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Development of cDNA-derived SSR markers and their efficiency in diversity assessment of *Cymbidium* accessions

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Abstract *Cymbidium* spp. are popular flowering plants. Assessment of the genetic diversity in cultivated *Cymbidium* facilitates conservation of germplasm and subsequent cultivar improvement. Thus, it is important to develop more efficient polymorphic DNA markers. Although more motifs (403) were identified and more primers (206) were designed in the genomic library compared to the cDNA library, a larger number of successful primers were obtained from the cDNA library (59.9%) than from genomic DNA library (51.1%). However, higher PIC and gene diversity were identified in genomic SSRs. The average allele number per locus was also higher in genomic SSRs (7.3) than EST-SSRs (5.2), among the 24 evaluated *Cymbidium* accessions. AT/TA was comparatively high in EST-SSRs, while this motif was not as common in genomic SSRs. The CTT/AAG/TCT/AGA/TTC/GAA and TGC/GCA/GCT/AGC/CTG/CAG motifs were the most abundant tri-nucleotide sequences in EST-SSRs, while GTT/AAC/TGT/ACA/TTG/CAA was the most frequent in genomic SSRs. The number of repeats ranged from 3 to 12 in EST-SSRs. Currently, 52 novel polymorphic SSR markers have been evaluated, which will be useful for germplasm assessments, core set construction, evaluation of genetic diversity, and marker assisted selection (MAS) based *Cymbidium* breeding.

Keywords: cDNA library, *Cymbidium*, enriched library, molecular diversity, SSR

INTRODUCTION

The genus *Cymbidium,* a member of the Orchidaceae, is terrestrial, epiphytic, and lithophytic, composed of 44 species and distributed in Northwest India, China, Japan, Korea, the Malay Archipelago, and North and East Australia (Du Puy and Cribb, 1988; Obara-Okeyo and Kako, 1998). The oriental *Cymbidium* is a popular ornamental orchid, and is important in the Korean floricultural industry. *Cymbidium spp.* have highly variable floral morphologies, pollinator relationships, and diversities in ecological habitats (Arditti, 1992; Judd et al. 1999). However, the genetic relationship between many of the *Cymbidium spp*. lineages remains unclear (Choi et al. 2006). It is important to properly characterize and evaluate the genetic diversity of *Cymbidium* to effectively conserve and use this species (Park et al. 2009). Thus, it is essential to identify novel polymorphic markers.

Parchman et al. (2010) discussed the advantages of 454 sequencing as a cost and time effective way to discover novel SSRs. Next-generation sequencing facilitates developing such markers, not only because enormous amounts of sequencing data are generated, but also because the novel markers are gene-based (Parchman et al. 2010). A 454 sequencer is a large-scale parallel pyrosequencing system, using a genome sequence (GS) FLX titanium instrument, with the ability to sequence 400-600 million base pairs per run with 400-500 base-pair read lengths. The GS FLX system includes a simplified sample preparation work-flow and emulsion (em) PCR automation. Pyrosequencing is a powerful tool that has been used in genome and functional expression analyses. It is also an attractive

approach to discover novel transcripts, unknown gene functions, sequences of high quality, base discrepancies, and alternative spice variants (Bainbridge et al. 2006). The combination of long, accurate reads and high throughput makes 454 pyrosequencing analysis on the FLX genome sequence well suited for detailed investigations (Jarvie and Harkins, 2008). Thus, 454 pyrosequencing is increasingly being used in many investigations.

Previous studies have focused on intra and inter-specific genetic diversity and/or evolutionary relationships in *Cymbidium* using isozymes, random amplified polymorphic DNA, amplified fragment length polymorphisms, inter-simple sequence repeats, and internal transcribed spacer sequence. Currently, simple sequence repeats (SSRs) or microsatellite markers have been used to study genetic diversity, phylogenetic relationships, classification, evolutionary processes and quantitative trait loci in many crops. They have some advantages, such as technical simplicity, relatively low cost, high genetic resolution power, and being highly polymorphic. Moreover, they are reliable and easy to score (Gupta and Varshney, 2000). They are clusters of short tandem repeat nucleotide bases distributed throughout the genome and are co-dominant, multi-allelic, and require a small amount of DNA for scoring. Therefore, they have been recognized as useful molecular tools for marker-assisted selection in various species (Agrama et al. 2007). Thus, Xia et al. (2008) designed and applied nine novel SSR markers in *Cymbidium sinensis.* However, *Cymbidium* genomic research has lagged behind other crop species due to a lack of polymorphic DNA markers. Thus, it is important to develop and identify polymorphisms of SSR markers in *Cymbidium*. In the past, SSRs were developed by screening several thousand clones through colony hybridization with repeat-containing probes. Presently, they are developed by constructing genomic and/or complementary DNA (cDNA) libraries and followed by sequencing. This study investigated and developed SSR markers, and discussed strategies to develop these markers from genomic DNA and cDNA libraries.

MATERIALS AND METHODS

Plant materials

DNA and RNA were extracted from the Korean *Cymbidium* species g*oeringii*. RNA was extracted from fresh green leaves to synthesize the cDNA library. To confirm that the novel SSR markers were polymorphic, we evaluated 24 *Cymbidium* accessions, including 20 *C. goeringii* collected from different geographical origins and four *C. sinensis* from the National Institute of Horticultural and Herbal Science of the Rural Development Administration, Republic of Korea (Table 1).

cDNA synthesis and library preparation

Total RNA isolation, mRNA purification, cDNA synthesis, fragmentation by nebulization, and adaptor ligation were performed prior to 454 sequencing. Total RNA was isolated using Trizol RNA isolation protocol (modified by D. Francis from Edgar Huitema) and the RNeasy Plant Mini kit (Qiagen, Valencia, CA, USA), following the manufacturer's protocol. Fresh green leaves (100 mg) of *C goeringii* were frozen in liquid nitrogen, ground into a powder, and total RNA was extracted. RNA concentration was determined using the NanoDrop ND-1000 (NanoDrop Technologies, Inc., Wilmington, DE, USA) and agarose gel electrophoresis. mRNAs were purified with the PolyATract mRNA Isolation System (Promega, Madison, WI, USA). The purified products were used as template to synthesize full-length cDNA using the ZAP-cDNA Synthesis kit (Stratagene, Santa Clara, CA, USA). The cDNA was fragmented by nebulization for library construction.

From the cDNA, a single-stranded template DNA library was generated. The cDNA was fragmented by nebulization using an Agilent 2100 bioanalyzer (Waldbronn, Germany) with a mean fragment size of approximately 600 bp. Approximately 1 µg of cDNA was used to generate a genome sequencing library using a FLX Titanium analyzer (Roche, Mannheim, Germany). The cDNA fragment ends were polished (blunted), and two short adapters were ligated to the ends according to standard procedures (Margulies et al. 2005). The adapters provided priming sequences for amplification and sequencing of the sample library fragments, as well as a "sequencing key," which is a short sequence of four nucleotides used by the system software for base calling. The sequencing key also releases the unbound strand of each fragment (with a 5' adaptor, A) following DNA repairs in the double-stranded library. The quality of the single-stranded DNA fragment library was assessed using the 2100

bioanalyzer. The library was also quantified to determine the optimal concentration of library to use for emulsion-based clonal amplification.

454 pyrosequencing of the cDNA library

Single copies of template species from the DNA library were hybridized to DNA capture beads. The immobilized library was re-suspended in amplification solution, and the mixture was emulsified, followed by PCR amplification. After amplification, the DNA-conjugated beads were recovered from the emulsion and enriched. The second strands of the amplification products were removed, leaving the amplified single-stranded DNA library bound to the beads. The sequencing primer was annealed to the immobilized amplified DNA templates. After amplification, a single DNA-carrying bead was placed into each well of a PicoTiterPlate (PTP) device. The PTP was inserted into the FLX genome titanium sequencer for pyrosequencing (Ronaghi, 2001; Elahi and Ronaghi, 2004), and sequencing reagents were sequentially flowed over the plate. Information from the PTP wells was captured simultaneously by a camera, and the images were processed in real time by an onboard computer. After sequencing, sequence assembly was performed using the GS De Novo Assembler software to get contigs and singletons. All sequence data was confirmed with references using GS Reference Mapper software. The resulting sequences were trimmed using SeqClean and the Lucy program.

Investigation of SSR motifs and designing DNA markers

All sequences generated by 454 pyrosequencing were investigated for SSR motifs using the ARGOS program 1.46 (SSRManager) at the default setting (Kim et al. 2007). Of the identified SSR motifs, only motifs having sufficiently large flanking sequences were used to design primer pairs. The SSRs detected were categorized as perfect di-, tri-, tetra-, penta-, or hexa-nucleotide motifs.

SSR Genotyping using the ABI (Applied BioSystems) genetic analyzer

PCR amplification was first confirmed using 4 different samples for each SSR primer. M13-tail PCR method was used to measure the size of the PCR products (Schuelke, 2000). Only primers yielding amplified product were used for the M13-tail PCR. PCR amplification conditions were as follows: 94ºC for 3 min, 30 cycles each at 94ºC for 30 sec, 55~60ºC for 45 sec, 72ºC for 1 min, followed by 10 cycles of 94ºC for 30 sec, 53ºC for 45 sec, 72ºC for 1 min, and a final extension at 72ºC for 10 min. SSR alleles were resolved on a ABI 3500 Genetic Analyzer (Applied Biosystems) using the GeneMapper 4.1 software and sized precisely using GeneScan installation Kit DS-33 and GeneScan 600 LIZ size standard v2.0. The GeneScan installation standard DS-33 consists of pooled PCR products labeled with 6-FAM, VIC, NED, and PET dyes.

Data analysis

Locus variability was measured in terms of the number of alleles, heterozygosity (H), major allele frequency (M_{AF}), gene diversity (GD), and polymorphic information content (PIC) using PowerMarker 3.25 (Liu and Muse, 2005). The UPGMA algorithm was used to construct a phylogram for the entire population using shared allele distances with the MEGA4 software (Tamura et al. 2007) embedded in PowerMarker.

RESULTS

Sequencing and evaluation of SSRs

A summary of the genomic and cDNA sequencing from *C. goeringii*, and SSR identification, is shown in Table 2. SSR-enriched genomic library results were taken from our previous published paper (Moe et al. 2010) and were also shown in Table 2. From a genomic library of 525 sequenced clones, we identified 56 clones (10.67%) that were redundant and 322 clones (61.33%) that had microsatellite repeating motifs. Four hundreds and three SSR motifs were indentified from 322 clones using the SSRManager, ARGOS program 1.46. In total, 206 (51.11% of total repeat motifs) primer pairs were designed from the flanking sequences of SSR-containing clones, and screened for polymorphisms in a

panel of 10 *C. goeringii* accessions, using the procedure described previously (Dixit et al. 2005). Only 14 primer pairs produced reproducible polymorphic bands (Moe et al. 2010), and these were further evaluated against 24 *Cymbidium* accessions.

In total, 4238 contigs and singletons were assembled *by De Novo* Assembly after 454 pyrosequencing from a cDNA library. Among them, 166 (3.9%) were found to be redundant and 4072 (96.1%) were found as unit express sequence tags (ESTs). Although 312 SSR motifs were investigated from unit ESTs, only 187 (59.93%) had sufficiently large flanking sequences to allow for primer design by ARGOS program 1.46 (Table 2 and Table 3). Currently, we have screened 70 (39.33% of the total designed primers) primer pairs for polymorphisms against the 24 *Cymbidium* accessions. We selected 70 primers proportionally to its designed primers each of di-, tri-, tetra- and others nucleotide motifs. Of the 70 primers tested, only 52 (74.3% of total tested primers) showed reproducible polymorphic bands against 24 tested accessions.

Comparisons of SSR repeat motifs

SSR development and its characteristic features are summarized in Table 4. Sequence analysis of all SSR-containing clones revealed a high number of di-nucleotide SSRs (82.88%), compared with tri-nucleotide SSRs (16.63%), from the genomic library. The CT/AG/TC/GA class of repeat motifs was most frequently identified (69.46% of the total di-nucleotide motif type) among the di-nucleotides, followed by the TG/CA/GT/AC class (27.54%). Among the tri-nucleotide SSRs, the GTT/AAC/TGT/ACA/TTG/CAA class of repeat motifs was predominant (56.72%), followed by CTT/AAG/TCT/AGA/TTC/GAA (19.41%) and TGC/GCA/GCT/AGC/CTG/CAG (14.93% of total tri-nucleotide motif type).

Different SSR motif distributions were found in EST-SSRs identified from the cDNA library. Similar proportions of di-nucleotide (47.44%) and tri-nucleotide (47.12%) SSRs were found. A small number of tetra-nucleotide (3.85%) and other motifs (1.6%) were also identified. Among the di-nucleotides, CT/AG/TC/GA (61.49%) was the most common SSR class, followed by TA/AT (33.78%) and TG/CA/GT/AC (4.73% of total di-nucleotide type). There was no GC/CG class of di-nucleotides found in either case. The CTT/AAG/TCT/AGA/TTC/GAA class was the most abundant (27.89%) among tri-nucleotide nucleotide motif type, followed by TGC/GCA/GCT/AGC/CTG/CAG (14.96% of total tri-nucleotide motif type).

Figure 1 presents the distribution of different repeat motifs (di-, tri-, others) and their respective number of repeats, regardless of the motif class. The highest value was observed for tri-nucleotides with 4 repeat motifs, followed by 5 and 3 repeat motifs. This trend was not consistent in the di-nucleotides. In this case, the highest number was still observed for 4 repeat motifs, but followed by 3, 6, 7, and finally 5 repeat motifs. The lowest numbers were seen at 9 repeat tri-nucleotide motifs, 12 repeat di-nucleotide motifs, and at 6 repeats of other motif types.

Designing SSR primers and screening for polymorphism

Primer pairs were designed for all available SSR motifs detected. However, not all SSR motifs can be designed as primer pairs, it can be designed only if the SSR motifs had sufficiently large flanking sequences. Among the 402 and 312 SSR motifs, only 51.11% (206) and 59.93% (187) of the motifs of genomic SSRs and EST-SSRs could be used for primer design, respectively. Respective SSR motifs and successfully designed primer pairs (EST-SSR primers) were presented in Table 3.

The 70 novel markers were classified according to the nucleotide type, di-, tri-, and others, in their polymorphism. In the screening analysis, 25 di-nucleotide markers, 40 tri-nucleotide markers, and 5 of other nucleotide types were included. Although 84% (21) of the di-nucleotides, 67.5% (27) of the tri-nucleotides, and 80% (4) of the other nucleotide types were polymorphic, it was difficult to deduce whether the di-nucleotides were more efficient in the polymorphism test (Table 5). There was no correlation between the number of motif repeats and the polymorphic efficiency in *Cymbidium* (data not shown).

The SSR markers were analyzed to confirm that they were polymorphic against 24 *Cymbidium* accessions. Variability at each SSR locus was measured in terms of the numbers of alleles,

heterozygosity, gene diversity, and PIC using the PowerMarker 3.23 software (Liu and Muse, 2005). In total, 102 alleles were detected, with an average of 7.3 alleles per locus, by 14 genomic SSRs (Table 6). The KNU-CC-32 locus had the highest number (15) of alleles, followed by KNU-CC-35 (9), while only two alleles were observed at KNU-CC-42. Allele size ranged from 105 to 380 bp. Heterozygosity values ranged from 0.000 to 1.000 (mean, 0.416). The average gene diversity and PIC values were 0.621 and 0.589, with ranges from 0.363 (KNU-CC-52) to 0.896 (KNU-CC-32), and from 0.323 (KNU-CC-42) to 0.887 (KNU-CC-32), respectively.

In total, 271 alleles, with an average of 5.2 alleles per locus, were detected by 52 EST-SSRs across 24 *Cymbidium* accessions (Table 7). The highest number of alleles was found in CG-cSSR-67 locus (13), followed by CG-cSSR-9 (11), while only two alleles per locus were observed at seven other loci. The alleles ranged from 72 to 449 bp. The heterozygosity value ranged from 0.000 to 1.000, with an average of 0.601. The average gene diversity and PIC values (0.545 and 0.497) identified from 52 EST-SSRs was slightly lower than the 14 genomic SSRs against 24 *Cymbidium* accessions. The gene diversity ranged from 0.117 (CG-cSSR-20) to 0.842 (CG-cSSR-27), while PIC ranged from 0.110 (CG-cSSR-20) to 0.824 (CG-cSSR-20) in EST-SSRs.

Genetic diversity

As described above, of the 70 markers evaluated, a total of 56 (80%) microsatellites amplified well in the survey panel, and these were used for the polymorphism survey in the germplasm panel and diversity analysis. In this final group of 56 amplified markers, 4 (5.71%) were monomorphic and 52 (74.29%) were polymorphic (Table 3).

A genetic distance-based analysis was performed to determine how useful these novel SSRs could be in studying genetic variations and phylogenetic relationships among 24 germplasm collections. A UPGMA dendrogram was constructed using the Mega 4.0 program (Tamura et al. 2007) embedded in the PowerMarker program (Liu and Muse, 2005). The greater value of genetic diversity (0.629) and PIC (0.589) across the 24 different *Cymbidium* accessions was revealed by genomic SSRs markers than by EST-SSRs. It reflected a high level of polymorphism was in genomic DNA. All *Cymbidium* accessions were clustered into two main groups (G1 and G2) in the UPGMA phylogram by 14 sets of genomic SSRs. When *Cymbidium* accessions were evaluated using 52 EST-SSRs, they were classified into 3 groups and one outstanding accession (G1, G2, G3 and KNU-085; Figure 2a and 2b). Group 1 (G1) included all *C. goeringii* accessions (20) and group 2 (G2) consisted of all (4) *C. sinensis* accessions, based on the genomic SSRs. Two *C. goeringii* accessions (KNU-017 and KNU-032) were separated from G1, and one *C. sinensis* accession (KNU-085) was separated from G2, when analyzed using the EST-SSRs.

DISCUSSION

Cymbidium spp. are popular flowering plants. Assessment of the genetic diversity in cultivated *Cymbidium* facilitates conservation of germplasm and subsequent cultivar improvement. However, few molecular studies focusing on the genetic diversity and conservation of these species have been performed. Only a limited number of genetic markers, such as 224 ISSR (Wang et al. 2009) and 38 SSR (Xia et al. 2008; Moe et al. 2010; Huang et al. 2011) have been developed. Thus, it is important to characterize novel, efficient polymorphic DNA markers. Previously, to develop novel markers, several thousand clones were screened through colony hybridization with repeat-containing probes. This method is extremely tedious and inefficient for plant species with low SSR frequencies (Zane et al. 2002). Some studies have reported construction of simple sequence repeat (SSR)-enriched genomic libraries to facilitate the development of SSR markers for crop plants (Gwag et al. 2006; Cho et al. 2010). Previously, we used a SSR-enriched *Cymbidium goeringii* genomic library to search for novel SSRs. In this study, we prepared a cDNA library from mRNA to develop *Cymbidium* genetic markers. Aside from marker development, cDNA libraries play an important role in gene separation and cloning. Target genes can be isolated from cDNA and used directly for expression. Thus, this library is a basic tool to evaluate and characterize novel genes (Jun, 2007). With advances in molecular biological technologies, the construction and use of cDNA libraries have improved. The use of next-generation cDNA sequencing has become popular to develop markers, not only because enormous amounts of sequence data are available from which markers can be identified, but also because the novel markers are gene-based (Parchman et al. 2010).

Previous studies have shown that microsatellite enrichment levels ranged from 11% to 99% (Zane et al. 2002; Ueno et al. 2003; Pandey et al. 2004; Zhao et al. 2005; Cho et al. 2010). The efficacy of SSR development in *C. goeringii* was higher (61.3%) than in other plants, for which enrichment efficiencies ranged between 10% and 22% (Ferguson et al. 2004; Moretzsohn et al. 2004). Primer pairs cannot always be designed from the SSR repeat motifs. Although more motifs (403) were identified and more primers (206) were designed in the genomic library compared to the cDNA library, a larger number of successful primers were obtained from the cDNA library (59.9%) than from genomic DNA library (51.1%). These values were slightly lower than the percentage of successful primers available in wheat (69.6%) (Gadaleta et al. 2010) and mungbean (65.2%) (Blairet al. 2011), but much higher than ginseng (8.59%) (Chengjun et al. 2008). It was surprising that in *Cymbidium* a high percentage of the EST-SSR markers (74.3%) were polymorphic, as opposed to genomic SSRs (6.8%). In other crops, such as mungbean 32.5% and 39.8%, and ginseng 60.5% of the EST-SSR markers are polymorphic, respectively (Chengjun et al. 2008; Blairet al. 2011). Although we used the same accession (*Cymbidium goeringii*) and program to design the SSR markers, a clear difference in polymorphic efficiency was observed. There are three possible reasons for these differences: (1) the use of different number of *Cymbidium* accessions during polymorphic screening, (2) only 39.3% of designed primers (not all primers) can be screened in the EST-SSRs, and (3) polymorphic primer pairs may be selected by chance while primer selection, although they were selected proportionally according to their repeat motifs types. In comparison, amplification with non-gene based microsatellites is prone to some pitfalls for AT-rich hybridization-derived genomic microsatellites (Blair et al. 2011). Differences between genic and other types of genomic microsatellites have been observed in marker sets of other crops (De Campos et al. 2007; Hanai et al. 2007).

Of the EST-SSRs, similar ratios of 47.4% and 47.1% di- and tri-nucleotide repeat motifs were identified, respectively. This differs from others crops, such as mungbeans, peanuts, and wheat, in which tri-nucleotides are the most common motif, followed by di- and other nucleotide types (Wang et al. 2009; Gadaleta et al. 2010; Teh et al. 2010; Blair et al. 2011). However, for genomic SSRs, di-nucleotides are the most abundant (82.9%), followed by tri-nucleotides (16.6%). This is not consistent with other crops, such as proso millet and Italian millet, in which tri-nucleotides are more common than di-nucleotides (Cho et al. 2010; Zhao et al. 2012). Although the AT/TA class was comparatively high in the EST-SSR di-nucleotides, it was less so in genomic SSR. It was previously reported that the most common di-nucleotide repeat in plants is TA (Tóth et al. 2000). However, this repeat is not suitable for hybridization because of its ability to auto-complement (Jia et al. 2009). The CTT/AAG/TCT/AGA/TTC/GAA and TGC/GCA/GCT/AGC/CTG/CAG motifs were the most frequent tri-nucleotide classes in EST-SSRs, while GTT/AAC/TGT/ACA/TTG/CAA motif class was the most frequent genomic SSR tri-nucleotide. The number of repeats ranged from 3 to 12 in EST-SSRs.

The average levels of polymorphism in the selected genomic SSRs were 0.589, while EST-SSRs were 0.497. The average gene diversities were 0.621 and 0.545 for genomic and genic SSRs, respectively. A high PIC and gene diversity was identified in genomic SSRs. It is believed that polymorphism should be primarily present at the genomic level, and less so at the EST level. The average number of alleles per locus was also higher in genomic SSRs (7.3) compared with EST-SSRs (5.2), among the 24 evaluated *Cymbidium* accessions. Generally, as the number of genetic markers increases, higher genotyping resolution can be expected. Thus, we completed accurate grouping of the 52 EST-SSRs, identifying KNU085 as a unique class. The 52 SSR markers, rich in polymorphisms, will be useful for germplasm assessments, core set construction, assessment of genetic diversity, MAS-based crop breeding, and other *Cymbidium* improvements programs.

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TABLES

Table 1. Cymbidium accessions used in this study showing the UPGMA results analyzed by 14 genomic SSR markers and 52 EST SSR markers.

No.	ID	Collection region	Grouped by 14 genomic SSR markers	Group by 52 EST-SSR markers
1	KNU-006	Jeonbuk sunchang-gun sunchang-eup folk village	1	1
2	KNU-008	Jeonnam gwangju-si moodeungsan	1	1
3	KNU-011	Jeonbuk namwon-si jucheon-myeon jangan-ri	1	1
4	KNU-012	Jeonnam wando-gun dangin-ri	1	1
5	KNU-013	Jeonnam hwasun-gun doam-myeon daecho-ri unjusa	1	1
6	KNU-017	Jeonnam gangjin-gun doam-myeon seokcheon-ri	1	3
7	KNU-018	Jeonbuk buan-gun gaeamsa	1	1
8	KNU-026	Gyeongnam sancheong-gun danseong-myeon jeongchon-ri	1	1
9	KNU-028	Gyeongnam namhae-gun changseon-myeon changseondo	1	1
10	KNU-029	Jeonnam yeongam-gun samho-eup maeja-ri	1	1
11	KNU-030	Jeonnam yeonggwang-gun beopseong-myeon beopseong-ri beopseongpo	1	1
12	KNU-031	Jeonnam goheung-gun oenarodo	1	1
13	KNU-032	Jeonnam yeonggwang-gun bulgap-myeon moak-ri bulgapsa	1	3
14	KNU-033	Jeonnam hampyeong-gun hampyeong-eup japung-ri	1	1
15	KNU-034	Gyeongnam masan-si gusan-myeon bandong-ri	1	1
16	KNU-035	Jeonnam jindo-gun seomang-ri phangmonkport	1	1
17	KNU-042	Jeju-do seogwipo-si citrus museum	1	1
18	KNU-044	Ulsan-si buk-gu jeongja-dong	1	1
19	KNU-064	Jeonbuk namwon-si snanae-dong jirisan	1	1
20	KNU-073	Jeju-do pyoseon-myeon hacheon-ri	1	1
21	KNU-084	National Institute of Horticultural & Herbal Science of RDA	2	2
22	KNU-085	National Institute of Horticultural & Herbal Science of RDA	2	Outstanding
23	KNU-086	National Institute of Horticultural & Herbal Science of RDA	2	2
24	KNU-087	National Institute of Horticultural & Herbal Science of RDA	2	2

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Table 2. A summary sequencing information and SSR marker designed by SSR Manager (ARGOS program).

	Genomic DNA library			cDNA library		
	Items	**Numbers**	**(%)**	**Items**	**Numbers**	**(%)**
1	Total reads				70272	
2	Totally and partially assembled by De Novo				42753	60.84
3	Number of sequenced clones	525		Total contigs and singletons	4238	
4	Redundant clones	56	10.67	Redundant identified by ARGOS	166	3.9
5	Clones that had SSR motifs	322	61.33	Unit contigs	4072	96.1
6	SSR motifs	403		SSR motifs	312	
7	Total Primer designed	206	51.11	Total Primer designed	187	59.93
8	Tested for Polymorphism with 10 accessions	206	100	Tested for Polymorphism with 24 accessions	70	39.33
9	Polymorphic markers	14	6.8	Polymorphic markers	52	74.3

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Table 3. Primer sequences and simple sequence repeat motif for new set of cDNA-derived (micorsatellite derived from cDNA sequence) series markers.

Sr No.	Primer Name	Left Primer	Right Primer	Motif	Expected Product Size	Amplification
1	CG-cSSR-51	CCTTATCCAAAACCAACCG	CGCCACCATAACCAGTGT	(ACC)5	227	Polymorphic
2	CG-cSSR-1	AAAACCACAGCTACAGGGC	ATGGCCCCAAGTTCAGAC	(TA)9	251	Polymorphic
3	CG-cSSR-52	TTTGGCGCCTCCTAGAAT	GACTATGGAGATCGGGGC	(GTT)5	265	Polymorphic
4		TCTATTCCTCTCACCTGTCGT	AAAACAGCGGCAAACAGA	(CT)5(GT)(CT)5	210	not tested
5	CG-cSSR-2	TGGCCAATCTCTTGCATT	AATCTCTTGCAACAATCACAAA	(TAA)2A(TAA)4	294	monomorphic
6	CG-cSSR-3	GAACGCATCCCCCTAAAC	CAGGCACTAAGGCATGAGA	(TA)6	293	no amplify
7		CCAATGTACCAGAGGCCA	TCGTCGTAAGCCTCCTGA	(GAG)3(AT)(GAT)2	236	not tested
8	CG-cSSR-53	AGCCAAAGGTCTGCCTTC	TCCAACATAGACGTCGCC	(CTT)5	265	Polymorphic
9		CTTCGAGACATTCGCAGC	TCCACTCACCAAAATAGGTTCT	(TTA)4	289	not tested
10		TGGGATCTTCAAGCTTCATT	TGGGGATTAGGATGAGCA	(TCT)3(TT)(TCT)2	156	not tested
11		AGCTATGGCAAACGGATG	GGGTTTTGGATCTGAGGG	(GCC)4	157	not tested
12		GCTGAGTTTCCAAGCGAA	CCCTCCTGGAAATCCACT	(CT)5	251	not tested
13		GGCCACCTTTTTAGTGCC	AAAAAGCAGGGGCTTGAG	(CT)4	288	not tested
14	CG-cSSR-4	CTTATGGCAACGAGCAGG	GTAACCTCCGTCGCTCCT	(TGG)8	245	Polymorphic
15		TTGGGAGATGAAGGAGGG	GTCCTGGACCTCCACCTC	(GA)4,(GA)4	298	not tested
16		ACGGTGCCTAAGGAGGAG	GGGCTGACATTTGCATTG	(TG)3,(TG)3	240	not tested
17		GATTTGGCTGAGCACTCG	GGACGAGGAACATGACGA	(ATC)4	170	not tested
18	CG-cSSR-5	TGCGGTGAATTTGAGCTT	GCAGTTTGCTGGTCGGTA	(GGC)5	292	Polymorphic
19		AGCTTGGCCTTCACAACA	GCTATTTCCACCGCCTTC	(GGC),(GGC)4	211	not tested
20		GAAACCAACGGTCGTTCA	GCTCTGGCCCTTCTCCTA	(TTC)5	170	not tested
21		CATCCACCTCACCTCCAA	TCCATCCACTCTGCCAAC	(CTT)4	184	not tested
22		CCGACTCAAGGCTCTGTG	TCAATGAACACGGCATCA	(TCT)(TC)(TCT)3	279	not tested
23	CG-cSSR-6	TCCGCTCTGTAGTTCCGA	AGCTGCAAGAAGCACATGTAT	(TATG), (TATG)3	252	monomorphic
24	CG-cSSR-7	GCCAACTGCTCGAAAATG	TTGGCATTACTGAGAGAATTGA	(TTC)7	245	no amplify
25		AGTGCTGGTCTTCCTCGC	CTGGTGAAGCTTTCGACG	(GA)3,(GA)3	267	not tested
26	CG-cSSR-8	CTGTAGCTGCAACCGGAC	CGGCCTCCATTCTCATCT	(AG)6(CG)(AG)3	247	Polymorphic
27		TTTCTTCGTTGAGCGCAT	GAGTTTCTCCCAGGCAGG	(CT)3(GT)(CT)3	277	not tested
28		AGCTTACACGACCGCAAA	AAGTCCTCCGCTCTCAGC	(CGG)4	213	not tested
29		GACTTACGAGGGCCGATT	GGGCTTGCTGCTATAAGGA	(TGG)3(TGT)(TGG)2	165	not tested
30		CTTGCGAAGAGGAGGGAT	CGCTTCGAGACCTTGTTG	(GA)5	191	not tested

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31		TTCTTGGCTTCACGCTTC	ATCCAGAAGAGGGCTCCA	(GGT)4	290	not tested
32		TTCGCCTCCTCCTTTCTC	ACGTTCTCCAGCGAGTTG	(GCG)5	228	not tested
33		TTGCTGCTAATACATGCCC	TCTCAGTCCCTCATGCGT	(TA)5	158	not tested
34		GGGTTCAAAAGAACAAGCA	CCAAAGAACCTCCCAAGG	(TCA)2(TCT)(TCA)3	233	not tested
35		CTGCCATTTTTGGAGTCG	GGAGGGGAAACACCACAC	(TCT)3(CT)(TCT)	291	not tested
36		AAGCTTGCCAGTACGCTG	AACCCCTGGCTTAGGAAA	(CT)5	282	not tested
37		ATCCAGTAAGGCTGGGGA	AGAGGACCGAGCCTCAAG	(TGC)5	179	not tested
38		GGATGCAAAACACAAACAT	GAGCTGAGAACGGAGGGT	(CA)3(GA)(CA)2	277	not tested
39		TGTGAAGCAAGTAGGCCG	TGGACAGCCGTCAAATTC	(GCA)3,(GCA)2	254	not tested
40		TCTTTGTGCCTGTTGGCT	CAACAATAGCAATGGGGC	(TA)6	237	not tested
41		ACTACCAGCGGCAAGGAT	TCATATGGCCGACGAGAG	(GCC)3(GCT)(GCC)2	274	not tested
42		GATCGGAAGCGAATCCTC	GGAGAGGGAGGAGGGAAT	(CCG)4	250	not tested
43		CCGGTCTCTCATGGCTT	CTCCGACACAACAACGGT	(CT)3(T)(CT)4	286	not tested
44	CG-cSSR-54	TTGCACCTCAAAGGATGG	TGCCACCACCTCTACCAC	(AG)2(GT)(AG)3	287	Polymorphic
45		CGCGCTACCTCTGTCTTG	TCAGCACTGGTCCCAATC	(CGC)3(GC)(CGC)	291	not tested
46		CCGCTTCATCCTCCTCTT	GCTCGGCACTCTAAGCAA	(TCG)4	262	not tested
47	CG-cSSR-9	CTCATCGCCTTGCTTGAG	TCGATTCTCAATGGCACC	(CTT)6	276	Polymorphic
48		ACCACAGCCATTCCCTCT	GCGATGGGATCTTCTTCC	(AAG)5	185	not tested
49	CG-cSSR-10	CGGAAGTCTATCGGGGAG	CGAAACGTTCGATGCAAT	(GCC)4	294	Polymorphic
50		ATTGGCCAAAATGCATCA	GGGCTAACAAGCCGATTT	(ATTTT)3	284	not tested
51	CG-cSSR-11	TTTTAGAGGAGGGCGGAG	CAAGCGACCTCAACTTGC	(AAG)6	223	Polymorphic
52		CAAAAGCAAACAGTTCAGCA	AGAGAAGGGCTCCATCCA	(TCA)3(TCT)(TCA)3	242	not tested
53		ACCATCCTGATGGAACCC	CGACGAAGTCTGGTGGAG	(CTT)4	235	not tested
54	CG-cSSR-12	TTGCATGGCTCAGGATCT	CATTCAAGGAGCAGCCAC	(GAA)10	175	no amplify
55		GCTTCTTGATGCACGCAC	CCCTAGCCGAATCCTTTG	(CTC)4	228	not tested
56		CTCGAATCCACCGAGAAA	CTCCAGCCTCAACGAGTG	(GCC)4	219	not tested
57		TTTGCTGCTCTTTCCTACC	TCTCTCCCGTTGAAGCAA	(TC)3,(TC)4	238	not tested
58		GCTCCTCTTTGGCGTTTT	CGGTCGGATCAGTCTCAA	(TTC)2(TTT)(TTC)3(TTT)(TTC)2	251	not tested
59		GAATCACCTCTCAGGCCC	TTGGCAAAGCTCTGCTTC	(GAA)4	270	not tested
60		CGCTAAGATTGCACCACC	GGAGTTGACTAAGCTGGCTG	(CTT)2(CGT)2(CTT)3	232	not tested

61		GGTGAGGCTACCAAAGGG	CGGTTGCAAGGTTGTAGG	(CTT)6	238	not tested
62		AGCTGCCAGCCTTCTTCT	GTGTGCTGCATGTGGCTA	(TTC)3,(TTC)2	254	not tested
63		TTTGATTGCGTGTATTTGGA	TTTTGATGTCCGGAGACG	(GCC)4	254	not tested
64	CG-cSSR-13	CCATGGTTGCACCTCAGT	TGTCTGCATTGCCTCTGA	(TGG)2,(TGG)4	239	no amplify
65		CTTCGCCATTGAGCTCTT	TTTCTTCTGAAGGCGACG	(TC)2(AGC)(TC)4	211	not tested
66		CTCTCGCAAATGCTCCAC	CCTCTAAGGACCAAAGCCA	(CAG)4	287	not tested
67		CGTCTCTCCTTCCAGCCT	GTTCTTCCCACTCGACCC	(CGG)3(TGG)(CGG)2	277	not tested
68		TTCTGGAAAGGATGTGCC	AGGCTCATGGAAGCAGAAC	(GCT)2,(GCT)3	297	not tested
69	CG-cSSR-14	TGAAGAGGCTTCTGCTGC	TTCATTACCACCAACGCC	(TGG)5	271	no amplify
70		GGCCGAAAGCAGCTTAGT	GCCATCTACTGAAAGAATTGATG	(ATGT)4	179	not tested
71		TATGCAGCAGAAGCGGAT	GTGCCGTGAACGGAATTA	(TC)5	170	not tested
72	CG-cSSR-15	CCGTCACGACTAGCGAAG	GGGAATCCTCGCCGT	(TG)9	264	Polymorphic
73	CG-cSSR-16	CGTTTCTGGTGAGGGACA	CCAAGGCTTCACATCCAA	(TGA)5	253	Polymorphic
74		TGCAGCCATTGATCCTTC	CGCAGAGGTGGGTAATCA	(GA)10	220	not tested
75		GTCCTGCACAGGATGTGG	ACCATGGGAGAAGGGTTT	(GA)2(GCT)(GA)4	261	not tested
76		TCGCTTGCCTCCTCATTA	AAGGAAAAGCTCATCGGC	(AG)6	252	not tested
77	CG-cSSR-55	CGAAGCATTGGGAGATCA	TCCGCGTCAATTGTCTTC	(GA)6	188	Polymorphic
78		GCAGAGGAAGGATTTGCC	GCACCTGCTATCCCAACA	(GCT)5	288	not tested
79	CG-cSSR-17	TAGCAGAAGCTATGCCCG	TCGGAGGAGAGACCACTG	(GCC)5	209	Polymorphic
80	CG-cSSR-18	GTGCCCAGACACAAGAGG	GTGACGCGGCTAATACCA	(ATG)4(AGG)(ATG)3	250	no amplify
81		GGTGTCGCCAGATACCAA	AAGCTAATAGCCGCCCTG	(AG)3(CAC)(AG)3	193	not tested
82		ACAACATCCTCACCACGC	GTGATCCGGAGAAGAGGG	(ACC)5	178	not tested
83		GCTCGCTTTTACCTGCAC	AGTCAAGCATCCGCTGAA	(TC)3(ACTGTT)(TC)4	213	not tested
84		GAGCCATTCGCAGTTTCA	ATTCTTTCCCCTCCCTCC	(TA)7	176	not tested
85		GGGTGAACACCAAGCAGA	GAAGTTGTTGCTGCCAGG	(TGG)3,(TGG)5	266	not tested
86	CG-cSSR-19	AATCTGGGAATGATCGCA	TCACAGCTCATAACAGAAGCA	(GA)11	277	Polymorphic
87	CG-cSSR-56	GAGCAACACTGCCTACCG	ACTTCCCTCGTCCAGCTC	(TTC)3	224	Polymorphic
88	CG-cSSR-20	GTTCCCGCTCTTCATTCC	CCTTCGCTTCGAGAAGGT	(TTCTTTC)3	193	Polymorphic
89		GTAGAGCTGCCCGCAAG	CCCATCGACATCATCATCA	(GAT)2(GAC)(GAT)4	247	not tested
90	CG-cSSR-21	GAATCAATCACATTCCAGGG	ATGAGGTTCCGAGCCATT	(ATC)8	173	monomorphic

91		TCATCACCATTCGATCCC	CTCGCATCTCCAAACCAA	(CTG)4	292	not tested
92	CG-cSSR-22	TCAGCTTCACCTGGCACT	TGAAGGATGTGGTTTGGC	(TATACA)5	280	Polymorphic
93		TAAAATGGGCGAGGTTCA	GCGAGTTTCTGATCTCTTGG	(GAA)6	260	not tested
94		TGCTTTGTCCTGGCAGAT	AAGATTAGCTGCGATGCG	(CCG)5	188	not tested
95	CG-cSSR-23	GCACCTCACTCCATCCAA	AAGAGGCATACCCAAGCC	(GAT)5	177	Polymorphic
96		GCGTCCACCACTGTGTCT	CGTCATCCTCCGATGTTC	(CGT)4	262	not tested
97	CG-cSSR-24	CGCTCTCGAGTGTGATCC	CGTTCACATGACAAGATACGA	(TC)5	215	Polymorphic
98		CTGAATACTTTCTTCTCACTGGG	TGAAGGATTCATGGCTCTG	(ATTT)4	242	not tested
99		CTTGACGCGAATGAGGAC	ACTGTACCCACCCGGAAT	(AGG)5	299	not tested
100		TCCAACAGCTCGCTCACT	GTGAAAACGTGGGCTGAA	(TTC)4	297	not tested
101		GGGTGTGAATGAGCGAAA	CACGCGAGAGAAACCAAC	(TA)6	259	not tested
102		GTTCATGCATGCCCAGTT	TGCTTTGTAAAAGCCAAACA	(AT)6	188	not tested
103		CACCTCCACCAGGCTACC	TCCACTCTTAGTCGCAGCA	(GCT)2(CTGT)(GCT)3	153	not tested
104		ACCCACCACAAATCCTCC	TGCAGGAGGTGACTCCAC	(CTC)4	282	not tested
105		AGGCAATGGAGACCCAAT	GGAAACCAATCTGGAGCA	(TTTC)4	283	not tested
106	CG-cSSR-25	CGTTGCTCTCTGTATGACCG	TCGACCAAATTGCCTGTC	(TAT)4	281	Polymorphic
107		TTCTTGCCGTCTTCTTGC	ACCCAGTTCCTCTGCCAT	(ATG)5	271	not tested
108		GCCAGCTCCAGCCTCTA	GTTTGCAGCGATCGAGAC	(TC)4,(TC)2	157	not tested
109		CTATCCCCGATCCGATGT	TACTCTCTCGCCTGCTCG	(GA)4,(GA)4	250	not tested
110	CG-cSSR-26	CTACTGCAGCCAGTTCGG	ATGCGCATGTTTCACTTT	(TA)7	183	Polymorphic
111	CG-cSSR-57	AGTGCCTGCAACTCAGGA	CGAAAACCAAGACAAGCG	(GA)3(TACAA)(GA)3	262	Polymorphic
112		GCTCCGCCACAGTATCAG	GGGTCACCAAACAACCAC	(TGC)6	276	not tested
113		GGAGATTGGTTGGGCTTT	CAGCAAACCTCCCTTTGTT	(CT)6	192	not tested
114		GATTGGCCGGAATTGAGT	AAGCTGGCAGGCAAAAAT	(CT)7	265	not tested
115		TCAAGGTTTGAAGAACAGCC	TACGATACCCGCAACGTC	(ATT)5	222	not tested
116		GGAGACATACCTTGGCCC	GCGGCGCAAAATACAG	(GA)4(CA)(GA)2	296	not tested
117		ATTGTCCAACCCCCACTT	CAGTTGGTAGAATGCCGC	(AGG)3(ACG)(AGG)2	236	not tested
118		AACCTCAGCCGTCTCCTC	AAAGCACACCCCTCCAGT	(CAG)4	249	not tested
119		ACTGAGGCACTCAGGCTCT	TGACTCCAACATCACACCAC	(AG)7	292	not tested
120	CG-cSSR-58	AGCTGGTCCGTGCTACAA	CCAGCCTCTCCACAGTTG	(GGC)3(GGT)(GGC)2	168	Polymorphic

121	CG-cSSR-59	TCATTTCAGGCGAGATGC	GCCTCCATTTGTTCACCA	(GCA)5	176	Polymorphic
122		AGCTCAGTAAGCTCGCCA	TTCAGTTCTGTTTATGAACTGTCA	(GAA)5	243	not tested
123		AAAAGGCGGACTGAAAGC	TTTTGAACAGCTCTGAACCA	(TA)3(TT)(TA)4	191	not tested
124		CCCCAGACATCTCGCATA	CAGAAATTAACAGCCACTGAAA	(TA)2(TT)(TA)4	258	not tested
125	CG-cSSR-27	TTGAGATTGTTCCGGCTG	CAAATCTGCAGCCTCTTGA	(TA)7	191	Polymorphic
126	CG-cSSR-60	GACGAATTCGACTCTGCG	AATGATGCTCCCGACCTT	(AGG)8	237	Polymorphic
127	CG-cSSR-28	AGGTGGAGGAAGCTTTGC	CAGATGCATGTACCCGCT	(GTG)4	226	monomorphic
128	CG-cSSR-61	TCCATTGGCTGGTGCTAC	TTCCACCATCAAGGCATC	(CTG)5	269	Polymorphic
129		GCTAGCCCAGCTCTCCTC	GTGTGCGCTTCCATGATT	(CT)2(TC)(CT)4	179	not tested
130		CCTCTGTGCCTTCTGGTG	CGAACAAACTGAAGCCCA	(GCT)3(CCT)(GCT)2	156	not tested
131		GACATCTCTTCGCGATCC	AAATGTCATAGTGGGAGCCA	(TTG)4	161	not tested
132		TTGGCCGGTTAACACATC	GACATCACATTAGCGTATTCCA	(TA)3,(TA)2,(TA)3	240	not tested
133		CTGACCAATTCAAGGCCA	CGTAGGCCTCCTGAGCTT	(GAG)3(AT)(GAG)2	267	not tested
134		CACCCCTTGAAACTGCTTT	GCCAAATGGTAAGTAATGAGGA	(TC)4(CC)(TC)2	202	not tested
135	CG-cSSR-29	TCTCGACATCCAACACCTG	TCCCGAGGTGTGAAGAAA	(AGCC)4	294	Polymorphic
136	CG-cSSR-30	GCTATGGCAGTGGCTACG	TCAACAGTAAGCGACGCA	(CGG)4	224	Polymorphic
137		CTCTCCATGCTCTGCCAC	CCAGTGCTGCTTCCAGAC	(CTT)4	150	not tested
138	CG-cSSR-31	CTTCTCCACCACCACTGC	GACGACCACAAGGCAGAA	(CTT)5	279	Polymorphic
139		TACGGTTTGTTGGCCGTA	CAGCCAGACCTCGGTACA	(GTCT)3	248	not tested
140	CG-cSSR-32	ACGGGTTGTTTGGTTTCC	AACTTCTGATCGGTACTCGG	(TC)10	181	Polymorphic
141		GGTGATTATGGATGCCCA	CTCCGGCCTTGTCCTT	(GGC)3,(GGC)3	173	not tested
142	CG-cSSR-62	AGCAAGCAGAATACAAACCA	TCATCTTGTACCGATTGAGTTCT	(TA)7	239	Polymorphic
143	CG-cSSR-33	ACTCACTCCCAAGGGCAT	AACCACCATGACCACCAA	(GCC)2(GCT)(GCC)4	154	no amplify
144	CG-cSSR-63	AGTGGTTACGGCGACCTC	GATCCGCTCTTCTCTGCTT	(AG)7	184	Polymorphic
145		CCCACAAAGGTGGTGAGA	GTTCACTGCCGTTCGGT	(ATTA)3(T)(ATTA)	266	not tested
146		AATGCGCTGATTGAAACG	TGCGAAGGGAACTCATGT	(CA)3(AA)(CA)2	214	not tested
147		CCATGCATCGAACAGTGA	TGGTGTTCCTTGGTTTGC	(TCA)5	224	not tested
148	CG-cSSR-34	GCTGGGCAAGTTGTCTGT	CTGCAACATCCCCATCAT	(CAT)3,(CAT)2	152	no amplify
149	CG-cSSR-35	GAAGGAGAAGAAGGCGGA	CGGCTTCTTGTTGTAGCG	(AAG)5	286	Polymorphic
150	CG-cSSR-64	GCCTCTTGGAGGCTTGTT	TGGCGAAGAGAATGAGGA	(AGA)5	270	Polymorphic

DOI: 10.2225/vol15-issue2-fulltext-4

151		TGGCAGAAGGAACCAGAG	TCCCTTAAATCTGGGCAT	(CA)2(TA)(CA)4	263	not tested
152		CAGCTTCTAAGCCAGCCA	TAATCGCCCTTCGTGCTA	(GAA)4	213	not tested
153		CGCTACTATCGGCCTCCT	TCCGGTAGCAAGAAAGCA	(TCC)(TC)(TCC)3	177	not tested
154	CG-cSSR-36	CATCTGCCTTTTCGCATC	AATAGCAAGTGCAGCGGA	(CAG)6	288	no amplify
155		TTAAAAATGCTGCCGGTG	CAGACATCGCCCTTTTTG	(GAA)4	282	not tested
156		TGGAAGGACATGGCAAAG	TCGCCAGCTCCATAAGAA	(GAA)3(GGA)(GAA)2	227	not tested
157		TGCGCTCTCAAGGACATT	ACAAAGCCCGATTGGTTT	(TA)5	173	not tested
158	CG-cSSR-37	TTCCCCGGTCTATCATCC	CCTCCACATATGAGCCGA	(TC)6	242	no amplify
159		TGAACGTTTGTGTCTATATGGC	AAGTTGGCTGATTCATTTATATC	(ATA)4	203	not tested
160		TTGGTGGAGTCATCTCCG	ATCGCGATGATGATGAGG	(TC)5	243	not tested
161	CG-cSSR-38	GATCAGCGGGCGAGA	AAGGCCACCCTTTGTTGT	(GA)8	248	Polymorphic
162	CG-cSSR-65	CCATTTGGCCACAGTCAC	TAGGAGCTGCAAGGCAAA	(TAA)5	195	Polymorphic
163	CG-cSSR-39	AACACAGCTCAGGCTCCA	TGTTTCCATTTCGCTGCT	(CAT)6	255	Polymorphic
164	CG-cSSR-40	AAGGGCCTTGCAGGTAGA	AACGCTAAAGAACATGCAA	(CTG)5	151	Polymorphic
165		TGATGAGAATAAATGCACGG	TTGTTTAATGCACGGTGATG	(CT)3(CC)(CT)4	261	not tested
166	CG-cSSR-66	TCCGAACCCAATTTTGAA	GAAGATTAGCATGGCCCC	(TA)4(AC)(TA)4	255	Polymorphic
167		AGCTTCCAGGTCGCTTTT	GGTTCAAATGTGGGAGCA	(GAA)3(GA)2(GAA)2	235	not tested
168	CG-cSSR-41	TGCTGTGTTATCTGGGGG	CACCAAAGCTTGGGAAAA	(CTG)5	269	no amplify
169	CG-cSSR-42	GCGCAGGTGTTCATTGTT	AATATGCGGTGAGCATGG	(AT)7	269	no amplify
170		GGCCACTGCTACCTCCTC	CTCCTTCACGGCTTCCTT	(CCTCTT)7	286	not tested
171	CG-cSSR-43	AACAGCCAAACCATAGGGA	CCATCTTCTGCAACCAGC	(TCA)6	231	Polymorphic
172	CG-cSSR-44	TGGATCAGATGATCAGAGAGG	TCACCACTCAGCATCCAA	(TCGAT)(CGA)(TCGAT)2	232	Polymorphic
173	CG-cSSR-67	AAAGCAACTGCGACCTGA	AAGTGGCAGGGAAAGGAG	(TC)11	200	Polymorphic
174		TGGAAACACCAGAGTGGG	AAGCAATAGAAACAGTCGAAGA	(TA)7	257	not tested
175	CG-cSSR-68	GCGTAAGGCAAAGCTTGA	GGGTTCTACAGGACGCTG	(AG)6	160	Polymorphic
176	CG-cSSR-45	TCTCCCTATCCTCCCTCG	GGCCAAACATTGGATTGA	(TGA)5	281	no amplify
177		GGTAGGAGCGGCTATGCT	CCTTTACAGGTCCGTGTCC	(TA)4(GA)(TA)2	227	not tested
178		CGCCTGTTGAAAGGAGTG	CCTGATCGATCTTGGCAC	(CTT)2(CAT)(CTT)4	204	not tested
179	CG-cSSR-46	GTTACGCATCGGTGTGCT	ATCTGTGAAATGGCTGCG	(AGC)3,(AGC)7	223	Polymorphic
180		GGCATCCAACAAATGAGG	CAGATGGCTTGGTATGAGG	(CAA)4	276	not tested
181	CG-cSSR-47	AGTCACCTGAATTGGCGA	TTGCTTTGAGGCTTCTCG	(GA)8,(GA)3	162	Polymorphic
182	CG-cSSR-69	CACGCGTTTCATAATGGG	CTTGGGACCTAAAGAAGAAACA	(GA)8	216	Polymorphic
183	CG-cSSR-48	TGGTTACTACCGCCCACA	AAGAAGCAAAGCTATGCCAA	(TA)6	183	Polymorphic
184		GATGGGATGAGGAGGAGG	ATGGCAGTCGACGAGGTA	(AAG)6	206	not tested
185	CG-cSSR-49	TCCCGATAAGGAGGATCG	TAAATCCGTCAGCCATGC	(AGG)4(TGG)(AGG)3	286	Polymorphic
186	CG-cSSR-50	TTCTTCGAAACCTTGGTCC	CGAAATAAAAGAGAGCTGCAC	(CT)12	286	no amplify
187	CG-cSSR-70	TGACCGGTAACAGCTCCA	TCATCAGTACGCTAATGCTTTT	(AT)7	200	Polymorphic

Table 4. Characteristics of the SSR sequences identified from genomic DNA library and cDNA library of *C. goeringii*.

Repeat unit	Repeat class	Genomic DNA library			cDNA library		
		Repeated motifs	(%)	Designed SSRs	Repeated motifs	(%)	Designed SSRs
Di	CT/AG/TC/GA	232	69.46		91	61.49	
	TG/CA/GT/AC	92	27.54		7	4.73	
	TA/AT	10	2.99		50	33.78	
	GC/CG	0			0		
	Total	334	82.88		148	47.44	67
Tri	ATT/AAT/TAT/ATA/TTA/TAA				18	12.24	
	CTT/AAG/TCT/AGA/TTC/GAA	13	19.41		41	27.89	
	GTT/AAC/TGT/ACA/TTG/CAA	38	56.72		5	3.40	
	CGG/CCG/GCG/CGC/GGC/GCC	1	1.49		19	12.92	
	TGG/CCA/GTG/CAC/GGT/ACC				9	6.12	
	AGG/CCT /GAG/CTC/GGA/ TCC	5	7.47		13	8.84	
	TCA/TGA/CAT/ATG/ATC/GAT				18	12.24	
	TGC/GCA/GCT/AGC/CTG/CAG	10	14.93		22	14.96	
	CGT/ACG/GTC/GAC/TCG/CGA				2	1.36	
	Total	67	16.63		147	47.12	108
Tetra	TATG/ATGT/TGTA/GTAT	1	50		2	16.67	
	TCGA/CGAT/GATC/ATCG				1	8.33	
	TTAA/TAAT/AATT/ATTA				1	8.33	
	TCTG/CTGT/TGTC/GTCT				1	8.33	
	AGCC/GCCA/CCAG/CAGC				1	8.33	
	TTTC/TTCT/TCTT/CTTT				1	8.33	
	ATTT/TTTA/TTAT/TATT	1	50		2	16.67	
	GTTT/TTTG/TTGT/TGTT				1	8.33	
	GAAA/AAAG/AAGA/AGAA				1	8.33	
	TAGA/AGAT/GATA/ATAG				1	8.33	
	Total	2	0.50		12	3.85	8
Others					5	1.60	4
	Total	403		206 (51.11%)	312		187 (59.93%)

DOI: 10.2225/vol15-issue2-fulltext-4

Table 5. Amplification and ploymorphism of 70 tested simple sequence repeat markers according to its repeated types using 24 *Cymbidium* accessions.

Repeats	Di			Tri			Others		
	Tested	Amplified	Poly	Tested	Amplified	Poly	Tested	Amplified	Poly
3	2	2	2	3	2	2	3	3	2
4	1	1	1	9	6	4	1	1	1
5	1	1	1	17	14	14	1	1	1
6	6	4	4	5	4	4			
7	6	5	5	2	1	1			
8	3	3	3	3	3	2			
9	2	2	2	0	0	0			
10	1	1	1	1	0	0			
11	2	2	2						
12	1	0	0						
Total	**25**	**21(84%)**	**21(84%)**	**40**	**30(75%)**	**27(67.5%)**	**5**	**5(100%)**	**4(80%)**
Grand total							**70**	**56(80%)**	**52(74.3%)**

DOI: 10.2225/vol15-issue2-fulltext-4

Table 6. Characterization of 14 simple sequence repeat markers identified as polymorphic markers using 24 cymbidium accessions.

Marker	Size Range	Major Allele Frequency	Allele No	Gene Diversity	Heterozygosity	PIC
KNU-CC-01	157-181	0.46	6	0.688	0.000	0.641
KNU-CC-25	166-266	0.55	6	0.620	0.000	0.569
KNU-CC-30	116-284	0.79	4	0.354	0.000	0.330
KNU-CC-32	202-238	0.17	15	0.896	0.913	0.887
KNU-CC-34	169-243	0.73	6	0.447	0.545	0.422
KNU-CC-35	173-225	0.27	9	0.808	0.455	0.781
KNU-CC-40	218-250	0.39	7	0.728	1.000	0.684
KNU-CC-42	207-209	0.72	2	0.405	0.478	0.323
KNU-CC-43	181-281	0.50	9	0.709	0.636	0.687
KNU-CC-52	236-260	0.79	7	0.363	0.375	0.350
KNU-CC-55	105-243	0.23	8	0.833	0.792	0.812
KNU-CC-71	105-243	0.60	8	0.597	0.500	0.569
KNU-CC-76	107-237	0.54	8	0.636	0.125	0.594
KNU-CC-203	138-380	0.59	7	0.616	0.000	0.590
Total			**102**			
Mean		**0.52**	**7.3**	**0.621**	**0.416**	**0.589**

DOI: 10.2225/vol15-issue2-fulltext-4

Table 7. Characterization of 52 simple sequence repeat markers identified as polymorphic markers using 24 *Cymbidium* accessions.

Marker	Size Range	Major Allele Frequency	Allele No	Gene Diversity	Heterozygosity	PIC
CG-cSSR-1	122-379	0.92	4	0.157	0.083	0.153
CG-cSSR-4	83-216	0.92	2	0.153	0.167	0.141
CG-cSSR-5	83-215	0.56	2	0.492	0.875	0.371
CG-cSSR-8	171-336	0.48	5	0.635	0.708	0.569
CG-cSSR-9	112-292	0.50	11	0.694	0.958	0.665
CG-cSSR-10	83-216	0.88	4	0.228	0.250	0.219
CG-cSSR-11	112-236	0.69	2	0.430	0.292	0.337
CG-cSSR-15	83-282	0.88	4	0.228	0.250	0.219
CG-cSSR-16	136-302	0.42	4	0.647	0.792	0.577
CG-cSSR-17	171-336	0.56	3	0.570	0.625	0.496
CG-cSSR-19	235-305	0.60	7	0.606	0.583	0.584
CG-cSSR-20	83-215	0.94	2	0.117	0.125	0.110
CG-cSSR-22	136-302	0.48	6	0.658	0.625	0.603
CG-cSSR-23	171-336	0.60	6	0.593	0.500	0.561
CG-cSSR-24	83-232	0.77	3	0.374	0.458	0.336
CG-cSSR-25	136-302	0.58	7	0.602	0.542	0.562
CG-cSSR-26	171-336	0.56	8	0.638	0.542	0.608
CG-cSSR-27	72-206	0.25	10	0.842	0.500	0.824
CG-cSSR-29	112-294	0.48	5	0.559	0.250	0.461
CG-cSSR-30	83-239	0.69	3	0.473	0.625	0.421
CG-cSSR-31	83-216	0.60	2	0.478	0.792	0.364
CG-cSSR-32	83-194	0.79	2	0.330	0.000	0.275
CG-cSSR-35	136-298	0.79	4	0.350	0.375	0.320
CG-cSSR-38	136-302	0.56	2	0.492	0.875	0.371

DOI: 10.2225/vol15-issue2-fulltext-4

CG-cSSR-39	171-273	0.69	8	0.505	0.333	0.484
CG-cSSR-40	136-302	0.50	5	0.603	0.792	0.528
CG-cSSR-43	171-250	0.67	4	0.490	0.500	0.431
CG-cSSR-44	136-302	0.52	3	0.536	0.875	0.430
CG-cSSR-46	171-336	0.56	4	0.609	0.625	0.558
CG-cSSR-47	136-302	0.60	4	0.533	0.625	0.458
CG-cSSR-48	171-336	0.65	7	0.548	0.458	0.518
CG-cSSR-49	171-336	0.58	5	0.569	0.625	0.507
CG-cSSR-51	230-240	0.75	3	0.404	0.250	0.367
CG-cSSR-52	83-283	0.53	3	0.604	0.563	0.533
CG-cSSR-53	236-346	0.48	7	0.677	0.870	0.630
CG-cSSR-54	136-302	0.38	7	0.723	0.875	0.676
CG-cSSR-55	171-336	0.28	6	0.773	0.750	0.736
CG-cSSR-56	83-254	0.50	5	0.664	0.783	0.616
CG-cSSR-57	236-449	0.63	4	0.497	0.708	0.410
CG-cSSR-58	179-302	0.37	6	0.767	1.000	0.734
CG-cSSR-59	227-285	0.83	6	0.313	0.200	0.303
CG-cSSR-60	236-351	0.74	6	0.437	0.522	0.418
CG-cSSR-61	83-165	0.93	3	0.141	0.150	0.136
CG-cSSR-62	83-216	0.48	5	0.664	1.000	0.610
CG-cSSR-63	136-302	0.37	4	0.692	0.957	0.635
CG-cSSR-64	88-336	0.33	10	0.809	0.870	0.787
CG-cSSR-65	209-224	0.43	7	0.684	0.550	0.631
CG-cSSR-66	289-303	0.48	5	0.653	0.913	0.594
CG-cSSR-67	83-229	0.29	13	0.838	1.000	0.820
CG-cSSR-68	151-302	0.48	5	0.653	0.958	0.592
CG-cSSR-69	211-302	0.33	10	0.799	0.900	0.774
CG-cSSR-70	171-336	0.30	8	0.816	0.826	0.793
Total			**271**			
Mean		**0.58**	**5.2**	**0.545**	**0.601**	**0.497**

DOI: 10.2225/vol15-issue2-fulltext-4

FIGURES

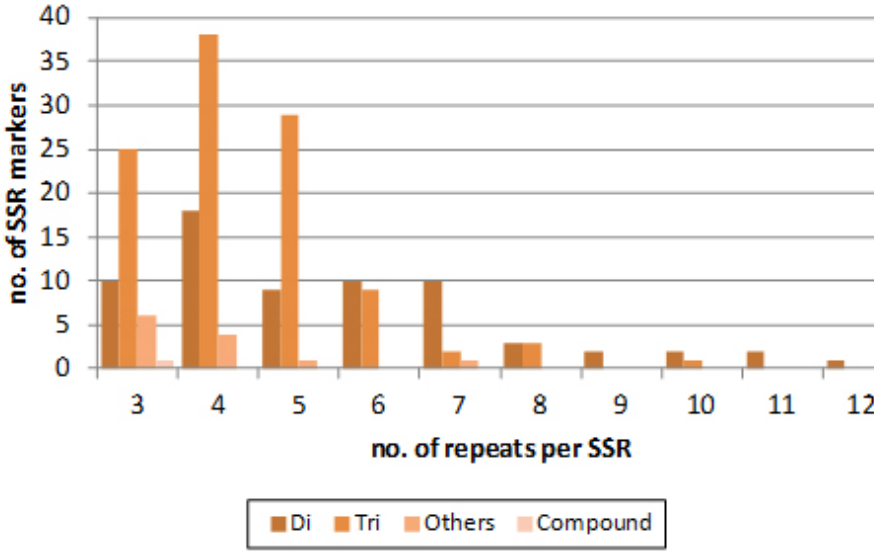

Fig 1. Distribution of EST-SSR marker sizes. Coloured bars show the number of markers from di-nucleotide, tri-nucleotide, and other (tetra-, penta- and hexa-nucleotide) categories with different numbers of repeats.

DOI: 10.2225/vol15-issue2-fulltext-4

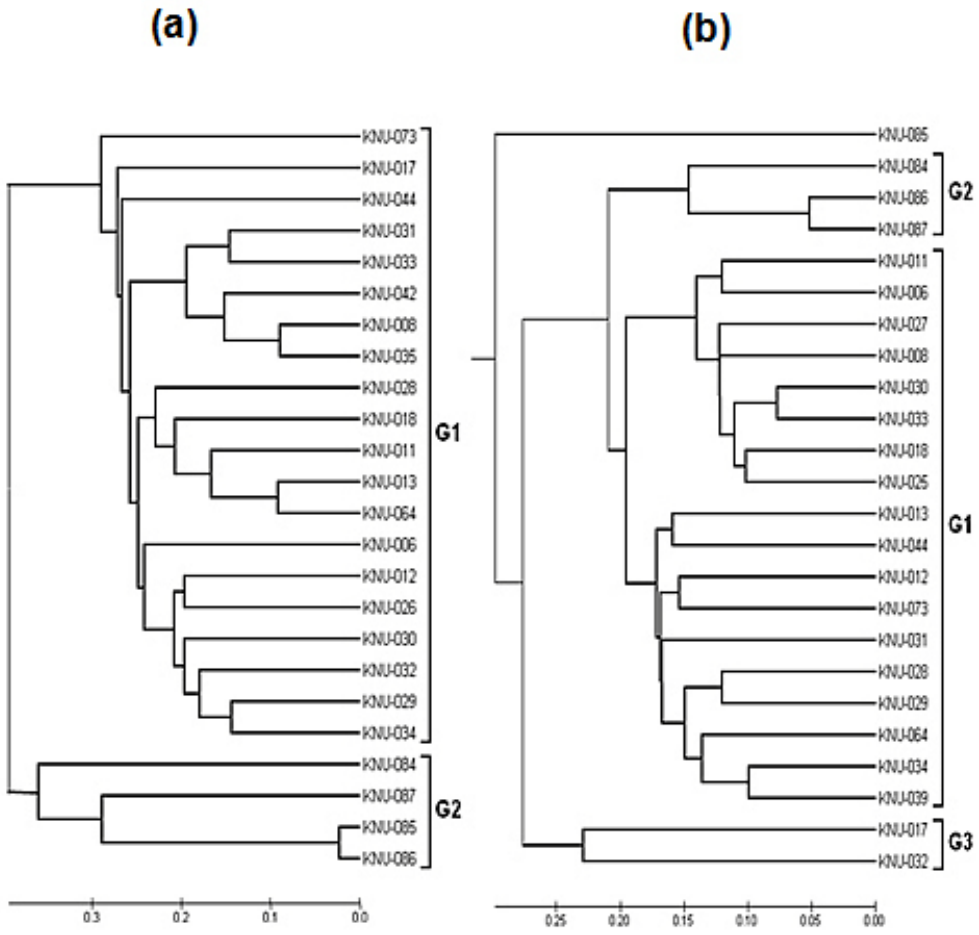

Fig 2. UPGMA dendrograms showing phylogenic relationships among the 24 *Cymbidium* accessions analyzed by (a) 14 genomic SSRs markers and (b) 52 EST-SSRs markers.