/night cycle, in fluorescent light and at 22°C. Tender leaf tissues were harvested and snap frozen in liquid nitrogen. The tissue was freeze dried for 7 days prior to DNA extraction.

DNA extraction

Genomic DNA was extracted from freeze dried tissue according to Porebski *et al.*, 1997 with minor modifications. The freeze dried tissue was ground using a Polytron and in presence of 2% CTAB

(cetyltrimethylammonium bromide) extraction buffer and incubated at 65°C.

Chloroform:octanol extraction was carried out once and the aqueous

phase was mixed with 0.2 volume of 5M NaCl, precipitated with 2 volumes

of cold 95% ethanol and refrigerated overnight at 4°C. The DNA pellets

were dissolved in TE (10mM Tris-HCl and 1 mM EDTA, pH 8.0) buffer.

After dissolving, DNA was treated with Rnase (100 µg/ml) for 1h at 37°C

and Proteinase K (1µg/ml) for 30 min at 37°C. The DNA was back

extracted with phenol:chloroform to remove Rnase and Proteinase K and

re-precipitated with 1/10 volume of sodium acetate and 2 volumes of 100%

ethanol. The solution was allowed to sit overnight at 4°C followed by

centrifugation. The DNA pellets were washed with 70% ethanol and

allowed to dry. Finally, the DNA was dissolved in TE (10mM Tris-HCl and

0.1 mM EDTA, pH 8.0) buffer.

DNA fingerprinting

The SSR-targeted primer pairs were tested across a panel of six

genotypes (*C. viscosissima* accession VS55, *C. lanceolata* f. *silenooides*

accession LN 193, *C.lanceolata* f. *typica* accession LN 204, *C.lanceolata*

accession LN 192, partial seed retaining line PSR23 and *C.lanceolata* f.

silenooides with partial autofertility accession LN 185). Twenty nine primer

pairs that produced amplicons with most of the above genotypes were

selected for SSR fingerprinting. The PCR was carried out in 10 µl reactions

with 1x buffer, 2.5 mM MgCl₂, 0.2mM of each dNTP, 0.2 µM of each primers, 0.075 units of Taq polymerase and 1 ng/µl of template DNA. A touchdown PCR protocol (Don *et al.*, 1991) was adapted to reduce non-specific priming. The parameters of the PCR program included an initial start up of 95°C for 3 minutes followed by 6 cycles of 94°C for 30 seconds, touchdown by 1°C from 64°C to 58°C and 72°C for 30 seconds. This was followed by 30 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds and a single cycle of final extension of 72°C for 15 minutes. Seven microliters of PCR product were tested on 1.5% agarose gel to confirm successful amplification. For fluorescent fragment analysis, different amplicons labeled with FAM, HEX or NED fluorescent tags were diluted 20 fold and multiplexed in a single well. GS 500 ROX was used as an internal lane standard. On excitation, the fluorescent dyes FAM, HEX, NED and GS 500 ROX produced blue, green, yellow and red colors respectively. The genotyping was performed on an ABI 377 Gene Analyzer with Gene Scan Analysis Version 2.1 (Applied Biosystems, Perkin Elmer, Foster City, CA). Genotyper Version 2.0 (Applied Biosystems, Perkin Elmer, Foster City, CA) was used for data collection and calculating the allele sizes.

Data analysis

Polymorphic Information Content, PIC, scores (Botstein *et al.*, 1980) reflect the relative value of each marker with respect to the degree of polymorphism exhibited for each polymorphic locus. PIC scores were estimated by the formula:

$$\text{PIC score} = 1 - \sum_{i=1}^k p_i^2$$

where p_i is the frequency of the i^{th} allele and k is the number of alleles.

Thus, PIC scores are essentially the same as heterozygosity values that were described by Ott (1991).

Standard deviation of molecular weight (SDMw) expressed in basepairs was calculated for each SSR marker. SDMw is an excellent measure of SSRs' potential for array contraction or expansion. It acts as a robust measure for microsatellite molecular diversity.

The maximum repeat count was estimated by the formula:

$$\text{MRC} = [(\underline{\text{MAMW}} - \underline{\text{RAMW}})] + \text{RRC}$$

x

where MRC is maximum repeat count, MAMW maximum allele molecular weight in base pairs, RAMW is reference allele molecular weight in base pairs as predicted by Primer 3 Software distribution, RRC is reference repeat count

and x equals two or three depending whether the motif is dinucleotide or trinucleotide respectively. (Goldstein and Clark, 1995; Cho *et al.*, 2000; Kishore *et al.*, 2002).

A survey of the data on allele sizes suggested microsatellites in *Cuphea* must not have evolved in a stepwise fashion. These microsatellites violate the assumption of methods computing genetic distance measures that are based on a stepwise model (Matsuoka, 2002). Hence proportion of shared alleles distances that is free of the stepwise mutation assumption was used. We used FITCH program with Fitch-Margoliash coefficient in the PHYLIP package (Felsenstein, 1993) with the log transformed proportion of shared alleles distances as implemented in the MICROSAT program (Minch *et al.*, 1997) to estimate genetic distance among *Cuphea* accessions. In FITCH, the J option was used to randomize the input order of samples.

Cluster analysis of the genetic distances was performed using the Treeview software (Page, 1996) version 1.6.0. Principal Component Analysis (PCA) was performed to evaluate associations among the 94 *Cuphea* accessions using SAS software version 6.12 (SAS Institute, Cary, NC).

Results

Development and characterization of simple sequence repeat markers

The inserts from 600 clones each from the (CA)_n, (GA)_n, and (GAA)_n enriched genomic DNA libraries were amplified by colony-PCR and checked for length on agarose. Of the 1,800 clones, 1,095 had inserts in the 300 to 1,000 bp range required for SSR marker development. The amplicons were purified, sequenced, and screened for the presence of SSRs using SSRIT, a web-based software program (<http://brie2.cshl.org:8082/gramene/searches/ssrtool>). SSRs were found in 1,035 of the DNA sequences. The DNA sequences were screened for redundancy using the GCG PILEUP routine (Madison, WI). We identified 610 redundant and 425 unique SSRs. The different repeat motifs identified were CA, GA, GAA, TA, ATC and ACA (Table 1).

DNA sequences harboring unique SSRs were searched for the presence of primer sites flanking the SSRs. SSR primers were designed for 271 unique SSRs. We could not identify primer sites in 154 unique SSRs because of short flanking DNA sequences, poor sequence quality in the flanking DNA sequences, or failure of the primers to meet the required design criteria. The efficacy and cross-taxa utility of the SSR primers were initially tested on a panel of six genotypes (VS55, LN193, LN204, LN192,

Table 1: Microsatellite motifs in *Cuphea* (CA), (GA) and (GAA) libraries

Motif	# of sequences
GAA	72
CA	24
GA	300
TA	12
ATC	1
ACA	16

PSR23, and LN185). The reference genotype (DNA source for constructing the SSR-enriched genomic DNA libraries) was PSR23, an inter-specific line originating from an interspecific hybrid between *C. viscosissima* and *C. lanceolata* f. *silenooides*; hence, the reference genotype for a particular SSR marker locus could be either taxon. Of the 271 SSR primers, 184 amplified alleles from one or more of the six genotypes (87 failed to amplify alleles from any of the genotypes). Table 2 summarizes the development and characterization of SSR markers in *Cuphea*.

The cross-taxa amplification rates of the SSR markers were tremendously variable. While 100% of the 184 SSR markers amplified alleles from the reference genotype (PSR23), 73 to 124 (40 to 67%) failed to amplify alleles (produced null alleles) from the other genotypes (Fig. 1). The cross-taxa amplification rate was greatest for *C. viscosissima* (VS55, 60%) and lowest for *C. lanceolata* f. *typica* (LN204, 23%). VS55, a *C. viscosissima* genotype, was one of the parents of the reference genotype PSR23 (Knapp and Crane 2000c), and, as such, was expected to have a high cross-taxa amplification rate (a high percentage of the reference DNA sequences should be of VS55 origin). *C. lanceolata* f. *typica*, on the basis of interfirma and interspecific hybrid sterility (Brandt and Knapp 1993; Ali and Knapp 1996a), seems to be the most genetically distant taxon of the

Table 2: Summary of SSR marker development

Category	Number
Clones sequenced	1095
SSR-containing sequences	1035
Unique SSR-sequences	425
Primer pairs designed and tested	271
SSR markers developed	184
SSR markers used for diversity study	29

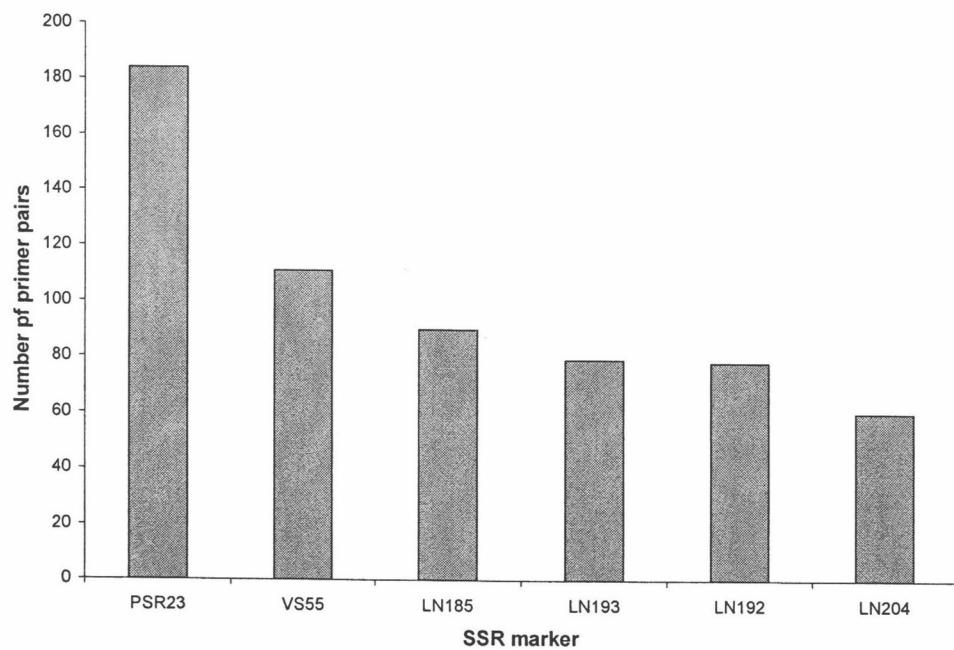


Figure 1. Cross taxa amplification by primer pairs that produced functional SSR markers when tested on VS55, LN193, LN204, LN192, PSR23, and LN185 DNA

three and, accordingly, had the lowest cross-taxon amplification rate.

DNA fingerprinting

Of the 184 SSR markers, the 11 markers (ORC 16, 18, 34, 187, 207, 210, 224, 225, 242, 251, 255) that amplified alleles from each of 6 genotypes and 18 markers (ORC 11, 14, 20, 22, 28, 30, 37, 47, 54, 58, 61, 144, 145, 180, 203, 221, 223, 247) from the set of 22 that amplified alleles from 5 genotypes were screened for cross-taxon and cross-genotype amplification and length polymorphisms among 74 *C. viscosissima*, 8 *C. lanceolata* f. *silenoides*, 8 *C. lanceolata* f. *typica*, and 4 unknown forma *C. lanceolata* germplasm accessions. The 29 primer pairs used for the DNA fingerprinting study are indicated with an asterisk in Table 3. On the basis of the number of amplicons observed on agarose, 28 SSR markers amplified one locus each and 1 SSR marker amplified two loci each.

Seven of the 29 SSR markers (ORC16, 58, 144, 146, 207, 225, and 226) were monomorphic in *C. viscosissima* (Fig. 2-3). The minimum, mean, and maximum number of SSR alleles amplified from *C. viscosissima* were 1, 4.76 ± 4.77 , and 20. By contrast, none of the SSR markers were monomorphic in *C. lanceolata*. The minimum, mean, and maximum number of SSR alleles amplified from *C. lanceolata* were 2, 10.97 ± 5.38 and 21. Similarly, the minimum, mean, and maximum PIC scores for SSR markers in *C. viscosissima* were 0.1, 0.42 ± 0.25 , and 0.93, respectively,

Tale 3. Two hundred and seventy one SSR markers developed for *Cuphea* species.

Primer label	Motif	Left Primer Sequence	Tm	Right Primer Sequence	Tm
ORC 1	(GA)5	TTCTTCTTCTTCGGCTTG	60.12	GAAAGCCTCGCCAAGTAAATCT	61.06
ORC 2	(CTT)10	TGGTAGGGGTTAGTGAGTGAGG	60.41	ACACGCTGTCTGTCTGTTCTGT	60.02
ORC 3	(GA)7(GA)12	TCACCATTGAACGAAGACAGA	60.67	TATGTCTATGTCACGCGCTCTC	60.44
ORC 4	(GA)7	CAGACAGCGTGTGTGTTG	61.45	CCCTCCACTGACCTTCTCTTC	61.51
ORC 5	(TG)7	GCCTCTCGTGAGTTGAATTTG	61.14	TCTGCACTCACTCATTACAGC	61.6
ORC 6	(GA)7	CAGACAGCGTGTGTGTTGTT	60.9	CCCTCCACTGACCTTCTCTTC	61.51
ORC 7	(GAA)7	GCAATTCAATACACAGAAGAACCA	60.4	GCAATTCAATACACAGAAGAACCA	59.88
ORC 8	(GT)10	ACGCTGCACAAAATGAGCTACT	61.34	CATACAATCTCTGCACCGCTTA	60.28
ORC 9	(CT)6	TCAAATGAATGTGCCCTTCTC	61.34	AACATTGATGGAATGGAATGGA	61.3
ORC 10	(GT)9	GCTCCAGTGTGATTGTTGA	60.15	TGATCCGACCACATCAATATCC	61.85
*ORC 11	(GA)5	CGTGTAAAGTCACGCGTTGTGA	62.09	CCTTCCTTCCCTTCCCTTCT	61.21
ORC 12	(GA)13	GGGGATGGTGTCTTCTCTT	60.69	CCCTCTCATTACAGATATCTCTCTC	59.38
ORC 13	(GT)5	TCTGGTCAAAGGGTTAAGATTGA	59.99	TTCTTCATACGCCTCCACATT	60.85
*ORC 14	(GT)6	TCTGGTCAAAGGGTTAAGATTGA	59.99	TTCTTCATACGCCTCCACATT	60.85
ORC 15	(GA)13	GGGGATGGTGTCTTCTCTT	60.69	CCCTCTCATTACAGATATCTCTCTC	59.38
*ORC 16	(CTT)9	GAGGAGGGTGAAGTGATGATGT	60.38	CACGCTGTGTCTGTTCTGTT	60.54
ORC 17	(CTT)8(GA)7	CTGAGGAGGGTGAAGTGATGA	59.99	CACCCCTCCACTGACCTTCTC	60.85
*ORC 18	(CTT)10	GGAGGAAGGGATGAAATATGCT	60.64	GGTTTCAGAGTTGAGGATTGC	60.12
ORC 19	(GAA)8	TGAAGGAGTTCGGAGAAAGTGA	61.28	CTCAGTTGCCCTCTGTCTCTGT	61.01
*ORC 20	(GT)5	GATGTTGAAGTGTCGCTTGG	62.92	TCAGCTCTGTTCTCCCTCTCAC	61.1
ORC 21	(CTT)10	GAGGAGGGTGAAGTGATGATGT	60.38	CACGCTGTGTCTGTTCTGTT	60.54
*ORC 22	(CTT)10	GAGGAGGGTGAAGTGATGATGT	60.38	CACGCTGTGTCTGTTCTGTT	60.54
ORC 23	(GA)6	AGCTCAGTGGTGAAGAGAGCA	59.31	CCCTTACTACTGGCTTGCTTG	60.3
ORC 24	(GAA)4(GAA)5	ATTTCAGGAACAAGCATCCA	60.99	CGTCTGGGACGATGATGATATT	61.09
ORC 25	(GT)5	GAGAATTAAATTGGGTGGGTGTG	60.46	CACGGTAGCTGCACAAATTAA	60.18
ORC 26	(CT)25	GCAATGTGTCGGTGTATGGAG	61.37	GGCTAACCCGGAGGTTTATC	61
ORC 27	(GAA)8	GCATTGCTGAGACAACACAC	59.95	GGGGGACTCCATACAGTAAGTCA	61.47
*ORC 28	(GA)5	CACCTAAGCCGATAACACCAA	61.22	GTTGCCTATTGCTTCCTTCT	60.94
ORC 29	(CT)6(CT)8	AACTATTGGGGTATTTGGATGGA	60.63	CGCAAGATTCGACATACCTCT	60.63
*ORC 30	(CTT)8	GCTTCTTCCCTTGTGAAA	59.87	GGCCACACTCTTCACTCTCT	60.06

(continued)

ORC 31	(GA)5(GAA)7	AGAAAAGCTCACAGAGACGGAGA	60.7	GGTTTCGCCCTAACCCCTAGATT	61.78
ORC 32	(GAA)10	AAGAGACATCGGAAGGAGAAAAA	60.58	AGTGATGTGCTGTTACCCATGA	60.44
ORC 33	(GT)14	ACGCATTAGATAACACCCCCAAC	62.17	GTATGCACACAAGCACGTTCAC	61.59
*ORC 34	(GAA)11	ATGCCACTGCCGACAGTAAAG	62.49	CTTCCATCCCACTGTTCTCCT	61.74
ORC 35	(CA)11	TTCGAGTTCTCAGATTATCAGTT	59.7	AAATCCACGTGATAGTATAGTCCTCAA	60.5
ORC 36	(GA)17	TCCCCACGTTCTCATTTATCTT	61.2	ATGAGTTGGTTTGGACACGAC	61.2
*ORC 37	(CA)12	TTTGGTGAACCGGATTAGAGTG	61.37	CGATCTCGGATGGAATCTTAGT	60.79
ORC 38	(GAA)5	ACCACAAGGGAGACCCAATATC	61.32	GTAAGCCAAGTGTTCGACCAGA	61.58
ORC 39	(GA)11	CACGTGAAGATAACGCTCACAA	61.24	TGGCATAAAATTTCACAAACAGG	61.02
ORC 40	(AG)27	TTGAGATCAAACCCCAGAGAAA	60.96	AGATGCACGCTAAGGTTGAGAA	61.29
ORC 41	(GA)6	TCAGGAGAGGGAGTGGTGGAGTA	61.21	CCGAGCTCTCTGCTCATACA	61.93
ORC 42	(GA)15	TAGAGAGACGGAGACGGTGATG	61.73	CACACAAGGAAACACGCTATGA	61.11
ORC 43	(GA)5	TCTTCTTCTTCTGGTCAAAGG	60.38	CCTCCACTGACCCCTCTTTCT	61.15
ORC 44	(GA)11(GA)10	GAGCATATTTCGCAATAAGTGGATG	61.01	GGTGGCAAGAGCTATCAGAAAAA	60.74
ORC 45	(CTT)20	GTCTTCTCCGGCTACTTGAT	60.96	CATTGCTGATGGTGAAGAGAGA	60.4
ORC 46	(GA)6(GA)7	GCGAATAGCTTTGATGATGGA	61.42	TTAAATATTGCGTGCACAT	60.39
*ORC 47	(GA)8	AGCAAGCAGAGAGGGAGAAAGA	62.01	ACCCCTCTAAATGTTGCGTTC	61.55
ORC 48	(GAA)19	CATTGTCGCTGCTTCTTACAAA	59.46	AAAGAAAAGGAGGGAAATAAGACG	60.33
ORC 49	(TC)29(GT)5	CCCTGTTAGACGAAAAGAGACG	60.29	TCACGCAAAATATCTCACTGTATG	60.42
ORC 50	(GA)5(GA)6	GTGTGTGTGAGAGTGAGCGTGA	62.59	ATAAAAAGAGAGCGCGTGTGTG	61.67
ORC 51	(TC)19	AAGAGGACGAAGACGAAGACG	60.94	TGCTGTTCTGTTCTCAT	59.78
ORC 52	(CT)9(CT)12	GCTAAGGTTGAGAAGGGAGATGG	59.37	TGCATTCTATTCGGGAGATG	60.05
ORC 53	(CT)15(CT)9	GCTTCCCCCTTAAGAAAAATAACG	60.63	CATGTGATAGATGCCGGAGATT	61.22
*ORC 54	(GA)21	GCACAAACACAGGTGAGAGAGG	62.22	TCCTTTATCTTGAACACCTAACGACC	60.27
ORC 55	(TC)24	TTCATGGACATGTTAGCTGGTG	60.94	TGCAATAAGTTATGGGAAACC	60.08
ORC 56	(GA)11	GCAATTGGAGTCGGAAGACAA	61.49	AGGAAAACTCAGGGCTTGGTG	62.77
ORC 57	(CTT)10(GT)6	TCAGAGCATCGATCTGAGGAG	61.06	TCTCTTCTTCATACGCCTCCA	60.86
*ORC 58	(TA)5	ATATTAGGTGACGGCGCTTAC	61.51	CATTGGGTTATGTGGATTATGATATG	60.42
ORC 59	(CT)6(CT)3	GGGTATGATGCTGAACGTAAAGG	60.88	TGGATGGATATGTTGATAGATACCG	61.11
ORC 60	(GAA)8	AGCTTCTTCATTGCTCTGTTGC	61.06	AAATAATGGCGAGGAAGACACG	61.36
*ORC 61	(GT)11	TGAAACTGTCAATGGAGCCAATA	61.73	GCGAGTTTGACATTGACGAT	61.39
ORC 62	(GT)13	TTCATTCTTGCCTTGTCAAAT	60.83	GTCCTCCATGTCGCTGTCTTC	61.09
ORC 63	(GA)29	CATTGTCGTTCATGTCTCG	60.83	AACACCGCAGGTAATTGAGAAA	60.88

(continued)

ORC 64	(TA)5(TA)6	TTTCCCTGGGTTCTGATTCT	61.15	CTTCTATGGTCCCTCCAACC	61.04
ORC 65	(CTT)10	ACATGAAAATGAGGGGCATAAA	60.55	AACGTCCAATTGCTTCCTGAT	60.87
ORC 66	(TC)25	CCTGATGGATGGCTGTAGACAG	61.98	TTTTGTTGCACGAAAGTCG	61.17
ORC 67	(GA)29	GCATGTGTAGTAACCAGTGATGCT	60.49	GCTCGTAGTGATGAAAATGACCA	61.4
ORC 68	(AG)14	GTCACCGTATGCCAGGTTTAA	61.11	TGAAGGGAAATACTTGCCAGT	60
ORC 69	(GA)4(GA)27	GAGGGAGAATGGGAGAGAGAGA	61.21	ATAATCCCCCAGACGATACACC	61.15
ORC 70	(CTT)10	GGAAGGAATAGGAATCAGAGCA	59.69	ATCTCCATCACACACGCAAAC	60.98
ORC 71	(AG)27	AATATGTGTTGGAACGGCATT	59.22	TGTATCTCCTGTCTCTCAAACG	59.35
ORC 72	(CTT)6	GAAAAATTCCGGTGTGAATG	60.56	AACAAATGAAGAAGTTCTGTGGTG	59.6
ORC 73	(CA)7	GAATCTGGTTCACCTGTTATCTAGAAA	60.28	TGGATGCTTCATTCACTCTTTG	60.25
ORC 74	(GA)14(GA)22	AGGGAAAATAAGCCACCCAAT	60.88	GTAAAACGACGGCCAGTGAAT	61.27
ORC 75	(CA)6(CA)8	CCTCTAACACAGAGCCCCTT	61.81	TGGGTATGAAGATGAGTGAGTGT	60.43
ORC 76	(GA)29	TAGCCCGCGAAATTAAAGAGAAT	61.33	CCCTGTTAGACGAAAAGAGACG	60.29
ORC 77	(GA)13	AGAAAACCCATCCTGGCTGT	61.22	CAGCAGTCATCTGGAATCACAA	61.24
ORC 78	(TG)6	GGGATGCTTCATTCACTGCTTTG	60.99	TCACTGAATCTGGTCACCTGTTA	60.93
ORC 79	(AG)18	GCACAGTTTAAGGGGCTAACAA	60.52	GGGTATGATGCTGAAGTGAAGG	60.88
ORC 80	(GT)11	GCCTTTTCAGCTACCAAAACA	60.62	CGAGAGAAAAGCAGGAACCTTT	61.21
ORC 81	(TC)8(TA)12	GGTATGATGCTGGAAC TGAAAGG	60.88	CCCAGAGTGTGAGGTTAAAGG	61.22
ORC 82	(GA)27	CCGAATGCAAGGTTTAAGG	60.5	GGGTATGATGCTGAAC TGAAAGG	60.88
ORC 83	(CTT)9	GATGATGTAAGTGGTATTGGGTTAATC	60.89	TCTTCATACGCCCTCACATTTC	61.36
ORC 84	(GT)11(GA)19	GCGCACACAGAAATTGAAAGAT	61.52	TATGACCATGATTACGCCAAC	62.08
ORC 85	(GA)16(GA)10	GGAAAATAAGCACCCGATTGAT	61.34	GGGTTTCCAGTCTCTGT	60.87
ORC 86	(TA)5(GA)20	TCCGTATGCACAGGTTTAAGG	61.22	GGGTATGATGCTGAAGTGAAGG	60.88
ORC 87	(GT)5	GCAGATCGAGAGAGAGAGAGA	60.38	GGCATGAAAATTAGCCCATTAA	60.17
ORC 88	(CT)33	GGGTATGATGCCACTGAAC TGAAAG	60.88	ACCCTCTCTCTGCCATATAAAA	59.67
ORC 89	(TA)4(TA)8	CTCCGAATGCACAGGTTTAAG	60.99	GAGAATGATGCTGAGCTGAAGG	61.42
ORC 90	(CA)11	GAAGTCTGCTCCTCAGCTCAA	61.2	TTTCCCCTTTCAATTTCACCG	61.29
ORC 91	(AT)5	TGCTTCCCTGCCATTATTCT	60.1	TTATCAGGGGGACAAACACATT	60.46
ORC 92	(CA)11(GA)6	TAAGGCGTCAGCAGAGACTCAA	62.49	GACCACTAAGATCGCAGGACAC	61.08
ORC 93	(GA)22	TCTATGACTCTCGCGATCTCT	59.77	CACTCGTGCAGAAATAACAAAAAA	60.16
ORC 94	(CTT)5	TGGGATGAATGAAGGAATGAAT	60.51	AAATCTCCATGACAGACGGAAA	60.86
ORC 95	(GA)5(GA)10	GGTGGATCTGTTCTGACAAAGG	60.91	TGCAATAACTCTCTCCATTTC	60.79
ORC 96	(AG)33	AGAATAGTCACCGAATGCAAGG	60.5	GGGTGTGATGCTGTACTGAAGG	61.88

(continued)

ORC 97	(CT)7	GATATTGGACGTGGCTGAAAAG	60.85	GCTAAACACAAGGCCAGTTAT	60.67
ORC 98	(AT)5	TCCAACCATGAATAACACACACA	59.74	GCATATCCACCATATTTACGTTCA	60
ORC 99	(GA)23	TTTCAAAACTAACCAACACACAG	59.98	TCTGTCCTTCTCTTGAAACACA	59.02
ORC 100	(GA)5	GAGGACCGAAATGAGAGGGAGAT	60.95	AGCTTGACAAC TG CAGGTAACG	61.75
ORC 101	(GT)5	AAATGGTTTCTGATCTAACAGG	60.72	GCACCGGTCA TTACACTTTGG	62.59
ORC 102	(GAA)8	CCTTCCTTGAGCCATCATATCTT	60.79	GGTTCTGTTGGATCTTGTCA	61.06
ORC 103	(GT)14	AAATGGGAGGAAGAAATCCAAA	60.97	CAAGCATGAAAATGACCACTGA	61.06
ORC 104	(GA)18	TTTGAGCTGATGATGACGATG	61.17	GGGAGAGCTATGTCAGTTCAA	60.63
ORC 105	(CA)5(CA)5	CACGGTTTGTACACGTTGTC	60.36	CCACTAAAGTGTGTCGAGT	60.27
ORC 106	(CT)12(CTT)8	TATGCTCTGGTGTCA TGT GTC	61.13	GGATCAGATTGAGGGAGGAGAA	61.81
ORC 107	(TC)11	AAGCGAAATCCAGAGTACAGAGC	61.25	CACCTTCTCTCTTAAATTCTCCAG	61.19
ORC 108	(TC)23	TTCATGGACATGTTAGCTGGT	60.94	TCGCGTGATTTACACACAGAG	61.24
ORC 109	(GA)31	CCTGTTTGCACCAAA TAGCAT	61.23	ACTTCTCGTGTGATGGGAAAAA	60.88
ORC 110	(GA)31	GGCACTCTGCCATAAGAAAGAA	60.74	GGGTATGATGCTGA ACTGAAGG	60.88
ORC 111	(GT)10	CTGGAAAACATCTTGCACATA	60.60	CCACTCAGGTAAAACATT CAGGA	60.39
ORC 112	(TA)5	ATATTAGGTGACGGCGGCTTAC	61.51	TGAACACATCACAAGCTGTCAA	60.35
ORC 113	(AG)29	GGCACTCTGCCATAAGAAAGAA	60.74	GGGTATGATGCTGA ACTGAAGG	60.88
ORC 114	(GA)31	GTCACCGAATGCAAGGTTT	59.98	GGCACTTTCATTAGTAGGGTATGA	58.66
ORC 115	(TA)13(TA)6(TA)4	CTCGTGAAGGATACCATGTGTG	60.43	ACGTATGAGACC GTGTGAGA	60.63
ORC 116	(TC)10(TC)6	TCTGCCAACGCCCTATGAATATC	60.43	GCCCTTGCATTTGCATACTTA	60.11
ORC 117	(GA)8(GA)5(GA)5	AGCTTCCATAAAATCACAGGA	60.99	GTAGACAGGT CGGGGAAAAACT	60.74
ORC 118	(GA)18	AACCGTTGGTAGAGTGAGGAA	60.03	GTGGCGTTAAAGAGCCATAC	60.86
ORC 119	(AG)22	TCTCGGTATTGGGAGTAAGAAA	59.17	ATAAACCA CGTACCCGAATCC	59.96
ORC 120	(CT)9(CT)7	GTATGCAGGTGCTATCGGAAAT	60.36	TCTATATACCCGGGCAATCC	62.03
ORC 121	(CT)28	GTTCTTCAGCCAGTCCCTTT	60.97	CGGTGTATATAGAAGGGTGTGT	59.26
ORC 122	(AG)30	AGAATAGTCACCGAATGCAAGG	60.5	GGAAGGAGAATGCACAAGTGG	61.96
ORC 123	(AG)7	CACATCGAACAGAACATTCCATA	60.89	CCGTGTGTCTCTCTCTCTCT	60.74
ORC 124	(GA)18	CTGCTGCTTCTGTTCTCAA	60.74	CAGCAGGATGAGATGAGAGGTT	60.79
ORC 125	(GA)18(GA)11	GTAGAACACCCGGGAAACAC	60.64	AAACGGTGTGACGTTAGTGGAT	60.7
ORC 126	(GA)8	GGAAGAGGAGGCAGAGAAGAAG	60.98	GTCCGGTCTCTGGAGTAGACAA	60.68
ORC 127	(GAA)29	ATGAACGAACCCATAGCTAAA	60.85	GCAGAGATCTGAAGAAAACCTGG	59.53
ORC 128	(AG)18	GGGAGTGAGTGAGTTGAGTTCTC	60.69	ATGTCAGAAACCAAACCCAACA	61.54
ORC 129	(GA)29	CCTGTTTGCACCAAA TAGCAT	61.23	GAGCAAGCACCAGCAAAGTAGT	60.98

(continued)

ORC 130	(GA)14	ATACATGAATGCAAGGGAGCAA	61.72	AGTCAAAGAAGCTCCTGGGTAA	61.48
ORC 131	(CT)15(CT)5	TCAGTCAGGAACTCAACTGCAC	60.49	GAAGTAACAAGGGAAAGCTCAGG	60.62
ORC 132	(GAA)10	AAGGAGGAGATGATGAGAAGCA	60.35	CCAAAGCATTAAACCCTCCCTA	61.46
ORC 133	(GA)6(GA)29	AATTTTAACAGTGTAGTGGCTTG	59.89	ATATCTGTGGGCAGTGACTCGT	60.96
ORC 134	(GA)19	GGTAGATAGGAGGGTGAGCA	60.52	CTTCACACATAGGGTTCCCTTC	59.86
ORC 135	(GAA)11	GCTGACCTCTGCAACAAAATTA	59.41	TTTGGTGCCAGTTTTAATCA	59.03
ORC 136	(CT)15	TCGTGATTTCAGCAGTCATCT	59.88	CTATGACCATGATTACGCCAAG	59.5
ORC 137	(GA)12	CCAATCTAATTGCAGGGAGAA	60.43	CATGGTCGACCTTTTAGCAAT	60.36
ORC 138	(GT)7(GA)31	TGAGAGAGGAGCTATCCATCC	59.81	TCTGTCTCTCCCACACACAC	61.25
ORC 139	(GAA)11	TGGAGGAAGGAGATGAGAGGA	61.26	AAAGGAAAGCAAAGGAAGCAGT	60.74
ORC 140	(GA)8(GA)28	GCGCAAGGGTATATGAGAGAGT	59.77	TCACTGAGAGATATCGGGTGAG	60.42
ORC 141	(AG)30	GGCACTCTGCCATAAGAAAGAA	60.74	GGAAGGAGAAATGCACAAGTGG	61.96
ORC 142	(GA)23	GATTGGGAGACTTCAGATCACG	61.02	CACCTGACACACACACAGAGA	62.29
ORC 143	(GA)17	CCAAAAGGGATGGAAGAAAAG	61.11	GCTCACAGAGGGCATAAAAGT	61.84
*ORC 144	(GA)5	CAGAGAAGTTCCGTCTGAAT	59.75	GACTGATTGCTTGATGGGTAA	59.95
*ORC 145	(GA)15	CTTGACCGCCCAATTCTATC	60.81	CCCCTGCTAGACGAAAAGAGA	60.87
ORC 146	(GA)16	AAATTTCACCTGGGTTCCCTC	60.55	ATATATCCTCAGTGGGCTCGAA	59.95
ORC 147	(GAA)11	CAGCCACGCATCTTGAGATAA	61.31	AAATGGCAGCATCAGTGTCA	61.25
ORC 148	(GA)19(GA)11	AGAGAGAGCGAGAGAGAGCTGA	60.32	ATTCCTCGTCCCACAACAGAG	61.46
ORC 149	(GT)12	AAGCCCAACACCACTAAAACA	60.78	AGCAATCCCACATAACCACCTG	61.14
ORC 150	(GA)5(GA)7	TCTCTCTCATCTCGTCTGTCA	59.34	GGGTTTCCCACTCTCTGT	60.87
ORC 151	(GA)25	AGAGTGACTIONGGGGAAA	62.11	GCTTCCAGCAGGTATCATCAAC	61.02
ORC 152	(GAA)9(GA)13	GGATCAGATTGAGGGAGGAGAA	61.81	TAACATGACCGGTTCACTAGCC	61.27
ORC 153	(GA)29	GATTGGGAGACTTCAGATCACG	61.02	CCCTGTTAGACGAAAAGAGACG	60.29
ORC 154	(AG)30	GGCACTCTGCCATAAGAAAGAA	60.74	GGAAGGAGAAATGCACAAGTGG	61.96
ORC 155	(GA)20(GA)17	TGCGATAATTGAGTTCTCTGC	61.61	CCCCACCCGTATAGACATGGTA	62.89
ORC 156	(GA)6(GA)14	AGCAAGACGCTTGGCAGTTAT	61.3	AAAGAGATCCCCTCCTCTCTC	60.68
ORC 157	(GA)14	ATGGGTTCTCTTCTCACCTT	60.35	TCCAAGATAGATTCCCTGCCTCA	61.08
ORC 158	(GA)30	TGTTGTGTGAGAAAGGACCA	61.58	AGCGGCAGTAAATCTCTCT	61.56
ORC 159	(GA)21	AGCATAGCGTGGAGGAATATCA	60.97	TCCTTATCTTGAACACCTAACGACC	60.27
ORC 160	(GA)14	AGATGAGGTGATGAAGGAAACG	60.5	AGAACTCACTGCACATCCAACA	60.75
ORC 161	(CTT)10(GT)6	ATCAGAGCATCGATCTGAGGA	59.93	CATACGCCTCCACATTCATC	60.18
*ORC 162	(TC)13(TC)5	ATCATAAGTGGAGGCCAGAAGG	59.61	CTTACCACGCCATTACCATCC	61.47

(continued)

ORC 163	(CTT)15	TCTCAACGAGACGCCATTATTT	60.98	GTGTTTGTTGAAATGGCTAGTG	59.97
ORC 164	(CTT)15	CGATGCCACACTTACAAGAA	61.2	CGACCGAACAGTTGAGATCAG	60.84
ORC 165	(CTT)10	TCTTCGGTGTCTTCTGTGT	61.07	AGAGTTGGTGGCTAAATCACG	60.53
ORC 166	(GAA)5	CTGTCAGTCATCATGGAGGTTG	60.57	TGAACGTTACCCAATAGAAATAAAG	59.03
ORC 167	(GA)32(CT)8(GA)6	GCAATTGTATTAGCATCAACC	60.72	CGCGCTCTCACATTATCTC	61.23
ORC 168	(GAA)9(GA)18	GAAAAGAGGGTTGGGATTTGT	59.67	TCATCTTCTCCCGAATTCTC	59.67
ORC 169	(GA)23	CACCCAATTCTATCCACGAGA	61.22	AGAGAACGACCCCCATTGTATC	61.57
ORC 170	(GAA)11	GTGTGAGAGAAGACCCAGTTGC	61.26	CAAAGGGTTGAAAGGAAAGGAA	61.61
ORC 171	(GAA)11	GCTTATCGGGATCATGTGTTG	61.59	TCTCAACGAGACGCCATTATTT	60.98
ORC 172	(CT)9	TGCCCTTCTCCACGAATATCT	62.14	GTACACCAGGATCAGCATAGGG	60.76
ORC 173	(CA)9	CGGCCTCATATCTCTACTCA	61.35	GACACCCTAGCGGAAGGAAAG	62.34
ORC 174	(GA)6(GA)5	TAGAGAGACGGAGACGGTGATG	61.73	AAAACCTGGCATACGGTGACTA	60.77
ORC 175	(AG)28	ATTCGGACTCATCCAAATTTC	61.37	AAGCTTCCACAGTCCCAGTATG	60.55
ORC 176	(GAA)9(GA)18	GAAAAGAGGGTTGGGATTTGT	60.55	TGTCACTCATCTTCTCCGAAT	60.83
ORC 177	(TA)28	CCACCCCTGTTCTACTGTACATC	59.8	GGGGAGGAGAACATAAGTGTGTC	59.3
ORC 178	(AG)12	TAACGATCAAAGGAAACCCAAC	60.22	TCTGTCTCTGTGGTTCTCCAG	59.49
ORC 179	(GA)29	GCATGTGAGTAACCAGTGATGCT	60.49	CACCACAAAACAGCTATGTCCA	60.97
*ORC 180	(GT)12	CCAATCTAATTGCAGGGAGAA	60.43	CGAATGTTCGTCAGTGGACTT	61.45
ORC 181	(CA)11	TCGAGTTCCCTCAGATTATCAGTTG	60.97	GCATATCCACCATATTACGTTCA	60
ORC 182	(AG)27	TCGAAGAAAACAAACACCAAGA	59.76	GCTAAGGTTGAGAAGGAGATGG	59.37
ORC 183	(CA)7	TCACTGAATCTGGTTCACCTGTTA	60.93	AATCCCAAGGATTCTGACCTGACC	61.41
ORC 184	(GA)5(GA)6	CATCAAATTGGCTTGGCTATC	60.8	AGTGAAGTGTGGCTCTCCATA	61.07
ORC 185	(GAA)5	TTGAGGAGTTGGTGGGTGTC	61.48	AGAAACCTCGTGCCCTGTGA	62.31
ORC 186	(GAA)5	AGTGATGGGACGATGATAATG	60.96	TCAGTGAAGCTGGACTATTGG	59.38
*ORC 187	(GAA)7	GTCGTACAGGTGGATTCAAGTT	61.38	ATCCCTCCCTCTTCTCCCT	60.9
ORC 188	(CTT)10	TGTCATTCTGCTGCTGAACC	61.37	AAGCTTGACTTCCCGACAT	61.02
ORC 189	(GAA)10(GA)18	GAGGGAGGGAGAGAGAAACGAT	62.29	AACCCATGAACCCATAACATGA	61.21
ORC 190	(GA)22	CTCCTAACCTTGTCCCTTCC	59.09	CTATCCGTGAAATTGCAAAC	59.65
ORC 191	(AT)10(GT)18	GATCATGAAGGGACGACGAGT	61.45	GGACTCGCATTGAAGAGGTAAA	60.61
ORC 192	(CT)18	CGACAAGACAAGACGACAAAAG	59.95	ACCCACAGAGACGGTAAGTGAA	60.96
ORC 193	(GA)7	CAGAAGCAAGAGCTGCAGATAG	59.56	TTTGGTCCCTAACCATCATT	60.05
ORC 194	(CTT)14	CCTAAGGAATTGCTCTAACGA	60.73	ATGACCATGATTACGGCCAAG	62
ORC 195	(TC)8(CT)7(CT)12	CGGGTTATATAGGGGGAGCTTA	60.51	CGAGAAGATTGGGAGACTTCAG	60.37

(continued)

ORC 196	(GA)28	CCCTTAGCCTGCGAAATTAAGA	61.81	GAGTAATGTGCGTGATGTATCTTG	59.97
ORC 197	(TC)25	CACCGCATTTCACCGAACATCA	61.88	TTTTGTTGCACGAAAGTCG	61.17
ORC 198	(GT)7	GAGCATTATCACCATACCGACA	61.1	AGTCGCCAACGCTAACACTATG	59.86
ORC 199	(GAA)6	GGAGGGACATTTCAAGAAAGC	61.28	AAATGCTAATTGGTGCCACCT	59.9
ORC 200	(CTT)10(GT)6	GAGGACAGAACCTCGTTGCAGAT	60.83	TTTCTTCATACGCCCTCCACATT	60.85
ORC 201	(CT)32(AC)13	TGAGCAAGCTAAATCGAGTACCA	61.25	CGCAAAAAGTATAACGCACACAC	60.6
ORC 202	(CTT)8	TGATTGTTCGTTGTTTCAAGCA	61.58	CCTCCCCAACATAGTATCTCCA	61.36
*ORC 203	(GAA)18	GCCTCCTATGGCTAGTGGAAAT	60.81	GAAAAGGAGGGAAATAAGACACG	60.34
ORC 204	(GAA)8(GA)15	GAAGGGAAAAGAGGGTTTGG	61.13	AACATGACCGGTTCACTAGGCC	61.31
ORC 205	(GA)32	ATACCTCCTTGCAGCATCAGT	60.16	AATTAAGGTCGGGAAGGAGAA	60.3
ORC 206	(AT)5(AT)6	AAGAATAAGCCCAACAAACACCA	60.75	GATGAAGGAGATGGACTGTGA	61.43
*ORC 207	(CT)8(CT)6	TCGGCATCACAGCCTACTAT	61.15	TGAATTCTCCATGTCCAGTC	61.25
ORC 208	(GAA)10	TTGAGGTTTCAATGTGACTGACG	61.12	TGTCCCTGGAAGAAATTCAAGA	60.96
ORC 209	(GA)32	ATACCTCCTTGCAGCATCAGT	60.16	AATTAAGGTCGGGAAGGAGAA	60.3
*ORC 210	(CTT)13(CTT)3	CGATTCCATTACAAAAACATCA	60.73	CAGATGATGCAGAACCTCTCG	62.22
ORC 211	(GA)16(GA)13	CAATGTTGGATTCCCTACTTGC	60.72	GGGTATGATGCTGAACGTGAGG	60.88
ORC 212	(CTT)8	CGAATCCAATCCTAGACGTTGA	61.35	GAACCTCGAAATTGACATGAGC	61
ORC 213	(CTT)13(CTT)3	CGATTCCATTACAAAAACATCA	60.73	CAGATGATGCAGAACCTCTCG	62.22
ORC 214	(GA)12	CCAACAAACCCCAGTCCCTATT	61.3	CCTTATTCCCTCGAAAAACTCC	60.3
ORC 215	(TG)9	TGAGAAGCTCAGCCATTATTCC	60.71	GGGGGTCATATCTCTCACTCAC	60.9
ORC 216	(AG)7	GGCACGGAAGTGAACAGAAATA	61.38	TTTCTTCGCACCATCGTAGATT	60.98
ORC 217	(TA)32(GT)5	TGAAATTAAAGGTCGGGAAGG	60.29	GGCAGAAATATACGTGTCCTTTCA	59.95
ORC 218	(CTT)11	TTCCCTTATAGTGCCTCCGATCC	61.61	GAACCTCCTTTCGGGTTCTT	60.81
ORC 219	(CTT)11	ATTTTGTACAGACCGAACATCAGC	60.46	GCCTCCCCCTTCTTCATGTTA	61.62
ORC 220	(TA)32	TTAAGGTCGGGAAGGGAGAATG	61.61	GGGGGAGGCAGAACATATACGT	60.41
*ORC 221	(GA)15	TTGCTGGAAAAGCACAGTTGAT	62.05	ACATTAGCCCCGCTCTCTTTTC	61.06
ORC 222	(GAA)7	TGATGAGGCTGTGGATGAGAGT	62.18	GCTTCCATCTCCTGTCTTC	60.74
*ORC 223	(GAA)5(GAA)6	TGCTGGCAATACTTTCTGAACC	61.51	CATCTTCATGTCCCTGCTCTTC	61.15
*ORC 224	(GAA)5	TGCTGAAATGCTGAAACTTATCC	60.61	AGCTTCCAGGAGCAAGACTAGG	61.39
*ORC 225	(CTT)17	GAAGACCCAACCGAACATCAG	61	GGCGAGAAAAAGAAAACACG	59.86
*ORC 226	(CTT)9	CGCGAAGGTACACACTATTG	60.69	TGCGGAAGGATTAGATGAGAAA	61.05
ORC 227	(GAA)8(GAA)8	AAGCAACATAGTGCACGTTGGAA	60.92	CAACATCAGGTATGAGGGTTGA	59.85
ORC 228	(CT)16(CTT)10	AACCCATGAACCCATAACATGA	61.21	GTAAGGGAAAGGGAAAGGGAAA	61.74

(continued)

ORC 229	(GAA)8	AGCTTGGACTTGGAAAAGTGAA	60.27	GGTTATCACACCTGGCTAGCTG	60.15
ORC 230	(CT)11	GGTTTCTTGTGCAGGTTGAGA	61.56	CGTCGCCACATATATAGATAGAGA	61.6
ORC 231	(CA)5	TGCACAATCATCATCAGAACATCA	60.08	TGATGAAAGAGGAAGAACGAGCTT	60.37
ORC 232	(GT)7	AAATGGGAGGAAGAACATCCAAA	60.97	TCGCCAAGCTTAACACTATGTACA	61.03
ORC 233	(GA)5(GA)6	GAGAGCCGTTGATGAGTTCTT	61.11	CTAGAGTTGGTAGCCCGAAA	59.79
ORC 234	(GAA)10(GA)11	GTAAGGGAAAGGGAAAGGGAAA	61.74	GTTGAACGCCAGGAAACACAT	62.24
ORC 235	(GAA)10	GGAAAATCGGAAGAAGGGAGAGG	62.53	GCCACATGATCAAATGTCAAAGT	61.15
ORC 236	(CT)9(GA)5(AT)5	GGTATGATGCGTGAACGTGAGG	61.8	GGCCGGTATCAAACACAATACT	60.14
ORC 237	(GA)32	ATACCTCCTTGCAGGCATCAGT	60.16	CAGTGTGCGCTCATCAGTGT	59.97
ORC 238	(GAA)10	GGCATGCAAATGCAAAGACTA	61.15	GGATCGCTACAATCCATCACAG	61.78
ORC 239	(GA)7	GAATCCTGAATCAATCGGCTAA	60.41	GTCCGGTCTCTGGAGTAGACAA	60.68
ORC 240	(GAA)7	CAAGTTCCAACCTGACTCCTC	60.15	GGGACAGTGAGGACATAGCTGA	61.61
ORC 241	(CTT)7(GT)7	AAAGAGACAGGAACCGAACGACC	61	TTTCTTCATACGCCCTCACATT	60.85
*ORC 242	(GA)29	ATGCGACCTTAACAAACATGA	59.52	GGGTATGATGCTGAACGTGAGG	60.88
ORC 243	(CA)5(CTT)10	ATAAATTACCCACCGAACATCACG	60.96	TTGAGGTTTATGTGACTGACG	61.12
ORC 244	(GAA)9	GAGCAATCGACGGAAAGTTAGAG	60.39	ACATTGTGCCTAACATCGAACAA	60.43
ORC 245	(CA)5(AC)5	TCTTCCCTTCTCCTCTGCTT	60.83	ATCAACCAGCTTCACCTTCAG	60.66
ORC 246	(CTT)12(CTT)3	TATCAACATCACCTCGCCTTC	61.36	CAGAACCTTCGGAGATCAAT	59.71
*ORC 247	(CA)9(AT)9	ATGGAAACCAAACACTGCGATATG	61.08	TACGTTAATTGTTCCCCCACA	61.31
ORC 248	(CTT)10(GA)5	CCAATACACGAAGAGACGGAAC	60.9	TTTCTTCATACGCCCTCACATT	60.85
ORC 249	(CTT)10(GT)6	CCAATACACGAAGAGACGGAAC	60.9	TTTCTTCATACGCCCTCACATT	60.85
ORC 250	(GT)9(GT)3	ACTGCCGTTCTGACATCTTC	60.67	TACAATATTGGCAAGGCATCC	61.03
ORC 251	(GA)26	ATCTACAATACCCCTGCGAGCTG	60.65	TCAACTGATACCACTGCGTTCTT	60.95
ORC 252	(GAA)6	GCCTTTTATCCTGCCATTACC	61.82	TCCCTCTGTGACTGTGTCTCT	60.49
ORC 253	(GA)29	CAGGTAGTTCATCCCCCTAGCC	61.18	TTGTTCTCTTATGGAGCAGT	61.18
ORC 254	(GAA)9(GA)19	CTTAGCTTGGGAGGACAAAGG	61.42	TGTCATCATCTCTCCGAAT	60.83
ORC 255	(GA)5	AGGAGGAAGAGATGGAAGATGG	60.94	CCACCTGATCAATTAAATGCAA	59.83
ORC 256	(GAA)13	GGTGAAGAGAACGAGGCATCAGA	60.9	TATCAACATCACCTTCGCCCTTC	60.83
ORC 257	(GT)13	CCTCTTATTATCCCGCGCTCTC	61.48	CAAGCATGAAAATGACCACTGA	61.06
ORC 258	(GAA)18	AGGAAGGAAGCAAATAGGCAAC	60.94	ACGTGTCTGGTTGGCTATC	61.31
ORC 259	(CA)13	ACATGCCCTTACACCCCAAAG	61.13	AACACGGAAAGAGCAGAACAGC	61.08
ORC 260	(GAA)5(TA)5	CTTGCTTCAGCTTACGTTCT	60.44	GCTTTCAGCATTGTGGTTAG	61
ORC 261	(CT)15(CT)6	GGCTAAGCTATTGCCATATT	59.63	TCTCATCGCTGATGAACGTGTT	59.88

(continued)

ORC 262	(GAA)13	GTCGCCATTAGAGAAGCAGAGA	61.02	GCCTTGTTAACCCCTATAACG	59.9
ORC 263	(CTT)10(GT)6	GACAGCCCTTGAATCTGAAGAA	60.74	GCCTCCACATTCATCCTCTCT	61.86
ORC 264	(GAA)7	GGAATTCATCTGGGCATAATCA	61.01	GGATCCCACATCTTCATCCTACC	60.87
ORC 265	(GA)27	ATGCGACCTTAACAAACATGA	59.52	TGAAATTAAAGGTTGGGAAGGA	59.82
ORC 266	(GA)24	TGAAGGCCACCAAATTAAAGAA	60.8	TCGCTAAACAGTGTGTGTCTC	60.41
ORC 267	(GA)24	TGAAGGCCACCAAATTAAAGAA	60.8	TCGCTAAACAGTGTGTGTCTC	60.41
ORC 268	(GAA)10(GA)7	TGGAGGCAGTCTGAGCTATTTC	60.89	TGTCATCATCTTCTTCCCGAAT	60.83
ORC 269	(CT)6(GA)8(GA)7	ACCAACTTCAACCTAGGGTTCC	60.62	CAGTCCCAGAAGTCAGTGTGTTT	60.62
ORC 270	(CT)13	TCTCTGGGACACATTGTTGTTG	60.01	CTTCCTCGAACGTGATGCTCT	60.91
ORC 271	(GA)25	GGAGGCTAATCACAGAACAG	61.16	AGCTTCCAGCAGGTATCATCAA	61.14

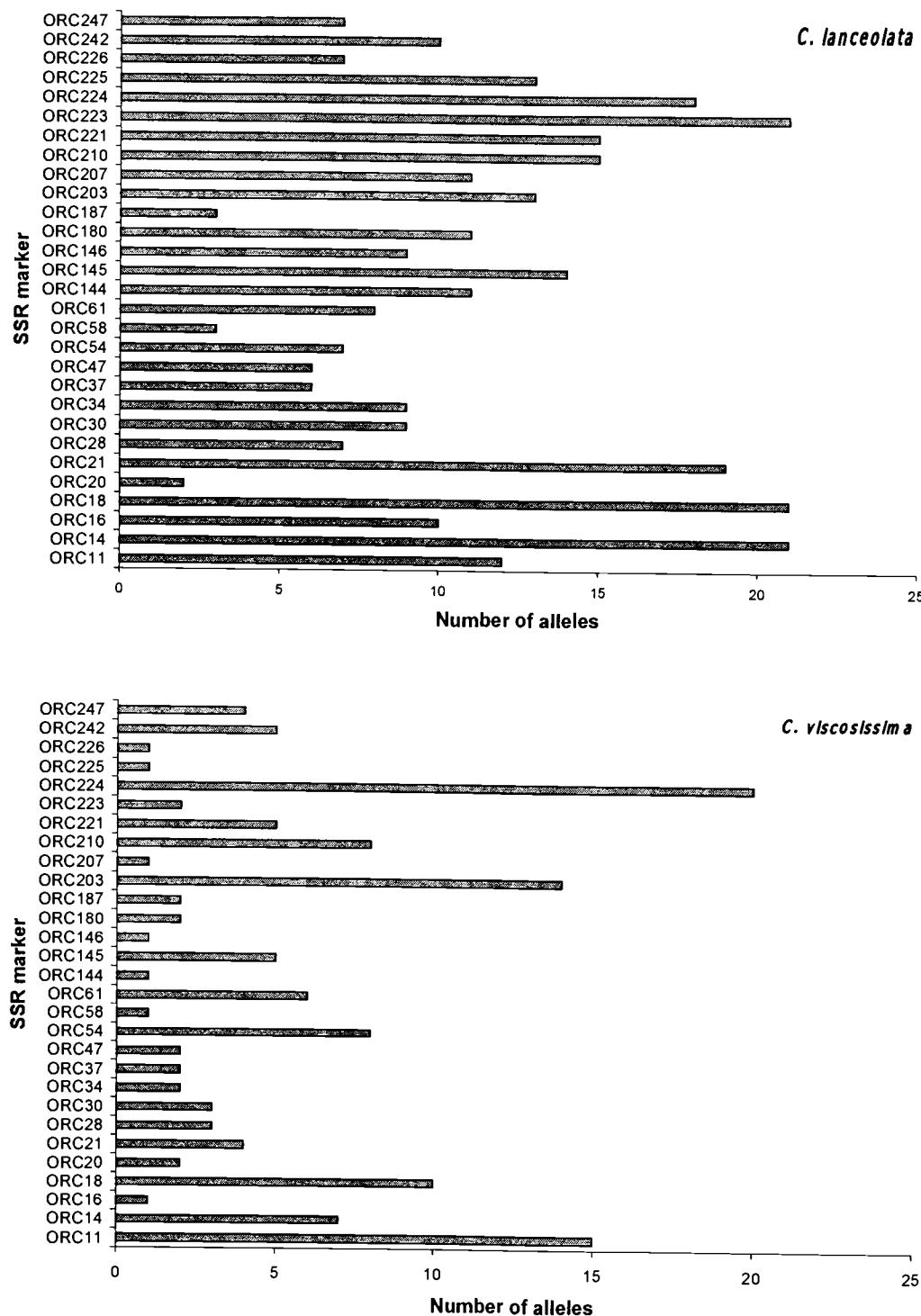


Figure 2. Number of alleles per locus amplified by 29 simple sequence repeat markers among 74 *C. viscosissima* and 20 *C. lanceolata* germplasm accessions.

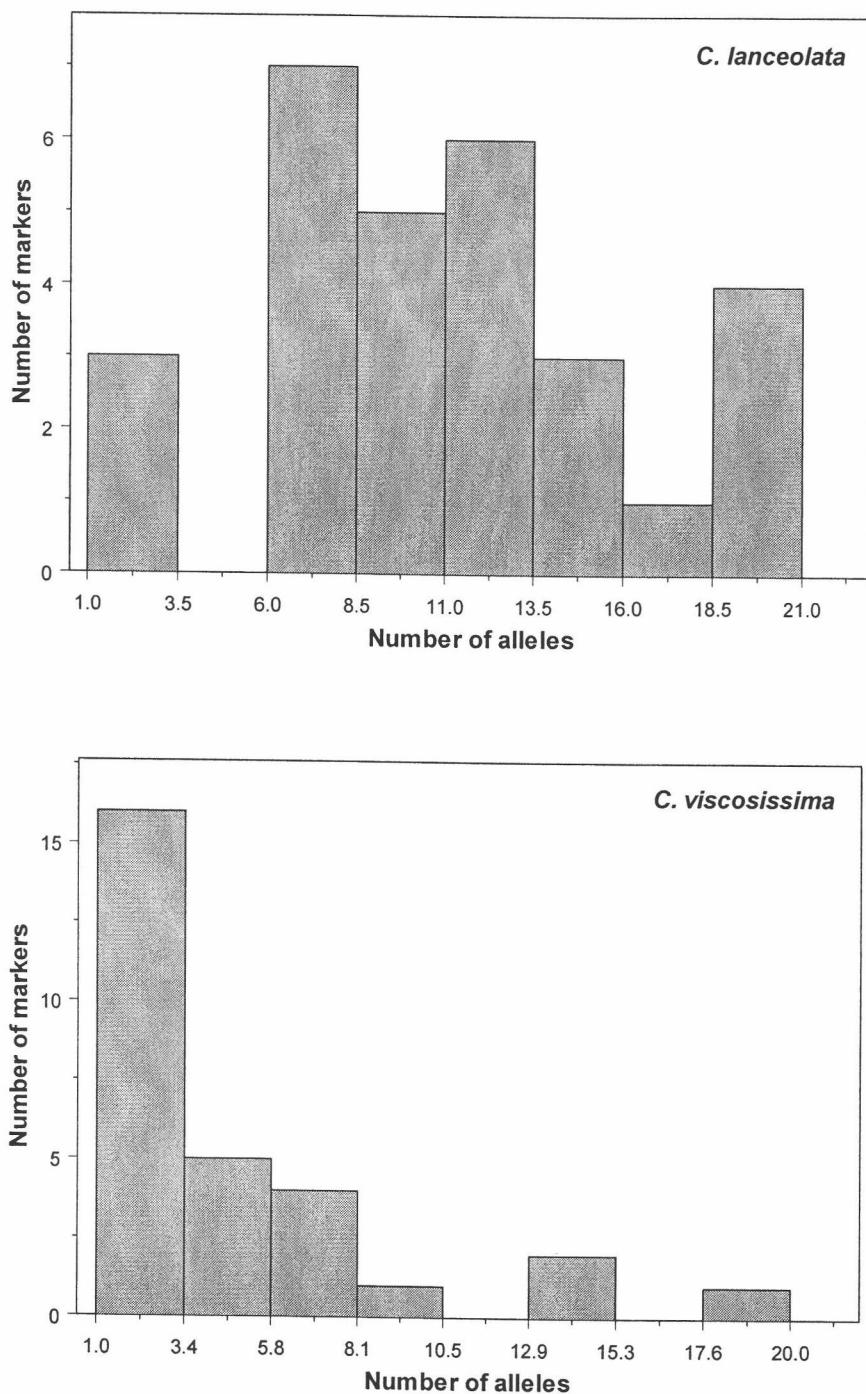


Figure 3. Distribution of the number of alleles per locus amplified by 29 SSR markers from 74 *C. viscosissima* or 20 *C. lanceolata*.

whereas the minimum, mean, and maximum PIC scores for SSR markers in *C. lanceolata* were 0.45, 0.74 ± 0.12 , and 0.94, respectively. The PIC scores for 31% of the SSR markers (9/29) were greater than 0.50 in *C. viscosissima*, whereas the PIC scores for 90% of the SSR markers (26/29) were greater than 0.5 in *C. lanceolata* (Fig. 4). The PIC score histogram for *C. lanceolata* had the classic right-skewed shape of an undomesticated, highly polymorphic, allogamous species (Fig. 4). Conversely, the PIC score histogram for *C. viscosissima* had a much flatter, uniform shape, aside from a spike in the 0.10-0.20 bin and sparseness in the upper half of the distribution. In summary, *C. lanceolata* was found to be significantly more polymorphic than *C. viscosissima*. Furthermore, because null allele frequencies were greater in *C. lanceolata* than *C. viscosissima*, allele number and PIC score differences between the species could be greater than reported.

For both species, a positive correlation was found between the number of alleles and MRC. However, the magnitude of correlation between number of alleles and MRC was higher for *C. viscosissima* ($r=0.70$) than *C. lanceolata* ($r = 0.40$) (Fig. 5). Similarly, in *C. viscosissima*, the SDMw demonstrated a stronger correlation ($r=0.78$) to MRC than in *C. lanceolata* ($r=0.72$) (Fig. 6).

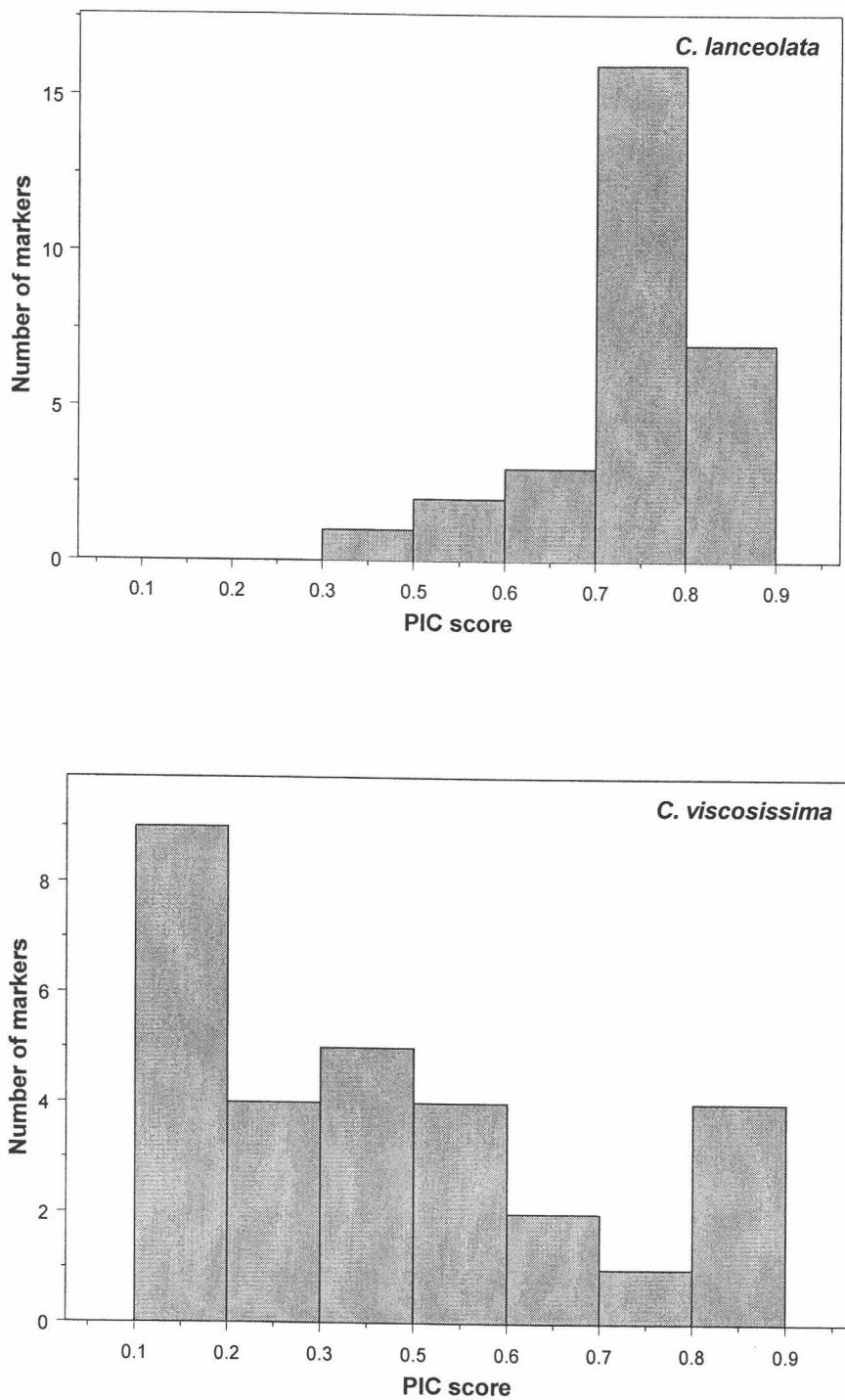


Figure 4. Distribution of PIC scores of 29 SSR markers for 74 accessions of *C. viscosissima* and 20 accessions of *C. lanceolata*.

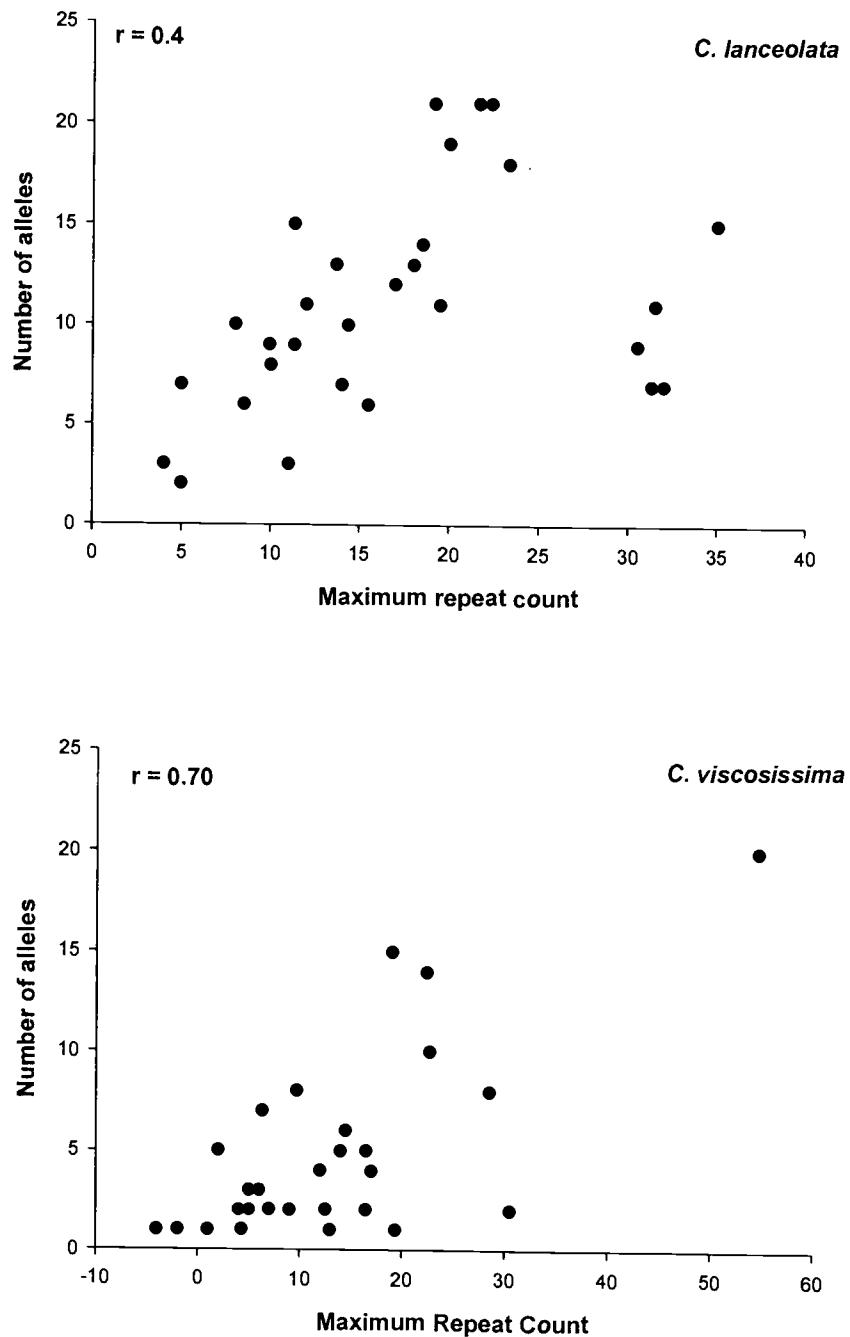


Figure 5. Correlation between the number of alleles and maximum repeat count for 29 SSR markers screened for polymorphisms among 74 *C. viscosissima* or 20 *C. lanceolata* germplasm accessions

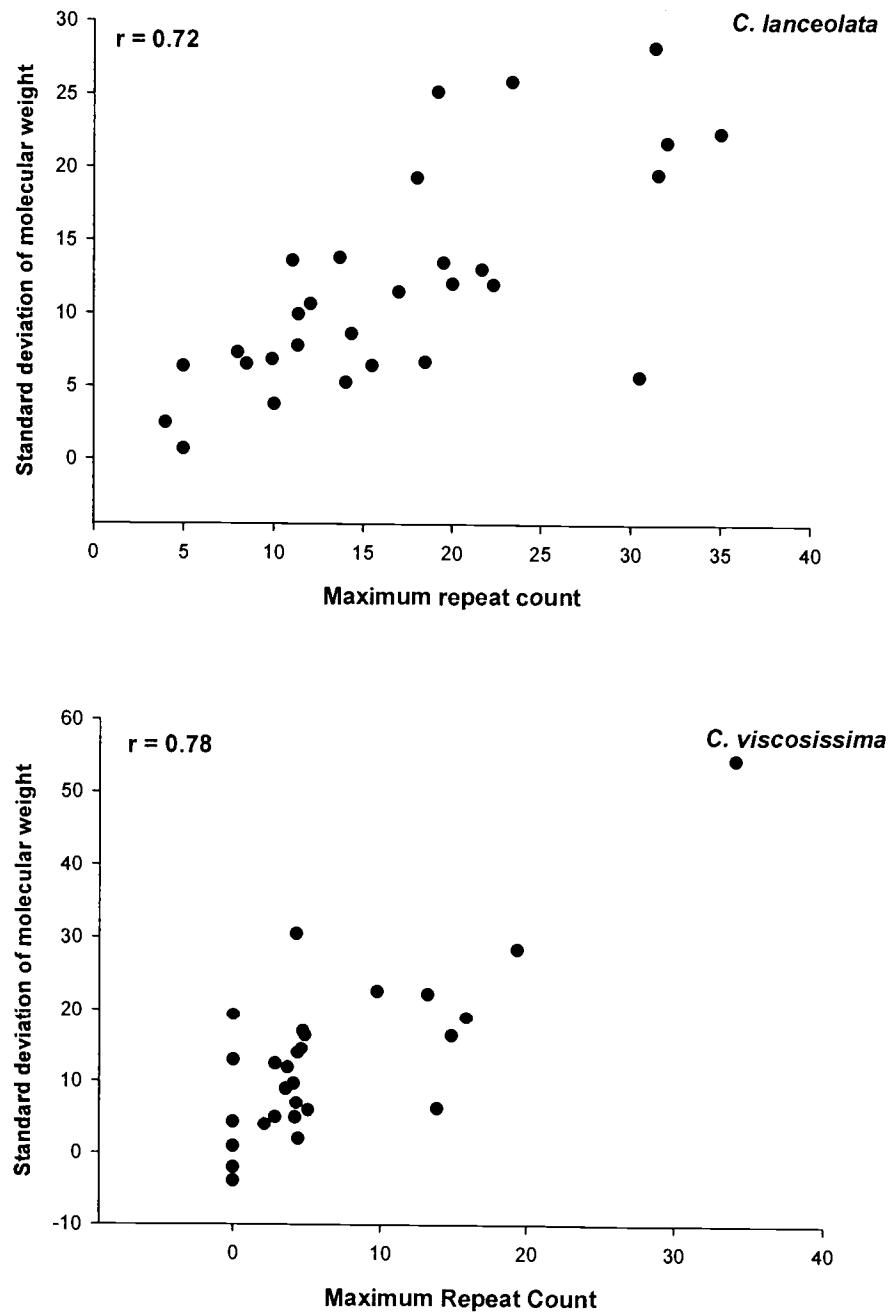


Figure 6. Correlation between standard deviation of molecular weight and maximum repeat count for *C. lanceolata* or *C. viscosissima*

Genetic Distance and Cluster Analysis

Genetic distance methods were used to search for patterns of genetic diversity within and between the three taxa (Fig. 7). Genetic distances ranged from 0.001 to 0.358 in *C. viscosissima*, 0.144 to 0.414 in *C. lanceolata* f. *silenoides*, and 0.088 to 0.521 in *C. lanceolata* f. *typica*. The shortest genetic distance was between VS352 and VS353 and the longest genetic distance was between LN198 and LN204. The two species formed distinct clades, and *C. lanceolata* f. *silenoides* and *C. lanceolata* f. *typica* formed distinct subclades within the *C. lanceolata* clade. LN186 is the only outlier from *C. lanceolata* f. *typica* that grouped with *C. lanceolata* f. *silenoides*. The four unclassified *C. lanceolata* populations, which may be interforma hybrids, grouped with both taxa. The clustering of *C. lanceolata* was in agreement with their geographic occurrence (Fig. 8). *C. lanceolata* f. *typica* and *C. lanceolata* f. *silenoides* are centered on Sierra Madre Occidental and Sierra Madre Plateau respectively. *C. lanceolata* populations and groups, as a whole, were separated from each other by significantly greater genetic distances than were *C. viscosissima* populations and groups (Fig. 7). Mean genetic distances in *C. viscosissima* and *C. lanceolata* were 0.11 and 0.30, respectively. While a few pairs of *C. lanceolata* populations (e.g., LN196-LN197 and LN200-201) were separated by shorter genetic distances than most other pairs or

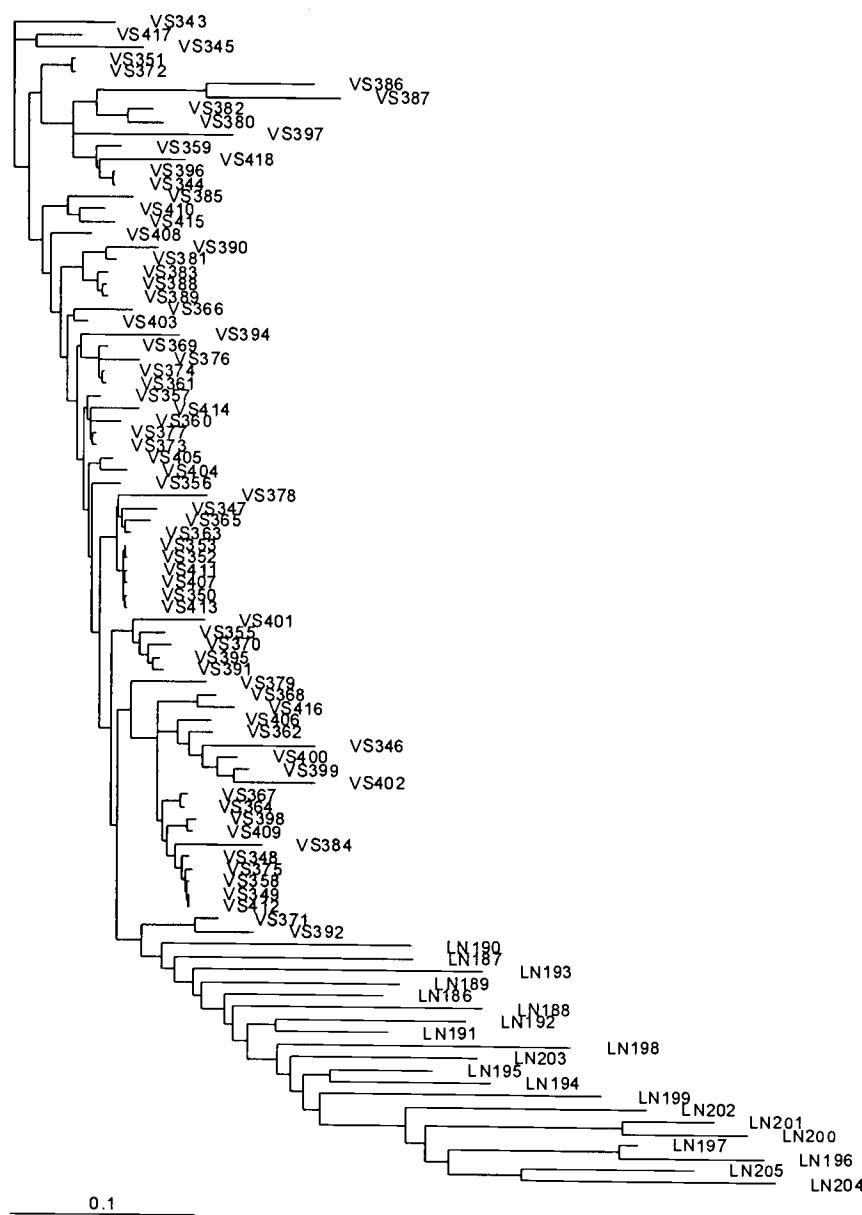


Figure 7. A phenogram produced by cluster analysis of logarithm of proportion of shared alleles estimated from 30 SSR fingerprints among 94 *Cuphea* accessions.

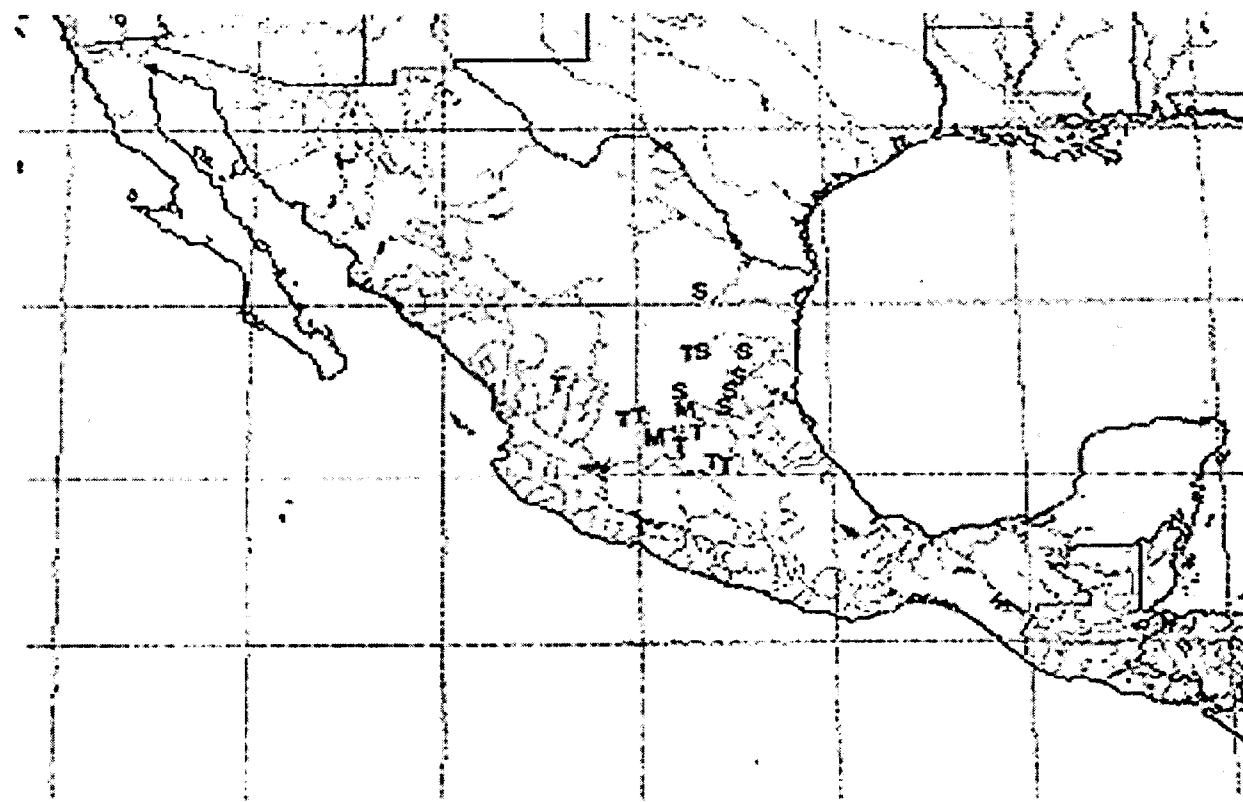


Figure 8. Geographical distribution of *C. lanceolata*.

groups of *C. lanceolata* populations, the opposite was observed in *C. viscosissima*. Most *C. viscosissima* pairs or groups were separated by short or very short genetic distances. The VS386-VS387 group, an outlier, was separated from other *C. viscosissima* populations by greater genetic distances than any other pairs or groups within the species (Fig. 7).

Principal Component Analysis

The first, second and third principal components accounted for 76.5, 6.8% and 4.3%, respectively, of the variability in the genetic distance matrix. The macropatterns of genetic diversity in *Cuphea* are clearly illustrated in the principal score plots (Fig. 9). The first axis (first principal component) separated *C. viscosissima* from *C. lanceolata* and the three *C. lanceolata* groups from each other. The dense cluster on the left-hand side was solely comprised of *C. viscosissima* populations. The two *C. viscosissima* outliers described earlier (VS386 and 387) were separated from the *C. viscosissima* group along the x-axis (first principal coordinate) and clustered with *C. lanceolata* f. *silenoides*. The *C. lanceolata* f. *typica* group, aside from one outlier (LNT186), was separated from *C. viscosissima* and the other two *C. lanceolata* groups by the greatest distance. The *C. lanceolata* f. *silenoides* group was closest to the *C.*

viscosissima group. Several *C. lanceolata* f. *silenoides* individuals (e.g., LNS187, 189, 190, 191, and 193) were closer to the *C. viscosissima* than the *C. lanceolata* f. *typica* group. The four unclassified *C. lanceolata* populations (LNU) fell between *C. lanceolata* f. *silenoides* and *C. lanceolata* f. *typica* closest to the right-hand edge of the *C. lanceolata* f. *silenoides* group.

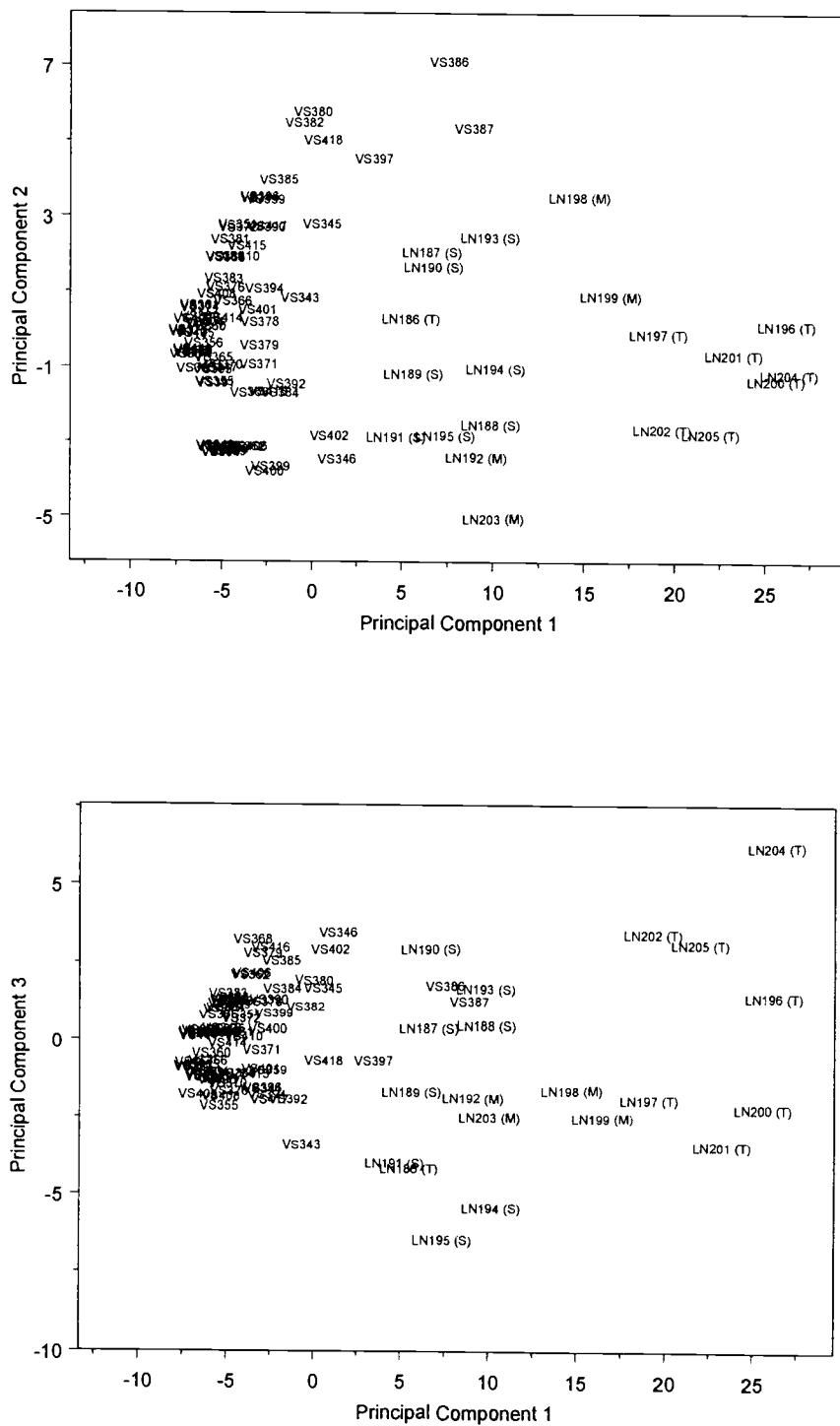


Figure 9. Principal component analysis of 94 accessions of *Cuphea*.

Discussion

The main objective of this study was to create a foundation for the domestication and breeding effort in *Cuphea* by developing high-throughput and robust co-dominant markers. Such markers can subsequently be used in linkage mapping, trait identification and marker assisted selection.

This study describes the development of 184 microsatellite markers for *Cuphea* and the use of a subset (29) of these markers to assess the genetic relatedness of the two *Cuphea* species, *C. viscosissima* and *C. lanceolata*. To characterize polymorphisms at the 29 microsatellite loci in the two species of *Cuphea*, we used a panel of 94 genotypes comprising the USDA germplasm collections of the two species. Several of these are currently being used in development of agronomic cultivars. This panel included 74 accessions of *C. viscosissima* and 20 accessions of *C. lanceolata*. The emphasis of the present investigation was on a comparative evaluation of marker polymorphisms and assessing the differences in genetic variability of microsatellite sequences. SSR libraries enriched for CA, GA and GAA motifs were constructed using genomic DNA

from an inter-specific line, PSR 23, developed from inter-mating *C. lanceolata* and *C. viscosissima* accessions. This strategy ensured that SSR motifs and flanking sequences from both species were present in the libraries.

Of the total clones sequenced, only 271 out of the 1095 sequences (24%) were amenable for primer design. Similar attrition rates have been reported earlier in many species e.g. sunflower (Yu *et al.*, 2001), barley (Ramsay *et al.*, 2000) and meadowfoam (Kishore *et al.*, 2001). The large attrition rate was primarily due to cloning redundancy within the libraries, presumably caused by selective amplification of certain sequences during enrichment or by the presence of SSRs within families of repeated sequences (Schimdt *et al.*, 1991). The success rate in primer amplification equaled 68% (184 out of the 271) pointing towards variability in sequences flanking the microsatellite loci and the difficulty in obtaining reliable sequences from SSR-containing clones. Kishore *et al.*, (2001) have reported similar success rates in meadowfoam. All of the 29 markers that were tested across the panel of 94 genotypes produced polymorphisms. While one marker amplified two loci, the remaining 28 markers amplified

single loci in the selfing *C. viscosissima* as well as outcrossing *C. lanceolata* accessions.

Microsatellites, being hypervariable, are generally more informative than other molecular markers, e.g. RFLP (Wu and Tanksley, 1993; Rongwen *et al.*, 1995; Taramino and Tingey, 1996). When *C. viscosissima* and *C. lanceolata* were considered as a single group, 21% (6 out of the 29) markers had a PIC score between 0.70 – 0.95. Segregating the two species in two different groups revealed an interesting fact. *C. viscosissima*, considered as a single group, produced 17% (5 out of the 29) markers with PIC scores between 0.70 – 0.95. On the contrary, *C. lanceolata* produced 79% (23 out of the 29) markers with PIC scores between 0.70 – 0.95. This observation is the molecular characterization of the mating behavior of both species. The PIC score distribution of both species considered as a group does not represent the actual heterozygosity distribution of each species. Rather, the distribution represents the ‘average’ of the two highly skewed PIC distributions of markers amplifying across the inbred *C. viscosissima* and the outbred *C. lanceolata*. This finding clearly indicates that molecular characterization of cross-pollinated and self pollinated species should be segregated to obtain a robust evaluation of amplifiable markers.

The molecular characterization of *C. viscosissima* as an autogamous and *C. lanceolata* as an allogamous species is further confirmed by the relative number of alleles produced by the functional markers across each of these species. As expected for a self pollinating species, 76% (22 out of the 29) markers produced 1-6 alleles. On the contrary, befitting a cross pollinating species, *C. lanceolata* 66% (19 out of the 29) markers produced 7-15 alleles.

SSR markers, like RFLPs, are locus specific, individually typeable and typically multiallelic (Yu *et al.*, 2001). However, unlike RFLPs, SSR markers contained more polymorphic information content in soybean (Akkaya *et al.*, 1992; Rongwen *et al.*, 1995), wheat (Plaschke *et al.*, 1995; Roder *et al.*, 1995), rice (Wu and Tanksley, 1993; Olufowote *et al.*, 1997), barley (Saghai – Maroof *et al.*, 1994) and rapeseed (Charters *et al.*, 1996; Kresovich *et al.*, 1995). SSR markers are high-throughput, and depending on the assay system and degree of multiplexing, have multiplex ratios 2 to 16 – fold greater than individually typed, single copy RFLP markers (Yu *et al.*, 2001). Molecular marker systems previously developed in *Cuphea* are limited to RFLP (Webb *et al.*, 1990), and SSCP (Slabuagh *et al.*, 1997). These marker systems are labor intensive and unsuitable for high-throughput molecular breeding and genomics research in *Cuphea*.

In earlier studies, it has been demonstrated that variability of simple sequence repeats is a positive function of the number of repeats in the tandem arrays (Goldstein and Clark, 1995). The linear positive, rather than exponential, correlation between standard deviation of molecular weight of each marker with the maximum repeat count was in confirmation with earlier studies on *Drosophila melanogaster* (Goldstein and Clark, 1995), rice (Cho, et al., 2000) and meadowfoam (Kishore et al., 2001). Standard deviation of molecular weight is a robust statistic to measure the variability of microsatellite markers. Since it is a derivative of number and frequency of alleles (the basis for PIC) on the one hand and the size range of the PCR fragments at each locus on the other, it provides a comprehensive measure of microsatellite diversity within a class (Cho et al., 2000).

It has been shown that mutation patterns at microsatellites are often complicated and thus use of step-wise mutation model may not be justified. In addition to these complexities, mutations in the region flanking microsatellite repeats can make the evolution of microsatellite loci even more complex (Matsuoka, 2002). Hence the present investigation was based on the assumption that the microsatellite loci may not have evolved in a stepwise manner. The data on allele sizes in maize and other crop species support the

hypothesis of non-step wise mutation model for evolution of microsatellites (Matsuoka, 2002). According to this assumption, the difference in allele sizes was not due to change within the repeat numbers in the sequence. On the contrary, the microsatellite polymorphisms are the direct consequence of insertions and deletions in the regions flanking the repeats. Several authors have reported that inter-specific and intra-specific size variation at microsatellites was caused by indels in the flanking regions (Angers and Bernatchez, 1997; Grimaldi and Crouas-Roy 1997; Buteler *et al.*, 1999; Colson and Goldstein, 1999). However, this assumption disagrees with the observation made in humans (Bowcock *et al.*, 1994) and horses (Vila *et al.*, 2001), where the evolution of microsatellites was based on stepwise mutation model.

The genus *Cuphea* comprises of nearly 260 species. The fertility of hybrids between *C. viscosissima* and *C. lanceolata* f. *silenoides*, coupled with the sterility of *C. lanceolata* f. *silenoides* × *C. lanceolata* f. *typica* and *C. viscosissima* × *C. lanceolata* f. *typica* hybrids, is a peculiar feature of this group (Brandt and Knapp, 1993). Earlier studies have revealed meiotic abnormalities in *C. lanceolata* f. *silenoides* and *C. lanceolata* f. *typical*. The inter-fertility between two of these species, *C. viscosissima* and *C. lanceolata* f. *silenoides*, is the motivation for molecular breeding in this genus. *C. viscosissima* is localized in the southeastern tracts of United

States and *C. lanceolata* is ubiquitous in the Central plateau region in Mexico.

The phenetic analysis of the 29 SSR fingerprints of 74 accessions of *C. viscosissima* and 20 accessions of *C. lanceolata* separated the two species into two distinct clusters. *C. viscosissima* was much more uniform with limited genetic diversity. As evident from the genetic distances, *C. lanceolata* had much higher genetic diversity than *C. viscosissima*. The pattern of diversity as illustrated in the phenogram resulting from cluster analysis was in agreement with geographical occurrence of the species. The cluster analysis of *C. lanceolata* demonstrated forma and elevation specific clustering. *C. lanceolata* f. *silenoides* that grows at lower elevation formed a cluster distinct from *C. lanceolata* f. *typica*, the latter being more common at higher elevations. The flower morphology of *C. lanceolata* f. *silenoides* and *C. lanceolata* f. *typica* is another feature that distinguishes the two forma from each other. The distinct grouping of *C. lanceolata* f. *silenoides* and *C. lanceolata* f. *typica* agrees with the taxonomical classification proposed earlier by Graham (1988). Despite geographical distance and difference in mating system, these two species share considerable genomic regions that constitute the microsatellites. This strongly suggests a common ancestor from which these species may have

evolved. Nonetheless, *C. viscosissima* seems to have passed through a severe evolutionary bottleneck accounting for its highly inbred behavior and tight grouping in cluster analysis. On the contrary, *C. lanceolata* has rapidly evolved to form an outcrossing species. We speculate that these two populations may have evolved from two distinct gene pools that were geographically far apart.

The principal component analysis revealed similar patterns as cluster analysis. *C. viscosissima* and *C. lanceolata* formed two distinct clusters with the first three components explaining 87% of the total variation. In a separate principal component analysis, the two species were segregated in two separate groups. In this analysis, *C. viscosissima* exhibited a tight clustering and the first three principal components explaining 87.5% of the total variation. In principal component analysis of *C. lanceolata*, the species showed sub-species specific clustering.

We have developed 184 markers for 94 *Cuphea* accessions. This study demonstrates the utility of SSRs for DNA fingerprinting and genomic research in under-privileged species. The present investigation also exemplifies the usage of SSR technology in domestication and molecular breeding of wild species.

Conclusions

Cuphea is a predominantly perennial and facultative annual oilseed plant. Inter-specific lines developed from *C. viscosissima* and *C. lanceolata* are under development as medium chain oilseed crops. *Cuphea* seeds contains 16–42% oil of total seed weight. In Lytheraceae, *Cuphea* has a unique profile of medium chain fatty acid (MCFAs) synthesis. MCFAs are primarily required in manufacturing soaps, detergents, surfactants and other related products (Thomson, 1989). The present need of MCFAs for the industrialized western world is met by imported sources like coconut (*Cocos nucifera* L.) and palm kernel (*Elaeis guinenes* Jacq.). The imported supplies are subject to periodic fluctuations in price and supply. The need to regularize the supply and develop indigenous source of MCFAs has fuelled the domestication of the crop.

Molecular markers are very powerful tools to expedite the process of crop improvement and cultivar development. Although an enormous amount of molecular marker data has been generated for most food and industrial crops, such information is very limited in *Cuphea*. In order to improve our efficiency of developing new cultivars for increasing crop productivity of

Cuphea, it is essential to understand the genome of *Cuphea* along with genetics of economically important traits. Hence it was deemed necessary to develop robust, co-dominant and multilocus markers for *Cuphea*. SSR markers have been established as a very effective tool for genomic diversity and evolutionary studies in humans (Bowcock *et al.*, 1994), mouse (Dietrich *et al.*, 1990), *Drosophila* (Goldstein and Clark, 1995), *Arabidopsis* (Innan *et al.*, 1997), rice (Yang *et al.*, 1994), sunflower (Yu *et al.*, 2001; Tang *et al.*, 2001) and meadowfoam (Kishore *et al.*, 2001).

In this study, we isolated and sequenced 1095 clones from genomic DNA libraries enriched for CA, GA and GAA repeats. Four hundred and twenty five clones harbored unique SSRs. Two hundred and seventy one clones (63.76%) were amenable for primer design. One hundred and eighty four primer pairs (67.89%) produced clean amplicons and yielded functional SSR markers. Twenty nine SSR markers were selected for the genetic diversity study. The PIC scores for these 29 SSR markers ranged from 0.11 to 0.95. A positive correlation was observed between standard deviation of molecular weight (SDMw) and maximum repeat count (MRC). Genetic distances among the *Cuphea* accessions ranged from 0.001 to 0.735. Cluster analysis and principal component analysis of genetic distance matrix uncovered patterns of diversity that agrees with species,

sub-species and elevation preference. The SSR markers developed will be very useful tool in molecular breeding of *Cuphea*. In essence this study presents:

1. Development and characterization of SSR markers for *Cuphea*
2. Estimation of genetic distances among various *Cuphea* accessions
3. Assessment of patterns of genetic diversity and relationships of *Cuphea* accessions

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APPENDICES

Appendix 1. Genetic distance matrix for 74 accessions of *Cuphea viscosissima* and 20 accessions of *C. lanceolata*

	VS343	VS344	VS345	VS346	VS347	VS348	VS349	VS350
VS343								
VS344	0.102							
VS345	0.159	0.13						
VS346	0.221	0.223	0.246					
VS347	0.162	0.139	0.155	0.118				
VS348	0.135	0.127	0.161	0.097	0.074			
VS349	0.133	0.124	0.159	0.102	0.079	0.007		
VS350	0.141	0.111	0.134	0.138	0.029	0.066	0.065	
VS351	0.153	0.057	0.146	0.194	0.084	0.124	0.124	0.059
VS352	0.142	0.111	0.134	0.138	0.029	0.065	0.065	0.003
VS353	0.142	0.111	0.134	0.138	0.028	0.066	0.065	0.002
VS355	0.126	0.109	0.148	0.155	0.079	0.06	0.053	0.059
VS356	0.134	0.105	0.131	0.166	0.058	0.07	0.066	0.031
VS357	0.117	0.099	0.111	0.153	0.054	0.058	0.052	0.03
VS358	0.135	0.124	0.159	0.102	0.079	0.007	0.002	0.067
VS359	0.122	0.023	0.145	0.224	0.139	0.127	0.124	0.112
VS360	0.126	0.099	0.113	0.173	0.07	0.078	0.073	0.046
VS361	0.103	0.089	0.115	0.178	0.072	0.084	0.08	0.045
VS362	0.178	0.174	0.175	0.073	0.05	0.059	0.065	0.065
VS363	0.145	0.119	0.132	0.133	0.025	0.061	0.062	0.013
VS364	0.154	0.145	0.153	0.106	0.058	0.027	0.026	0.062
VS365	0.161	0.128	0.131	0.14	0.032	0.071	0.076	0.023
VS366	0.121	0.085	0.134	0.186	0.104	0.093	0.088	0.078
VS367	0.157	0.145	0.152	0.108	0.061	0.029	0.029	0.064
VS368	0.182	0.173	0.182	0.12	0.077	0.056	0.057	0.063
VS369	0.103	0.094	0.113	0.173	0.071	0.081	0.075	0.047
VS370	0.134	0.132	0.15	0.174	0.072	0.08	0.077	0.058
VS371	0.168	0.165	0.195	0.155	0.071	0.112	0.118	0.07
VS372	0.151	0.057	0.144	0.194	0.084	0.124	0.124	0.059
VS373	0.119	0.088	0.112	0.165	0.055	0.069	0.063	0.028
VS374	0.102	0.089	0.114	0.178	0.072	0.084	0.08	0.045
VS375	0.138	0.129	0.163	0.098	0.079	0.008	0.006	0.071
VS376	0.106	0.092	0.118	0.201	0.095	0.106	0.101	0.069
VS377	0.12	0.088	0.112	0.165	0.055	0.068	0.063	0.029
VS378	0.187	0.152	0.174	0.178	0.073	0.11	0.11	0.054
VS379	0.169	0.154	0.164	0.16	0.12	0.073	0.069	0.098
VS380	0.145	0.079	0.176	0.249	0.147	0.189	0.19	0.126
VS381	0.124	0.092	0.12	0.203	0.1	0.111	0.108	0.075
VS382	0.14	0.058	0.155	0.266	0.161	0.174	0.172	0.134
VS383	0.148	0.127	0.142	0.167	0.071	0.1	0.1	0.049
VS384	0.176	0.168	0.202	0.148	0.127	0.056	0.055	0.117
VS385	0.159	0.126	0.155	0.213	0.115	0.155	0.156	0.099
VS386	0.219	0.158	0.283	0.333	0.262	0.258	0.259	0.237
VS387	0.226	0.242	0.24	0.326	0.286	0.249	0.245	0.266
VS388	0.152	0.117	0.143	0.177	0.071	0.11	0.11	0.049
VS389	0.149	0.116	0.141	0.178	0.073	0.111	0.11	0.049
VS390	0.154	0.12	0.141	0.225	0.122	0.136	0.132	0.099
VS391	0.148	0.127	0.143	0.161	0.054	0.072	0.071	0.052
VS392	0.168	0.172	0.223	0.151	0.11	0.124	0.13	0.11
VS394	0.123	0.11	0.166	0.209	0.131	0.12	0.115	0.108
VS395	0.148	0.118	0.143	0.169	0.059	0.073	0.071	0.044
VS396	0.102	0.002	0.13	0.223	0.138	0.127	0.124	0.111

VS397	0.14	0.108	0.234	0.271	0.195	0.216	0.217	0.179
VS398	0.155	0.147	0.179	0.077	0.062	0.022	0.027	0.064
VS399	0.188	0.188	0.192	0.088	0.091	0.08	0.086	0.094
VS400	0.179	0.178	0.184	0.106	0.101	0.066	0.065	0.104
VS401	0.161	0.13	0.157	0.21	0.105	0.119	0.115	0.081
VS402	0.227	0.225	0.227	0.098	0.112	0.12	0.124	0.132
VS403	0.099	0.067	0.12	0.156	0.071	0.062	0.062	0.047
VS404	0.103	0.096	0.141	0.143	0.066	0.074	0.074	0.043
VS405	0.12	0.088	0.139	0.135	0.05	0.063	0.063	0.026
VS406	0.166	0.177	0.183	0.086	0.067	0.051	0.056	0.071
VS407	0.142	0.111	0.134	0.138	0.029	0.066	0.065	0.003
VS408	0.098	0.065	0.121	0.179	0.097	0.084	0.081	0.074
VS409	0.153	0.145	0.177	0.078	0.059	0.022	0.027	0.062
VS410	0.128	0.094	0.148	0.172	0.093	0.109	0.109	0.076
VS411	0.14	0.111	0.135	0.138	0.03	0.066	0.066	0.003
VS412	0.133	0.124	0.159	0.102	0.079	0.007	0	0.065
VS413	0.142	0.111	0.135	0.138	0.029	0.066	0.065	0.002
VS414	0.143	0.109	0.132	0.185	0.078	0.092	0.087	0.052
VS415	0.101	0.066	0.126	0.201	0.124	0.109	0.107	0.102
VS416	0.18	0.171	0.206	0.101	0.077	0.052	0.052	0.087
VS417	0.082	0.08	0.083	0.213	0.141	0.124	0.123	0.118
VS418	0.138	0.055	0.128	0.257	0.177	0.172	0.172	0.152
LN186	0.144	0.19	0.252	0.292	0.226	0.218	0.218	0.206
LN187	0.247	0.185	0.304	0.276	0.217	0.211	0.214	0.208
LN188	0.294	0.298	0.317	0.254	0.267	0.221	0.22	0.261
LN189	0.241	0.221	0.283	0.237	0.205	0.203	0.201	0.199
LN190	0.258	0.252	0.246	0.245	0.227	0.197	0.197	0.213
LN191	0.182	0.223	0.26	0.215	0.208	0.181	0.178	0.202
LN192	0.232	0.274	0.305	0.238	0.24	0.219	0.223	0.235
LN193	0.283	0.25	0.297	0.324	0.261	0.276	0.273	0.248
LN194	0.245	0.248	0.318	0.311	0.266	0.26	0.258	0.254
LN195	0.182	0.228	0.285	0.268	0.239	0.216	0.214	0.228
LN196	0.364	0.422	0.41	0.46	0.426	0.416	0.418	0.413
LN197	0.293	0.338	0.398	0.406	0.351	0.352	0.353	0.335
LN198	0.259	0.293	0.296	0.384	0.329	0.314	0.31	0.314
LN199	0.313	0.325	0.33	0.391	0.332	0.325	0.322	0.318
LN200	0.35	0.413	0.47	0.459	0.434	0.4	0.397	0.42
LN201	0.327	0.39	0.447	0.444	0.414	0.383	0.38	0.4
LN202	0.33	0.363	0.346	0.346	0.359	0.291	0.287	0.345
LN203	0.236	0.284	0.341	0.226	0.269	0.205	0.204	0.265
LN204	0.414	0.452	0.408	0.409	0.425	0.39	0.393	0.414
LN205	0.318	0.388	0.407	0.322	0.386	0.324	0.326	0.375

VS351	VS352	VS353	VS355	VS356	VS357	VS358	VS359	VS360
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0.059								
0.059	0.001							
0.114	0.058	0.059						
0.08	0.033	0.032	0.061					
0.072	0.032	0.032	0.048	0.022				
0.124	0.065	0.065	0.055	0.067	0.054			
0.057	0.111	0.111	0.113	0.106	0.099	0.124		
0.073	0.046	0.046	0.066	0.038	0.022	0.073	0.1	
0.062	0.047	0.047	0.073	0.038	0.028	0.081	0.089	0.032
0.121	0.065	0.065	0.106	0.094	0.081	0.065	0.175	0.1
0.068	0.011	0.011	0.059	0.042	0.036	0.06	0.12	0.045
0.121	0.062	0.061	0.074	0.063	0.05	0.025	0.145	0.069
0.078	0.021	0.021	0.076	0.051	0.05	0.075	0.129	0.06
0.091	0.077	0.077	0.075	0.073	0.065	0.089	0.087	0.064
0.12	0.062	0.062	0.076	0.064	0.051	0.028	0.145	0.068
0.122	0.064	0.064	0.105	0.092	0.078	0.057	0.173	0.098
0.068	0.049	0.049	0.066	0.041	0.025	0.077	0.094	0.032
0.112	0.058	0.059	0.03	0.057	0.052	0.076	0.136	0.065
0.124	0.07	0.07	0.119	0.075	0.092	0.118	0.168	0.105
0.003	0.06	0.06	0.111	0.081	0.072	0.124	0.059	0.076
0.061	0.03	0.029	0.055	0.022	0.011	0.064	0.088	0.019
0.062	0.047	0.047	0.071	0.039	0.028	0.081	0.089	0.033
0.129	0.07	0.07	0.057	0.072	0.059	0.006	0.129	0.078
0.078	0.071	0.071	0.093	0.061	0.05	0.102	0.092	0.053
0.061	0.03	0.03	0.057	0.021	0.011	0.064	0.088	0.019
0.105	0.052	0.052	0.107	0.082	0.08	0.111	0.154	0.091
0.127	0.099	0.099	0.116	0.091	0.071	0.069	0.154	0.083
0.073	0.128	0.128	0.185	0.142	0.138	0.192	0.097	0.143
0.085	0.077	0.077	0.103	0.067	0.056	0.11	0.093	0.061
0.081	0.133	0.133	0.167	0.125	0.12	0.173	0.076	0.123
0.072	0.051	0.05	0.095	0.067	0.048	0.102	0.128	0.058
0.171	0.116	0.116	0.105	0.114	0.102	0.054	0.169	0.121
0.106	0.099	0.099	0.151	0.117	0.105	0.156	0.133	0.109
0.193	0.239	0.239	0.254	0.234	0.235	0.261	0.17	0.242
0.284	0.268	0.268	0.258	0.251	0.239	0.247	0.252	0.251
0.064	0.049	0.049	0.105	0.067	0.058	0.111	0.118	0.061
0.065	0.05	0.049	0.105	0.069	0.058	0.111	0.116	0.063
0.108	0.1	0.099	0.125	0.093	0.08	0.133	0.121	0.08
0.107	0.051	0.051	0.026	0.053	0.046	0.07	0.13	0.061
0.161	0.11	0.111	0.089	0.118	0.128	0.13	0.179	0.139
0.121	0.11	0.11	0.064	0.103	0.091	0.117	0.114	0.097
0.098	0.043	0.043	0.027	0.046	0.045	0.07	0.121	0.059
0.057	0.111	0.111	0.111	0.104	0.099	0.124	0.023	0.099

0.127	0.178	0.178	0.165	0.2	0.194	0.217	0.126	0.195
0.123	0.064	0.064	0.079	0.09	0.077	0.027	0.147	0.098
0.15	0.094	0.094	0.089	0.118	0.105	0.085	0.192	0.122
0.16	0.104	0.104	0.074	0.105	0.092	0.065	0.182	0.11
0.109	0.082	0.082	0.063	0.074	0.065	0.115	0.134	0.07
0.179	0.13	0.13	0.135	0.161	0.148	0.123	0.229	0.161
0.063	0.048	0.048	0.055	0.047	0.036	0.062	0.067	0.04
0.068	0.046	0.045	0.067	0.065	0.049	0.075	0.096	0.055
0.061	0.027	0.027	0.058	0.047	0.038	0.064	0.089	0.049
0.13	0.071	0.071	0.109	0.096	0.083	0.056	0.178	0.103
0.059	0.003	0.003	0.058	0.033	0.031	0.067	0.111	0.046
0.083	0.074	0.075	0.073	0.068	0.056	0.081	0.066	0.059
0.121	0.062	0.062	0.077	0.088	0.075	0.027	0.146	0.096
0.083	0.076	0.075	0.099	0.098	0.085	0.109	0.095	0.089
0.059	0.003	0.004	0.059	0.032	0.032	0.068	0.111	0.046
0.124	0.065	0.065	0.053	0.066	0.052	0.002	0.124	0.073
0.059	0.004	0.003	0.06	0.031	0.029	0.067	0.112	0.046
0.085	0.052	0.052	0.078	0.046	0.036	0.087	0.092	0.043
0.111	0.101	0.102	0.094	0.096	0.083	0.107	0.08	0.086
0.142	0.086	0.086	0.104	0.116	0.102	0.054	0.172	0.123
0.129	0.12	0.12	0.111	0.113	0.099	0.124	0.098	0.103
0.1	0.151	0.151	0.158	0.154	0.149	0.172	0.076	0.149
0.225	0.208	0.208	0.168	0.21	0.198	0.22	0.206	0.205
0.196	0.209	0.209	0.213	0.187	0.207	0.214	0.19	0.219
0.302	0.261	0.262	0.25	0.25	0.245	0.22	0.31	0.257
0.237	0.199	0.199	0.165	0.199	0.197	0.201	0.229	0.21
0.24	0.213	0.212	0.231	0.235	0.216	0.198	0.238	0.231
0.243	0.203	0.204	0.169	0.201	0.185	0.179	0.231	0.2
0.278	0.235	0.236	0.225	0.252	0.236	0.223	0.283	0.251
0.258	0.247	0.247	0.254	0.246	0.241	0.272	0.261	0.241
0.272	0.252	0.253	0.23	0.24	0.249	0.257	0.259	0.252
0.259	0.229	0.23	0.207	0.203	0.208	0.214	0.236	0.217
0.443	0.414	0.415	0.372	0.425	0.407	0.419	0.432	0.42
0.361	0.336	0.336	0.302	0.344	0.335	0.354	0.349	0.344
0.332	0.313	0.313	0.259	0.308	0.294	0.309	0.285	0.298
0.348	0.316	0.317	0.281	0.294	0.312	0.321	0.335	0.322
0.447	0.421	0.422	0.396	0.41	0.399	0.398	0.427	0.411
0.426	0.401	0.402	0.38	0.391	0.378	0.381	0.397	0.39
0.378	0.344	0.344	0.335	0.342	0.325	0.286	0.367	0.335
0.305	0.264	0.264	0.232	0.254	0.249	0.202	0.286	0.26
0.44	0.412	0.413	0.436	0.402	0.406	0.392	0.457	0.413
0.405	0.374	0.374	0.372	0.382	0.364	0.325	0.394	0.372

VS361	VS362	VS363	VS364	VS365	VS366	VS367	VS368	VS369
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0.107								
0.054	0.06							
0.077	0.041	0.055						
0.067	0.068	0.017	0.064					
0.059	0.138	0.081	0.108	0.09				
0.079	0.043	0.054	0.006	0.062	0.106			
0.104	0.059	0.059	0.05	0.074	0.136	0.052		
0.008	0.102	0.051	0.072	0.068	0.062	0.074	0.099	
0.053	0.103	0.052	0.072	0.069	0.101	0.074	0.103	0.049
0.087	0.084	0.07	0.098	0.075	0.142	0.101	0.118	0.091
0.063	0.121	0.07	0.121	0.079	0.09	0.122	0.122	0.068
0.019	0.091	0.037	0.06	0.049	0.055	0.062	0.088	0.022
0.002	0.107	0.054	0.077	0.067	0.058	0.079	0.104	0.007
0.085	0.065	0.063	0.025	0.076	0.092	0.029	0.06	0.08
0.024	0.13	0.076	0.097	0.091	0.077	0.099	0.127	0.028
0.019	0.091	0.038	0.06	0.049	0.055	0.062	0.088	0.022
0.095	0.107	0.053	0.105	0.061	0.077	0.101	0.108	0.096
0.084	0.106	0.103	0.068	0.116	0.119	0.07	0.096	0.082
0.112	0.183	0.139	0.188	0.145	0.16	0.188	0.188	0.119
0.05	0.134	0.082	0.106	0.095	0.085	0.106	0.132	0.055
0.112	0.197	0.143	0.169	0.149	0.139	0.167	0.195	0.119
0.059	0.098	0.055	0.098	0.069	0.098	0.098	0.098	0.059
0.127	0.113	0.113	0.074	0.123	0.135	0.075	0.108	0.125
0.102	0.147	0.103	0.152	0.116	0.135	0.15	0.105	0.105
0.212	0.294	0.248	0.28	0.254	0.232	0.28	0.248	0.219
0.229	0.29	0.27	0.264	0.283	0.247	0.264	0.248	0.23
0.054	0.108	0.056	0.107	0.067	0.091	0.106	0.109	0.059
0.052	0.109	0.057	0.107	0.068	0.088	0.106	0.108	0.058
0.075	0.157	0.101	0.128	0.11	0.055	0.123	0.154	0.077
0.065	0.088	0.045	0.055	0.056	0.093	0.058	0.098	0.061
0.125	0.115	0.105	0.133	0.116	0.151	0.136	0.154	0.123
0.067	0.164	0.115	0.137	0.128	0.088	0.139	0.165	0.07
0.06	0.096	0.045	0.063	0.051	0.085	0.065	0.098	0.06
0.089	0.174	0.119	0.145	0.128	0.085	0.145	0.173	0.094

0.188	0.227	0.185	0.236	0.194	0.182	0.236	0.192	0.193
0.104	0.043	0.059	0.034	0.067	0.113	0.037	0.053	0.101
0.129	0.043	0.087	0.063	0.097	0.155	0.066	0.084	0.122
0.115	0.055	0.097	0.043	0.107	0.144	0.047	0.088	0.108
0.069	0.14	0.088	0.11	0.101	0.101	0.112	0.14	0.071
0.171	0.071	0.123	0.102	0.134	0.157	0.103	0.117	0.166
0.028	0.107	0.054	0.083	0.067	0.039	0.085	0.106	0.035
0.037	0.096	0.049	0.096	0.066	0.065	0.098	0.097	0.034
0.048	0.086	0.036	0.085	0.047	0.057	0.086	0.087	0.052
0.092	0.034	0.065	0.036	0.072	0.141	0.039	0.056	0.088
0.046	0.065	0.012	0.062	0.023	0.076	0.064	0.063	0.046
0.049	0.131	0.078	0.102	0.094	0.054	0.103	0.131	0.053
0.102	0.039	0.056	0.029	0.063	0.108	0.031	0.048	0.097
0.081	0.126	0.079	0.129	0.095	0.083	0.13	0.083	0.082
0.044	0.066	0.013	0.064	0.024	0.078	0.066	0.065	0.046
0.08	0.065	0.062	0.026	0.076	0.088	0.029	0.057	0.075
0.046	0.065	0.014	0.063	0.023	0.077	0.064	0.064	0.046
0.047	0.113	0.055	0.082	0.067	0.049	0.079	0.11	0.047
0.079	0.154	0.104	0.127	0.12	0.08	0.128	0.107	0.08
0.129	0.07	0.084	0.075	0.098	0.138	0.077	0.03	0.126
0.079	0.169	0.122	0.144	0.139	0.098	0.146	0.171	0.078
0.141	0.211	0.158	0.193	0.167	0.133	0.191	0.212	0.146
0.179	0.253	0.21	0.237	0.224	0.195	0.24	0.261	0.179
0.199	0.241	0.212	0.223	0.214	0.222	0.225	0.26	0.204
0.245	0.231	0.258	0.21	0.27	0.284	0.215	0.254	0.242
0.204	0.217	0.2	0.206	0.204	0.203	0.208	0.25	0.204
0.231	0.208	0.214	0.213	0.227	0.236	0.213	0.219	0.228
0.201	0.196	0.199	0.185	0.211	0.2	0.187	0.219	0.196
0.251	0.217	0.231	0.229	0.243	0.25	0.23	0.253	0.245
0.247	0.279	0.247	0.262	0.237	0.233	0.26	0.253	0.247
0.254	0.284	0.252	0.267	0.249	0.251	0.268	0.303	0.256
0.218	0.243	0.228	0.225	0.241	0.223	0.23	0.268	0.217
0.401	0.429	0.413	0.43	0.426	0.392	0.432	0.454	0.398
0.318	0.374	0.339	0.367	0.35	0.311	0.37	0.39	0.32
0.299	0.35	0.312	0.325	0.326	0.296	0.327	0.363	0.3
0.318	0.355	0.321	0.334	0.327	0.317	0.338	0.375	0.321
0.392	0.43	0.421	0.41	0.435	0.412	0.415	0.451	0.392
0.371	0.414	0.401	0.394	0.415	0.39	0.398	0.434	0.371
0.34	0.33	0.342	0.303	0.355	0.338	0.305	0.342	0.339
0.266	0.235	0.261	0.213	0.273	0.269	0.218	0.259	0.265
0.418	0.393	0.407	0.383	0.421	0.438	0.385	0.361	0.417
0.378	0.359	0.368	0.341	0.365	0.348	0.342	0.369	0.375

VS370	VS371	VS372	VS373	VS374	VS375	VS376	VS377	VS378
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0.092								
0.11	0.125							
0.055	0.091	0.061						
0.052	0.089	0.062	0.019					
0.079	0.12	0.129	0.068	0.085				
0.073	0.11	0.079	0.042	0.025	0.105			
0.056	0.09	0.061	0.003	0.019	0.069	0.042		
0.104	0.115	0.107	0.078	0.095	0.115	0.118	0.079	
0.119	0.16	0.127	0.075	0.084	0.071	0.105	0.073	0.146
0.164	0.165	0.074	0.13	0.114	0.196	0.114	0.128	0.168
0.1	0.132	0.086	0.051	0.052	0.114	0.051	0.049	0.121
0.164	0.186	0.084	0.111	0.114	0.178	0.114	0.11	0.171
0.097	0.112	0.073	0.051	0.06	0.106	0.08	0.049	0.098
0.127	0.166	0.171	0.112	0.127	0.057	0.147	0.111	0.162
0.148	0.154	0.108	0.101	0.104	0.161	0.104	0.1	0.141
0.258	0.251	0.195	0.228	0.214	0.265	0.217	0.226	0.275
0.265	0.273	0.286	0.244	0.23	0.25	0.209	0.243	0.296
0.102	0.112	0.066	0.051	0.055	0.116	0.075	0.049	0.095
0.104	0.113	0.065	0.051	0.053	0.116	0.073	0.049	0.095
0.126	0.159	0.11	0.074	0.076	0.137	0.078	0.073	0.099
0.019	0.096	0.104	0.048	0.063	0.071	0.085	0.049	0.096
0.086	0.046	0.159	0.129	0.125	0.13	0.145	0.13	0.155
0.068	0.147	0.118	0.083	0.066	0.12	0.08	0.084	0.155
0.02	0.098	0.095	0.042	0.058	0.072	0.082	0.043	0.089
0.134	0.165	0.057	0.088	0.089	0.129	0.092	0.088	0.152

0.185	0.229	0.127	0.184	0.187	0.221	0.193	0.185	0.217
0.101	0.1	0.123	0.088	0.104	0.028	0.127	0.088	0.108
0.087	0.109	0.147	0.113	0.127	0.084	0.15	0.115	0.136
0.07	0.117	0.157	0.101	0.113	0.064	0.134	0.103	0.147
0.059	0.139	0.106	0.055	0.067	0.119	0.091	0.054	0.13
0.135	0.154	0.178	0.155	0.17	0.123	0.192	0.157	0.134
0.079	0.105	0.064	0.028	0.03	0.066	0.049	0.027	0.096
0.073	0.089	0.068	0.046	0.036	0.079	0.058	0.046	0.094
0.082	0.09	0.061	0.033	0.049	0.069	0.072	0.031	0.076
0.089	0.076	0.13	0.094	0.093	0.052	0.115	0.094	0.113
0.057	0.071	0.059	0.028	0.046	0.07	0.07	0.03	0.053
0.096	0.129	0.085	0.048	0.05	0.085	0.051	0.048	0.098
0.098	0.099	0.121	0.086	0.102	0.027	0.124	0.086	0.103
0.123	0.134	0.083	0.077	0.08	0.113	0.083	0.078	0.121
0.056	0.069	0.059	0.029	0.045	0.071	0.068	0.03	0.055
0.077	0.118	0.124	0.063	0.08	0.006	0.101	0.063	0.11
0.06	0.071	0.059	0.028	0.046	0.071	0.07	0.028	0.053
0.079	0.115	0.087	0.03	0.046	0.09	0.069	0.031	0.072
0.116	0.151	0.11	0.077	0.079	0.111	0.082	0.078	0.144
0.127	0.139	0.142	0.113	0.129	0.058	0.152	0.113	0.13
0.115	0.149	0.127	0.096	0.079	0.127	0.081	0.096	0.161
0.18	0.198	0.1	0.138	0.141	0.177	0.144	0.138	0.187
0.171	0.193	0.221	0.193	0.177	0.222	0.185	0.194	0.231
0.212	0.16	0.197	0.204	0.2	0.216	0.219	0.204	0.249
0.235	0.212	0.3	0.252	0.245	0.221	0.236	0.252	0.288
0.182	0.175	0.234	0.193	0.203	0.201	0.22	0.194	0.235
0.26	0.22	0.24	0.222	0.231	0.201	0.244	0.222	0.238
0.19	0.199	0.24	0.192	0.199	0.179	0.21	0.194	0.224
0.244	0.26	0.275	0.243	0.249	0.223	0.267	0.245	0.24
0.256	0.267	0.257	0.235	0.245	0.274	0.252	0.237	0.248
0.248	0.243	0.272	0.243	0.253	0.26	0.246	0.244	0.281
0.226	0.223	0.257	0.214	0.217	0.216	0.206	0.214	0.264
0.379	0.44	0.44	0.411	0.4	0.42	0.412	0.413	0.429
0.305	0.36	0.358	0.328	0.317	0.355	0.33	0.33	0.353
0.281	0.349	0.332	0.294	0.299	0.313	0.283	0.294	0.326
0.299	0.344	0.346	0.306	0.317	0.324	0.31	0.307	0.339
0.401	0.425	0.446	0.403	0.392	0.4	0.378	0.405	0.44
0.386	0.371	0.424	0.382	0.371	0.383	0.357	0.384	0.42
0.361	0.392	0.38	0.33	0.341	0.285	0.33	0.33	0.384
0.254	0.247	0.303	0.255	0.266	0.2	0.258	0.255	0.304
0.433	0.436	0.44	0.41	0.417	0.391	0.429	0.411	0.449
0.398	0.417	0.407	0.369	0.377	0.323	0.39	0.37	0.392

VS379	VS380	VS381	VS382	VS383	VS384	VS385	VS386	VS387
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0.145								
0.063	0.082							
0.127	0.033	0.064						
0.056	0.09	0.037	0.099					
0.07	0.184	0.106	0.165	0.1				
0.113	0.086	0.054	0.097	0.058	0.152			
0.245	0.132	0.186	0.131	0.207	0.244	0.158		
0.235	0.228	0.203	0.218	0.225	0.229	0.186	0.132	
0.065	0.082	0.032	0.09	0.01	0.11	0.054	0.202	0.23
0.065	0.082	0.029	0.088	0.013	0.11	0.053	0.199	0.228
0.087	0.112	0.034	0.091	0.063	0.131	0.085	0.214	0.225
0.113	0.177	0.095	0.159	0.094	0.119	0.143	0.271	0.278
0.196	0.206	0.169	0.226	0.149	0.178	0.191	0.266	0.283
0.148	0.168	0.106	0.168	0.124	0.163	0.159	0.243	0.26
0.112	0.168	0.092	0.15	0.092	0.117	0.142	0.263	0.278
0.154	0.077	0.092	0.056	0.127	0.167	0.126	0.156	0.24

0.252	0.139	0.194	0.146	0.197	0.259	0.143	0.18	0.275
0.088	0.184	0.126	0.189	0.094	0.071	0.149	0.265	0.263
0.128	0.219	0.159	0.222	0.13	0.134	0.181	0.314	0.308
0.109	0.227	0.148	0.212	0.14	0.114	0.192	0.314	0.295
0.124	0.18	0.1	0.161	0.1	0.162	0.151	0.278	0.294
0.164	0.246	0.199	0.258	0.164	0.173	0.207	0.358	0.343
0.091	0.131	0.056	0.116	0.061	0.109	0.102	0.204	0.227
0.105	0.119	0.083	0.146	0.051	0.123	0.104	0.209	0.231
0.102	0.128	0.077	0.136	0.049	0.112	0.1	0.217	0.246
0.093	0.176	0.135	0.198	0.104	0.104	0.157	0.279	0.276
0.1	0.128	0.077	0.135	0.051	0.117	0.1	0.239	0.267
0.112	0.134	0.055	0.115	0.086	0.126	0.106	0.21	0.226
0.092	0.185	0.128	0.191	0.096	0.074	0.15	0.266	0.264
0.142	0.137	0.087	0.147	0.088	0.155	0.053	0.179	0.207
0.1	0.126	0.077	0.135	0.05	0.117	0.1	0.237	0.266
0.069	0.19	0.108	0.172	0.1	0.055	0.156	0.259	0.245
0.097	0.125	0.074	0.133	0.048	0.117	0.098	0.237	0.265
0.102	0.154	0.077	0.132	0.079	0.136	0.125	0.246	0.262
0.139	0.142	0.083	0.122	0.113	0.151	0.062	0.164	0.182
0.119	0.204	0.156	0.218	0.119	0.105	0.122	0.247	0.246
0.156	0.124	0.099	0.123	0.128	0.165	0.135	0.203	0.165
0.205	0.111	0.146	0.088	0.169	0.212	0.172	0.163	0.2
0.258	0.234	0.205	0.235	0.226	0.261	0.238	0.26	0.308
0.269	0.225	0.236	0.223	0.243	0.258	0.277	0.258	0.331
0.256	0.333	0.285	0.332	0.286	0.267	0.333	0.387	0.331
0.262	0.291	0.233	0.275	0.241	0.25	0.285	0.348	0.371
0.192	0.238	0.198	0.242	0.178	0.194	0.211	0.3	0.249
0.233	0.29	0.225	0.271	0.229	0.191	0.257	0.301	0.267
0.259	0.325	0.276	0.323	0.263	0.234	0.309	0.358	0.386
0.285	0.3	0.266	0.274	0.282	0.271	0.254	0.269	0.306
0.31	0.318	0.275	0.294	0.289	0.268	0.307	0.317	0.315
0.267	0.29	0.237	0.271	0.248	0.236	0.282	0.297	0.264
0.463	0.458	0.432	0.464	0.433	0.455	0.463	0.461	0.481
0.398	0.375	0.351	0.379	0.362	0.394	0.384	0.382	0.415
0.354	0.361	0.31	0.332	0.329	0.36	0.349	0.383	0.315
0.373	0.394	0.34	0.373	0.353	0.319	0.381	0.398	0.339
0.426	0.459	0.424	0.454	0.438	0.427	0.467	0.452	0.422
0.429	0.436	0.402	0.431	0.417	0.415	0.445	0.426	0.399
0.31	0.43	0.359	0.408	0.365	0.337	0.401	0.456	0.444
0.261	0.355	0.288	0.335	0.291	0.253	0.336	0.378	0.353
0.412	0.479	0.432	0.463	0.432	0.392	0.409	0.454	0.462
0.371	0.445	0.394	0.426	0.396	0.326	0.428	0.465	0.466

VS388	VS389	VS390	VS391	VS392	VS394	VS395	VS396	VS397
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0.006								
0.055	0.052							
0.097	0.097	0.119						
0.152	0.154	0.196	0.09					
0.118	0.114	0.129	0.082	0.119				
0.092	0.092	0.115	0.009	0.096	0.08			
0.117	0.116	0.12	0.128	0.173	0.112	0.119		

0.189	0.189	0.221	0.183	0.204	0.164	0.177	0.108	
0.104	0.104	0.151	0.085	0.113	0.139	0.092	0.147	0.215
0.14	0.14	0.182	0.073	0.089	0.146	0.08	0.189	0.222
0.15	0.15	0.17	0.057	0.097	0.132	0.064	0.18	0.232
0.1	0.101	0.124	0.055	0.136	0.084	0.051	0.132	0.186
0.171	0.173	0.185	0.12	0.138	0.191	0.127	0.226	0.246
0.055	0.053	0.08	0.072	0.119	0.065	0.068	0.067	0.162
0.057	0.058	0.108	0.086	0.1	0.073	0.085	0.096	0.166
0.048	0.05	0.101	0.075	0.107	0.086	0.069	0.088	0.159
0.114	0.114	0.158	0.091	0.112	0.151	0.098	0.177	0.243
0.051	0.051	0.1	0.05	0.11	0.108	0.043	0.111	0.179
0.08	0.078	0.082	0.09	0.139	0.077	0.087	0.065	0.166
0.106	0.106	0.15	0.083	0.111	0.137	0.09	0.145	0.212
0.083	0.081	0.114	0.116	0.143	0.107	0.114	0.094	0.112
0.05	0.051	0.102	0.051	0.109	0.107	0.044	0.111	0.179
0.11	0.11	0.132	0.071	0.13	0.115	0.071	0.124	0.217
0.048	0.048	0.098	0.053	0.112	0.108	0.045	0.111	0.18
0.076	0.076	0.069	0.071	0.153	0.109	0.065	0.11	0.205
0.11	0.107	0.113	0.112	0.155	0.103	0.111	0.066	0.116
0.129	0.131	0.18	0.121	0.152	0.165	0.121	0.171	0.181
0.127	0.125	0.129	0.129	0.152	0.101	0.128	0.079	0.175
0.16	0.159	0.169	0.174	0.203	0.161	0.165	0.055	0.136
0.222	0.218	0.23	0.184	0.165	0.159	0.185	0.191	0.19
0.242	0.241	0.258	0.218	0.175	0.235	0.216	0.185	0.269
0.292	0.293	0.306	0.241	0.21	0.239	0.246	0.299	0.391
0.239	0.237	0.249	0.168	0.145	0.203	0.167	0.222	0.288
0.184	0.183	0.221	0.252	0.232	0.266	0.252	0.251	0.314
0.237	0.235	0.243	0.181	0.178	0.215	0.186	0.224	0.242
0.269	0.269	0.296	0.236	0.244	0.27	0.24	0.275	0.301
0.277	0.276	0.257	0.244	0.28	0.287	0.239	0.251	0.264
0.284	0.286	0.296	0.238	0.225	0.263	0.233	0.249	0.263
0.253	0.252	0.261	0.221	0.217	0.231	0.224	0.228	0.272
0.437	0.435	0.433	0.39	0.405	0.377	0.394	0.423	0.401
0.356	0.353	0.35	0.316	0.33	0.295	0.316	0.338	0.319
0.329	0.328	0.331	0.277	0.313	0.275	0.28	0.293	0.288
0.349	0.351	0.368	0.289	0.318	0.319	0.284	0.326	0.337
0.442	0.442	0.449	0.411	0.414	0.411	0.414	0.413	0.452
0.421	0.42	0.427	0.396	0.368	0.394	0.398	0.39	0.433
0.371	0.368	0.376	0.352	0.399	0.374	0.353	0.363	0.411
0.297	0.296	0.31	0.242	0.224	0.273	0.247	0.285	0.323
0.436	0.436	0.453	0.426	0.46	0.435	0.428	0.451	0.431
0.399	0.397	0.385	0.389	0.419	0.409	0.391	0.388	0.427

VS398 VS399 VS400 VS401 VS402 VS403 VS404 VS405 VS406

0.068									
0.071	0.027								
0.138	0.123	0.109							
0.104	0.052	0.073	0.166						
0.08	0.129	0.124	0.077	0.169					
0.073	0.123	0.132	0.095	0.158	0.03				
0.062	0.116	0.127	0.084	0.15	0.023	0.02			
0.034	0.058	0.059	0.143	0.097	0.11	0.087	0.094		
0.065	0.092	0.102	0.081	0.13	0.048	0.044	0.028	0.071	
0.104	0.151	0.14	0.097	0.189	0.028	0.055	0.05	0.135	
0.007	0.065	0.069	0.137	0.1	0.078	0.071	0.06	0.034	
0.108	0.152	0.164	0.125	0.179	0.053	0.055	0.053	0.137	
0.066	0.093	0.103	0.081	0.132	0.048	0.043	0.028	0.071	
0.027	0.086	0.065	0.115	0.124	0.062	0.074	0.063	0.056	
0.064	0.095	0.105	0.081	0.132	0.047	0.044	0.026	0.071	
0.112	0.135	0.123	0.082	0.152	0.055	0.073	0.058	0.116	
0.13	0.168	0.159	0.122	0.204	0.057	0.082	0.079	0.158	
0.049	0.107	0.111	0.164	0.122	0.104	0.094	0.083	0.078	
0.144	0.182	0.172	0.14	0.222	0.075	0.079	0.096	0.156	
0.185	0.223	0.222	0.18	0.257	0.114	0.138	0.13	0.215	
0.232	0.232	0.227	0.196	0.278	0.172	0.174	0.189	0.242	
0.219	0.258	0.249	0.253	0.3	0.198	0.201	0.202	0.228	
0.23	0.211	0.199	0.27	0.244	0.262	0.259	0.27	0.215	
0.211	0.187	0.181	0.206	0.225	0.189	0.205	0.194	0.227	
0.186	0.235	0.248	0.274	0.265	0.204	0.2	0.195	0.217	
0.191	0.18	0.175	0.224	0.217	0.186	0.197	0.195	0.207	
0.215	0.208	0.223	0.269	0.244	0.231	0.23	0.228	0.23	
0.288	0.274	0.268	0.259	0.285	0.247	0.27	0.258	0.29	
0.273	0.268	0.262	0.262	0.306	0.236	0.258	0.245	0.295	
0.23	0.24	0.232	0.24	0.281	0.203	0.217	0.216	0.251	
0.42	0.406	0.411	0.414	0.428	0.394	0.386	0.401	0.425	
0.359	0.35	0.352	0.331	0.373	0.312	0.31	0.32	0.365	
0.331	0.326	0.313	0.295	0.359	0.28	0.3	0.296	0.357	
0.339	0.332	0.322	0.32	0.377	0.3	0.322	0.308	0.365	
0.415	0.426	0.417	0.387	0.467	0.392	0.392	0.406	0.422	
0.399	0.416	0.406	0.371	0.457	0.371	0.371	0.386	0.406	
0.306	0.345	0.329	0.382	0.378	0.32	0.338	0.332	0.325	
0.215	0.216	0.204	0.275	0.247	0.248	0.264	0.256	0.229	
0.397	0.397	0.399	0.457	0.429	0.42	0.432	0.427	0.389	
0.334	0.368	0.364	0.42	0.378	0.353	0.367	0.362	0.351	

VS407 VS408 VS409 VS410 VS411 VS412 VS413 VS414 VS415

0.074								
0.062	0.103							
0.075	0.058	0.105						
0.002	0.074	0.063	0.076					
0.065	0.081	0.027	0.109	0.066				
0.003	0.074	0.062	0.076	0.004	0.065			
0.051	0.073	0.107	0.102	0.053	0.087	0.051		
0.102	0.055	0.127	0.031	0.102	0.107	0.102	0.101	
0.088	0.128	0.049	0.077	0.088	0.052	0.087	0.137	0.105
0.118	0.072	0.142	0.103	0.117	0.123	0.119	0.119	0.075
0.151	0.117	0.184	0.139	0.151	0.172	0.152	0.156	0.121
0.207	0.155	0.23	0.2	0.206	0.218	0.207	0.216	0.187
0.21	0.21	0.22	0.239	0.208	0.214	0.209	0.226	0.232
0.26	0.264	0.23	0.305	0.259	0.22	0.262	0.273	0.292
0.197	0.2	0.207	0.234	0.198	0.201	0.2	0.21	0.228
0.213	0.198	0.187	0.22	0.215	0.197	0.211	0.226	0.238
0.201	0.19	0.186	0.212	0.202	0.178	0.203	0.209	0.2
0.234	0.228	0.211	0.261	0.234	0.223	0.236	0.261	0.267
0.247	0.248	0.285	0.231	0.249	0.273	0.249	0.228	0.216
0.253	0.247	0.272	0.263	0.253	0.258	0.255	0.263	0.249
0.228	0.209	0.229	0.241	0.227	0.214	0.23	0.23	0.224
0.413	0.405	0.415	0.422	0.413	0.418	0.414	0.407	0.418
0.334	0.324	0.354	0.342	0.335	0.353	0.335	0.326	0.337
0.314	0.281	0.329	0.313	0.314	0.31	0.315	0.296	0.291
0.317	0.313	0.338	0.337	0.317	0.322	0.319	0.332	0.324
0.421	0.396	0.413	0.428	0.419	0.397	0.422	0.428	0.409
0.4	0.374	0.397	0.406	0.399	0.38	0.401	0.398	0.386
0.347	0.327	0.307	0.361	0.347	0.287	0.346	0.349	0.351
0.265	0.252	0.217	0.291	0.265	0.204	0.266	0.266	0.278
0.414	0.431	0.397	0.397	0.414	0.393	0.415	0.422	0.39
0.374	0.362	0.332	0.388	0.376	0.326	0.375	0.359	0.382

VS416 VS417 VS418 LN186 LN187 LN188 LN189 LN190 LN191

0.17									
0.211	0.068								
0.259	0.177	0.222							
0.256	0.231	0.223	0.258						
0.27	0.266	0.32	0.289	0.289					
0.251	0.228	0.256	0.23	0.207	0.217				
0.215	0.199	0.229	0.271	0.286	0.319	0.253			
0.219	0.207	0.246	0.207	0.286	0.242	0.217	0.288		
0.255	0.274	0.31	0.249	0.381	0.281	0.282	0.341	0.166	
0.273	0.283	0.282	0.328	0.351	0.342	0.32	0.414	0.228	
0.3	0.278	0.286	0.259	0.255	0.32	0.287	0.354	0.199	
0.265	0.228	0.262	0.226	0.266	0.226	0.257	0.323	0.174	
0.454	0.406	0.444	0.308	0.45	0.487	0.413	0.465	0.358	
0.39	0.326	0.359	0.226	0.372	0.429	0.336	0.403	0.299	
0.361	0.241	0.26	0.304	0.421	0.387	0.3	0.303	0.299	
0.372	0.289	0.31	0.329	0.387	0.424	0.336	0.319	0.275	
0.448	0.397	0.443	0.338	0.418	0.376	0.39	0.453	0.347	
0.431	0.374	0.419	0.277	0.358	0.32	0.318	0.391	0.334	
0.337	0.369	0.408	0.315	0.414	0.412	0.385	0.342	0.332	
0.253	0.291	0.328	0.252	0.319	0.201	0.244	0.333	0.215	
0.382	0.455	0.477	0.405	0.468	0.455	0.49	0.465	0.399	
0.366	0.391	0.416	0.331	0.43	0.446	0.408	0.399	0.338	

LN192 LN193 LN194 LN195 LN196 LN197 LN198 LN199 LN200

0.263									
0.257	0.268								
0.214	0.279	0.144							
0.384	0.502	0.389	0.343						
0.35	0.436	0.32	0.283	0.088					
0.387	0.434	0.327	0.296	0.435	0.358				
0.326	0.377	0.23	0.258	0.413	0.356	0.229			
0.295	0.45	0.302	0.252	0.299	0.259	0.409	0.364		
0.317	0.461	0.287	0.235	0.342	0.287	0.364	0.361	0.118	
0.352	0.468	0.386	0.336	0.298	0.326	0.408	0.329	0.295	
0.225	0.339	0.235	0.155	0.397	0.34	0.337	0.344	0.323	
0.43	0.448	0.379	0.345	0.3	0.317	0.521	0.399	0.373	
0.364	0.419	0.347	0.32	0.3	0.256	0.432	0.36	0.335	

LN201 LN202 LN203 LN204

0.376			
0.308	0.315		
0.407	0.289	0.376	
0.358	0.286	0.298	0.232

Appendix 2. Cuphea diversity screen and OSU plant identification numbers

Parent accession	OSU number	Parent description	Latitude		Longitude		State/Locality	Country
			Degrees	Minutes	Degrees	Minutes		
Ames 22404	VS343	<i>Cuphea viscosissima</i>	NA	NA	NA	NA	Oregon	USA
PI 534726	VS344	<i>Cuphea viscosissima</i>	NA	NA	NA	NA	Virginia, Ohio	USA
PI 534730	VS345	<i>Cuphea viscosissima</i>	NA	NA	NA	NA	Missouri	USA
PI 534734	VS346	<i>Cuphea viscosissima</i>	NA	NA	NA	NA	Kansas	USA
PI 534735	VS347	<i>Cuphea viscosissima</i>	38	25	94	47	Kansas	USA
PI 534736	VS348	<i>Cuphea viscosissima</i>	38	5	94	49	Kansas	USA
PI 534737	VS349	<i>Cuphea viscosissima</i>	37	38	94	48	Kansas	USA
PI 534738	VS350	<i>Cuphea viscosissima</i>	38	38	94	49	Kansas	USA
PI 534739	VS351	<i>Cuphea viscosissima</i>	37	26	94	57	Kansas	USA
PI 534740	VS352	<i>Cuphea viscosissima</i>	37	11	94	38	Kansas	USA
PI 534741	VS353	<i>Cuphea viscosissima</i>	37	3	94	37	Kansas	USA
PI 534742	VS354	<i>Cuphea viscosissima</i>	37	3	94	38	Missouri	USA
PI 534743	VS355	<i>Cuphea viscosissima</i>	37	6	94	29	Missouri	USA
PI 534744	VS356	<i>Cuphea viscosissima</i>	37	6	94	29	Missouri	USA
PI 534745	VS357	<i>Cuphea viscosissima</i>	37	27	93	47	Missouri	USA
PI 534746	VS358	<i>Cuphea viscosissima</i>	37	27	93	18	Missouri	USA
PI 534747	VS359	<i>Cuphea viscosissima</i>	36	33	93	56	Missouri	USA
PI 534748	VS360	<i>Cuphea viscosissima</i>	36	35	93	51	Missouri	USA
PI 534749	VS361	<i>Cuphea viscosissima</i>	36	32	93	45	Missouri	USA
PI 534750	VS362	<i>Cuphea viscosissima</i>	36	35	93	27	Missouri	USA
PI 534751	VS363	<i>Cuphea viscosissima</i>	36	22	92	25	Arkansas	USA
PI 534752	VS364	<i>Cuphea viscosissima</i>	36	30	92	8	Arkansas	USA
PI 534753	VS365	<i>Cuphea viscosissima</i>	37	3	91	40	Missouri	USA
PI 534754	VS366	<i>Cuphea viscosissima</i>	37	3	91	38	Missouri	USA
PI 534755	VS367	<i>Cuphea viscosissima</i>	37	17	91	24	Missouri	USA
PI 534756	VS368	<i>Cuphea viscosissima</i>	37	31	90	52	Missouri	USA
PI 534757	VS369	<i>Cuphea viscosissima</i>	37	27	90	35	Missouri	USA
PI 534758	VS370	<i>Cuphea viscosissima</i>	37	58	89	40	Illinois	USA
PI 534759	VS371	<i>Cuphea viscosissima</i>	38	9	89	32	Illinois	USA

(continued)

PI 534760	VS372	<i>Cuphea viscosissima</i>	38	21	89	26	Illinois	USA
PI 534761	VS373	<i>Cuphea viscosissima</i>	37	29	88	44	Illinois	USA
PI 534762	VS374	<i>Cuphea viscosissima</i>	37	35	88	26	Illinois	USA
PI 534763	VS375	<i>Cuphea viscosissima</i>	37	52	88	24	Illinois	USA
PI 534764	VS376	<i>Cuphea viscosissima</i>	38	24	87	48	Illinois	USA
PI 534765	VS377	<i>Cuphea viscosissima</i>	38	21	87	50	Illinois	USA
PI 534766	VS378	<i>Cuphea viscosissima</i>	38	37	88	40	Illinois	USA
PI 534767	VS379	<i>Cuphea viscosissima</i>	39	6	89	27	Illinois	USA
PI 534768	VS380	<i>Cuphea viscosissima</i>	39	17	90	40	Illinois	USA
PI 534769	VS381	<i>Cuphea viscosissima</i>	39	17	90	40	Illinois	USA
PI 534770	VS382	<i>Cuphea viscosissima</i>	39	25	90	47	Illinois	USA
PI 534771	VS383	<i>Cuphea viscosissima</i>	40	52	92	21	Iowa	USA
PI 534911	VS384	<i>Cuphea viscosissima</i>	NA	NA	NA	NA	Arizona (?)	USA
PI 560084	VS385	<i>Cuphea viscosissima</i>	37	1	88	31	Kentucky	USA
PI 560085	VS386	<i>Cuphea viscosissima</i>	36	52	89	6	Kentucky	USA
PI 560086	VS387	<i>Cuphea viscosissima</i>	36	27	87	27	Tennessee	USA
PI 560087	VS388	<i>Cuphea viscosissima</i>	35	57	82	8	North Carolina	USA
PI 560088	VS389	<i>Cuphea viscosissima</i>	36	16	82	10		Tennessee
PI 560089	VS390	<i>Cuphea viscosissima</i>	NA	NA	NA	NA	Virginia	USA
PI 560090	VS391	<i>Cuphea viscosissima</i>	36	49	81	34	Virginia	USA
PI 560091	VS392	<i>Cuphea viscosissima</i>	37	51	79	3	Virginia	USA
PI 560092	VS393	<i>Cuphea viscosissima</i>	37	47	79	25	Virginia	USA
PI 560093	VS394	<i>Cuphea viscosissima</i>	38	0	79	33	Virginia	USA
PI 560094	VS395	<i>Cuphea viscosissima</i>	37	54	80	6	Virginia	USA
PI 560095	VS396	<i>Cuphea viscosissima</i>	37	54	80	16	West Virginia	USA
PI 560096	VS397	<i>Cuphea viscosissima</i>	39	31	78	18	West Virginia	USA
PI 560098	VS398	<i>Cuphea viscosissima</i>	39	31	78	15	West Virginia	USA
PI 560099	VS399	<i>Cuphea viscosissima</i>	39	29	78	23	West Virginia	USA
PI 560100	VS400	<i>Cuphea viscosissima</i>	39	18	78	38	West Virginia	USA
PI 560101	VS401	<i>Cuphea viscosissima</i>	39	2	81	24	West Virginia	USA
PI 560102	VS402	<i>Cuphea viscosissima</i>	39	4	81	25	West Virginia	USA
PI 560103	VS403	<i>Cuphea viscosissima</i>	38	53	84	22	Kentucky	USA

(continued)

PI 560104	VS404	<i>Cuphea viscosissima</i>	38	23	84	43	Kentucky	USA
PI 560105	VS405	<i>Cuphea viscosissima</i>	38	7	85	34	Kentucky	USA
PI 560106	VS406	<i>Cuphea viscosissima</i>	38	5	85	50	Kentucky	USA
PI 560107	VS407	<i>Cuphea viscosissima</i>	38	12	86	16	Indiana	USA
PI 560108	VS408	<i>Cuphea viscosissima</i>	38	14	86	17	Indiana	USA
PI 560109	VS409	<i>Cuphea viscosissima</i>	38	26	86	8	Indiana	USA
PI 560110	VS410	<i>Cuphea viscosissima</i>	38	30	86	5	Indiana	USA
PI 560112	VS411	<i>Cuphea viscosissima</i>	NA	NA	NA	NA	OSU	USA
PI 560113	VS412	<i>Cuphea viscosissima</i>	38	26	86	24	Indiana	USA
PI 560114	VS413	<i>Cuphea viscosissima</i>	38	45	86	24	Indiana	USA
PI 561499	VS414	<i>Cuphea viscosissima</i>	37	9	91	26	Missouri	USA
PI 561500	VS415	<i>Cuphea viscosissima</i>	37	9	86	24	Indiana	USA
PI 566734	VS416	<i>Cuphea viscosissima</i>	39	22	93	29	Missouri	USA
PI 578179	VS417	<i>Cuphea viscosissima</i>	38	34	90	40	Missouri	USA
PI 578180	VS418	<i>Cuphea viscosissima</i>	38	36	90	44	Missouri	USA
PI 561486	LN186	<i>Cuphea lanceolata</i>	NA	NA	NA	NA	Queretaro	Mexico
PI 594934	LN187	<i>Cuphea lanceolata</i>	25	21	100	10	Neovo Leon	Mexico
PI 594935	LN188	<i>Cuphea lanceolata</i>	23	38	99	12	Tamaulipas	Mexico
PI 594936	LN189	<i>Cuphea lanceolata</i>	22	53	99	27	Tamaulipas	Mexico
PI 594937	LN190	<i>Cuphea lanceolata</i>	22	29	99	33	San Luis Potosi	Mexico
PI 594938	LN191	<i>Cuphea lanceolata</i>	22	4	99	38	San Luis Potosi	Mexico
PI 594939	LN192	<i>Cuphea lanceolata</i>	21	59	100	40	San Luis Potosi	Mexico
PI 594940	LN193	<i>Cuphea lanceolata</i>	23	37	100	28	San Luis Potosi	Mexico
PI 594941	LN194	<i>Cuphea lanceolata</i>	23	35	100	35	San Luis Potosi	Mexico
PI 594942	LN195	<i>Cuphea lanceolata</i>	22	25	100	44	San Luis Potosi	Mexico
PI 594943	LN196	<i>Cuphea lanceolata</i>	20	20	99	56	Queretaro	Mexico
PI 594944	LN197	<i>Cuphea lanceolata</i>	20	21	99	39	Hidalgo	Mexico
PI 594945	LN198	<i>Cuphea lanceolata</i>	21	6	101	26	Guanajuato	Mexico
PI 594946	LN199	<i>Cuphea lanceolata</i>	21	16	101	40	Guanajuato	Mexico
PI 594947	LN200	<i>Cuphea lanceolata</i>	21	42	102	16	Aquascalientes	Mexico
PI 594948	LN201	<i>Cuphea lanceolata</i>	21	46	101	56	Jalisco	Mexico
PI 594949	LN202	<i>Cuphea lanceolata</i>	22	39	103	58	Jalisco	Mexico
PI 596736	LN203	<i>Cuphea lanceolata</i>	21	15	100	18	Guanajuato	Mexico

PI 596737	LN204	<i>Cuphea lanceolata</i>	20	52	100	51	Guanajuato	Mexico
PI 596738	LN205	<i>Cuphea lanceolata</i>	20	23	99	56	Queretaro	Mexico