Botanical Sciences 99(1): 124-131. 2020
DOI: 10.17129/botsci.2619

Received: April 16, 2020, Accepted: August 10, 2020
On line first: October 27, 2020

Genetics / Genética

Development of SSR markers and their application to genetic diversity analysis of *Curcuma alismatifolia* varieties

Desarrollo de marcadores SSR y su aplicación al análisis de la diversidad genética de variedades de *Curcuma alismatifolia*

Lihui Mao[1], Jianxin Liu[1,2*], Huaqiao Ding[1], Qingcheng Zou[1], Danqing Tian[1]

[1]Zhejiang Institute of Landscape Plants and Flowers, Hangzhou, Zhejiang, P.R. China
[2]Yuanpei College, Shaoxing University, Shaoxing, Zhejiang, P.R. China

*Corresponding author email: liujianxin@usx.edu.cn

Abstract

Background: *Curcuma alismatifolia* is an ornamental cultivar with several varieties introduced into China from Thailand within the past fifteen years. *Curcuma alismatifolia* is widely used as cut flowers and potted flowers and is also used in flower beds.
Questions and/or Hypotheses: However, limited genetic and genomic information is available for this species, which has impeded studies on the enhancement of its ornamental value and stress resistance.
Studied species *Curcuma alismatifolia*
Study site and dates: Zhejiang, 2018
Methods: single-cell sequencing technology of PCR and polyacrylamide gel electrophoresis
Results: (A/T)n accounted for 43.6 % of the SSRs, the maximum proportion, and mononucleotide and trinucleotide repeats were the two most abundant repeat types. A total of 3,637 primer pairs flanking SSR sequences were successfully designed, and 70 sets of primers were randomly selected for validation in 10 varieties. Forty-one (59 %) of the 70 primer pairs successfully amplified alleles, of which 35 were identified as polymorphic markers and used to assess the level of genetic diversity and genetic relationships among the 10 varieties. The genetic diversity analysis showed that the number of alleles (Na) at each locus ranged from 2 to 8, with an average of 3.97, and that PIC had a mean of 0.524 and ranged from 0.095 to 0.795.
Conclusions: The genetic distance between 10 varieties varied from 0.30 to 0.96, and the dendrogram clustered all varieties into three groups.
Keywords: Full-length mRNA sequencing, ornamental plant, primer development, unigenes.

Resumen

Antecedentes: *Curcuma alismatifolia* es un cultivar ornamental con muchasvarias variedades introducidas en China desde Tailandia en los últimos quince años. *C. alismatifolia* se usa como flor cortada, flor en maceta o para hacer ramilletes de flores.
Preguntas y/o Hipótesis: Se dispone de información genética y genómica limitada para esta especie, lo que impide estudios sobre el aumento de su valor ornamental y la resistencia al estrés.
Especies estudiadas: *Curcuma alismatifolia*
Lugar de estudio y fechas: Zhejiang, 2018
Métodos: Tecnología de secuenciación unicelular de PCR y electroforesis en gel de poliacrilamida.
Resultados: (A/T)n representó el 43.6 % de los SSR, la proporción máxima, y las repeticiones de mononucleótidos y trinucleótidos fueron los dos tipos de repeticiones más abundantes. Se diseñaron con éxito un total de 3,637 pares de cebadores que flanquean las secuencias de SSR y se seleccionaron al azar 70 conjuntos de cebadores para su validación en 10 variedades. 41 (59 %) de los 70 pares de cebadores amplificaron con éxito los alelos, de los cuales 35 se identificaron como marcadores polimórficos y se utilizaron para evaluar el nivel de diversidad genética y las relaciones genéticas entre las 10 variedades. El análisis de diversidad genética mostró que el número de alelos (Na) en cada locus varió de 2 a 8, con un promedio de 3.97, y que el PIC tenía una media de 0.524 y osciló entre 0.095 y 0.795.
Conclusiones: La distancia genética entre las 10 variedades varió de 0.30 a 0.96 y en el dendrograma variedades forman tres grupos.
Palabras clave: Secuenciación de mRNA de longitud completa, desarrollo de primers, unigenes, planta ornamental.

Simple sequence repeat (SSR) markers are widely used for analyses of genetic diversity and relationships and molecular breeding due to their abundance in the genome, polymorphism, codominant inheritance and ease of detection by PCR (Kuleung *et al*. 2004, Li *et al*. 2018).

RNA sequencing is an effective way of obtaining a large amount of sequence data for SSR mining. The development of SSR markers for many plants based on transcriptome data resources has rapidly progressed (Hodel *et al*. 2016). In recent years, many SSR markers have been developed for plants of economic importance and for endangered species, such as elephant grass (López *et al*. 2018), *Torreya grandis* Fort. (Zeng *et al*. 2018), peanut (Bosamia *et al*. 2015), tobacco (Bindler *et al*. 2011), rubber tree (Li *et al*. 2012), and *Myracrodruon urundeuva* (Allemão) Engl. (Souza *et al*. 2018). Single-molecule long reads that capture the entire RNA molecule can provide insight into the transcriptome; thus, many studies aimed at SSR marker development and other objectives based on full-length mRNA sequencing have been carried out (Chen *et al*. 2018, Yi *et al*. 2018).

C. alismatifolia originated in Thailand and was introduced into China in recent years. During its introduction into China, it was cultivated in southern provinces such as Hainan, Guangdong and Guangxi, which have warmer winters than more northern provinces. It has been planted in the Yangtze River basin, where its bulbs are harvested and stored over the winter (Liu *et al*. 2017, Liu *et al*. 2013). This summer flowering plant has become an important urban garden flower in summer in Zhejiang, Jiangsu, Anhui and adjacent areas. Although it has become a popular ornamental plant, research on this plant at the molecular level remains lacking.

The genetic relationships among the ten most common *C. alismatifolia* varieties remain unclear and require elucidation to improve breeding programs. Previous studies on the genetic diversity of *C. alismatifolia* have been based mainly on dominant and universal markers, such as RAPD, ISSR and AFLP (Syamkumar & Sasikumar 2007, Das *et al*. 2011). However, SSRs, as codominant markers that are more stable than other marker types, are needed for research on molecular breeding and the genetic relationships of germplasm resources.

In this work, a large number of SSR primers were designed based on single-cell sequencing data. Seventy primer pairs were selected at random for testing, and 35 (50 %) displayed polymorphism within the 10 selected varieties. The genetic diversity and relationships of the 10 varieties were assessed based on the newly developed SSR markers. The present study provides a public resource and information that can aid future genetic studies and breeding programs in *Curcuma alismatifolia*.

Materials and methods

Plant materials. A mature plant of *C. alismatifolia* 'Chiang Mai pink' cultivated in the Flower Research and Development Center of Zhejiang Academy of Agricultural Sciences was selected for PacBio single-molecule long-read sequencing. Ten varieties (Figure 1), with one individual per variety, were selected to test the validity and polymorphism of 70 SSR primers. High quality DNA was extracted from leaves of each variety according to previous methods (Doyle & Doyle 1987) and stored in refrigerator at -20 °C for later use.

Full-length mRNA sequencing of C. alismatifolia. High-quality RNA of leaf, scape, fertile bract, sterile bract (ornamental bract) and flower was extracted and mixed in a proportion of 1:1:1:2:2 for cDNA library construction using the Clontech SMARTer cDNA synthesis kit (Takara, Japan). We performed size selection using the BluePippin Size Selection System protocol and produced three libraries corresponding to fragments of 1-2, 2-3 and 3-6 kb in length. The three libraries were sequenced on three cells with the PacBio Sequel system (PacBio, CA, USA). Long reads produced by the PacBio sequencer were processed with the PacBio IsoSeq pipeline (github.com/PacificBiosciences/IsoSeq_SA3nUP) to generate full-length refined consensus transcripts. The reads were filtered using standard protocols with the SMRT Analysis software suite (www.pacb.com/support/software-downloads/) (Yi *et al*. 2018).

SSR mining and character analysis. The software MISA (a microsatellite identification tool) was used to search the SSRs in all of the unigenes. For mononucleotide repeats, nucleotide sequences with fewer than ten repeats were excluded. For di-, tri-, tetra-, penta- and hexanucleotides, a minimum of six repeats was adopted as a filtering criterion. For compound microsatellites, a cutoff value of 100 bp was chosen as the maximum length of bases interrupting two SSRs (Bosamia *et al*. 2015).

SSR primer development and SSR-PCR amplification. Unigenes containing SSRs were used to design primers from sequences flanking SSR loci with Primer 3.0. All SSR loci except mononucleotide repeats were used for primer design. The criteria used for primer design were as follows: primer length, 20-25 bp; temperature, 50-60 °C; GC content, 40-60 % and product size range, 95-295 bp.

Seventy randomly selected SSR primer pairs were used for validation testing in the 10 *C. alismatifolia* varieties (Supplemental material S1). Each 20 µl SSR-PCR reaction mixture consisted of 1 µl of Taq DNA polymerase with 1 × reaction buffer, 0.4 µl dNTPs, 0.3 µmol/L each of forward and reverse primer, and 50 ng DNA template. PCR

Figure 1. Photos of ten varieties of *C. alismatifolia* used in the study. Code, variety name, Colour and shape of ornamental bracts: (A)C1, Splash, rose red and glossy, ovate; (B)C2, Sunset, dark rose red, shortly acuminate; (C)C3, KimonoRose, dark rose red, oval; (D)C4, Scarlet, rose red, ovate; (E)C5, Chiang Mai pink, pink, ovate; (F)C6, Swift, pink, oval; (G)C7, Emerald ChocoZebra, green, ovate; (H)C8, Snowwhite, white, shortly acuminate; (I)C9, Sunrise, light pink, shortly acuminate; (J)C10, Purple fairy, light purple, oval

amplification was performed with an initial denaturation at 94 °C for 5 min; followed by 10 cycles of 94 °C for 30 s, 60 to 50 °C for 30 s and 72 °C for 40 s, with a 1 °C decrement in annealing temperature per cycle; 25 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 40 s; and a final extension at 72 °C for 3 min. Electrophoresis was performed at 2,000 V for 1.5 h with a vertical polyacrylamide gel and stained with GoldenView (Gajjar *et al*. 2014). The gels were imaged with an automated gel documentation system (GelDoc XR+ Imager, USA) and scored for marker amplification. The alleles of the 10 varieties amplified by each primer pairs were named A, B, C, according to length from short to long.

Data analysis. The polymorphic primers were selected for further analysis. POPGENE software version 1.31 (Yeh *et al*. 1999) was used to calculate the number of alleles (Na), the number of effective alleles (Ne), the observed heterozygosity (Ho), and Shannon's information index (I).

The polymorphic information content (PIC) of the alleles was calculated by the formula PIC = 1-Σ(Pi)², where Pi is the frequency of the ith SSR allele. The genetic distances across the varieties were calculated using POPGENE software version 1.31 (Yeh *et al*. 1999). A cluster analysis of the 10 varieties based on Nei's unbiased measure of genetic distance was carried out using the unweighted pair-group method with arithmetic average (UPGMA), and a dendrogram was constructed by NTSYS-pc version 2.11V (Rohlf 2004).

Results

SSR mining and feature analysis. Out of the 64,471 unigenes subjected for SSR screening, 15,891 were found to contain SSRs. A total of 19,902 SSRs were identified among these 15,891 unigenes, with an average of one SSR per 2.06 kb; 3,155 unigenes contained more than one SSR (Table 1). The CT motif was the most common SSR motif in *C. alismatifolia*. The frequencies of di- to hexanucleotide SSRs were calculated, and the top 20 most frequent motifs, including 6 di- and 14 trinucleotides, are shown in Figure 2

Table 1. Feature of microsatellites identified by MISA in unigenes of *C. alismatifolia* transcriptome.

Feature	Values
Total number of sequences examined	64,471
Total size of examined sequences (Mb)	132.8
Total number of identified SSRs	19,902
Number of SSR containing sequences	15,891
Number of sequences containing more than one SSR	3,155
Number of SSRs present in compound formation	1,132

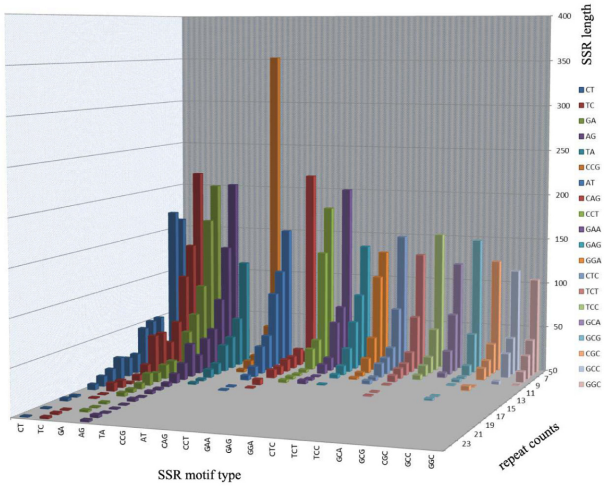

Figure 2. Frequency distribution of SSRs by motif and repeat length in *C. alismatifolia* 'Chiang Mai pink'.

Design of novel primer sets and validation. A total of 3,637 primer pairs were designed (Supplemental material S2), of which trinucleotides (54.88 %) showed the highest frequency, followed by di- (28.84 %), compound- (10.53 %), tetra- (3.38 %), hexa- (1.48 %) and pentanucleotides (0.88 %). The remaining SSRs contained sequences that failed to generate primer pairs, due either to the unavailability of flanking sites for primer design or due to nonconformance with the primer design parameters. Most *Curcuma* plants have medicinal and ornamental value; however, few SSR markers for this genus have been developed. As EST-SSR markers are usually transferable among distantly related species, these newly developed markers could be used with other *Curcuma* species for which little SSR and EST information is available.

Seventy primer pairs were selected at random to test their validity, of which 41 (59 %) were successfully used to amplify PCR products and 35 (50 %) displayed polymorphism within the 10 selected varieties.

Genetic diversity and relationships of C. alismatifolia varieties. The thirty-five SSR markers verified as polymorphic were then used to assess the genetic diversity and genetic relationships of the 10 core *C. alismatifolia* varieties used as garden flowers in China. A total of 139 alleles were detected in the 10 varieties, of which 49 were determined to be variety specific. The Na, Ne, Ho, PIC and *I* values for each SSR marker are listed in Table 2.

The genetic distance among the ten varieties ranged from 0.30 to 0.96 (Table S2). The largest genetic distance was observed between 'Scarlet' and 'Emerald ChocoZebra', and the smallest was observed between 'Splash' and 'Swift'. The white, green, light purple or light pink, and pink or

Table 2. The amplification characters of 35 valid primer pairs.

Primer Name	Sequence Name	Motif	Ho	No. of alleles	Length of product	Anneling temperature (℃)	Pic value
P2	c13778	(AGA)6	0.4	4	131	54	0.345
P5	c18662	(AGA)7	0.4	3	170	56	0.545
P11	c28839	(CTCTC)5	0.4	4	266	54	0.3475
P12	c9314	(GAGAT)5	0.9	5	245	52	0.635
P13	c3424	(TGAGC)5	0.5	3	295	56	0.485
P14	c27068	(CT)13	0.4	4	210	54	0.595
P16	c3374	(CAG)9	0.6	5	166	54	0.485
P17	c9346	(CTC)9	0.11	3	157	54	0.290
P18	c36716	(GA)14	0.2	5	158	54	0.64
P19	c19380	(GAAG)7	0.6	4	183	56	0.475
P21	c18940	(AGC)10	0.8	4	184	56	0.64
P22	c30792	(CAA)5(CTA)5	0.3	2	283	55	0.375
P23	c30453	(CGATGG)5	0.6	3	189	57	0.58
P25	c32105	(CGATGG)5	0.6	3	188	57	0.58
P26	c33727	(TAAA)5(AT)6	0.33	4	270	55	0.725
P27	c30007	(TG)7(T)17	0.3	4	259	54	0.615
P30	c5429	(GAA)11	0.8	8	183	54	0.665
P31	c1628	(TTC)11	0.2	4	187	53	0.475
P39	c33612	(GAA)11	0.8	6	164	54	0.655
P40	c74290	(GAG)6	0.4	4	195	57	0.345
P47	c35063	(AG)14	0.44	6	156	54	0.772
P48	c21222	(GAAG)7	0.6	4	184	54	0.685
P52	c17820	(CAG)10	0.5	4	278	54	0.625
P54	c27844	(GAAAGG)5	0.6	4	193	53	0.65
P55	c2465	(GAGAA)6	0.9	4	292	53	0.565
P58	c6985	(TC)17	0.1	4	126	52	0.345
P60	c28299	(TATC)9	0.89	5	157	54	0.728
P61	c19402	(CTGCTC)9	0.1	2	156	56	0.095
P62	c13037	(TCC)6	0.5	2	237	53	0.455
P63	c2023	(CCA)6	0.7	4	144	57	0.705
P64	c43017	(TCT)9	0.2	3	141	52	0.445
P66	c2969	(GCA)5(ACA)9	0.4	6	281	52	0.795
P67	c32672	(TC)25	0.1	4	141	52	0.475
P69	c5196	(ACG)5(ACC)5act(ACC)6	0.1	2	192	57	0.095
P70	c84245	(TCC)8tt(CTC)9	0.5	3	286	53	0.405

rose-red flower varieties can be distinguished by P23 and P25, indicating that these two markers might be related to flower color. The UPGMA clustered the 10 varieties into three groups; 'Emerald ChocoZebra' formed one group, 'Scarlet' and 'Chiang Mai pink' formed another group, and the remaining 7 varieties formed the third group (Figure 3).

Discussion

The number of alleles at each SSR locus of *C. alismatifolia* was found to range the number of alleles at each SSR locus of *C. alismatifolia* was found to range from 1 to 12, with an average of 3.77. Research on peanut (Bosamia *et al*. 2015) yielded similar results regarding allele number as our study on *C. alismatifolia*. The CT motif was the most common SSR motif in *C. alismatifolia*, as also observed in *Hevea brasiliensis* (Li *et al*. 2012) and *Corchorus capsularis* (Saha *et al*. 2017). In contrast, the AT motif was found to be most common for *Cryptomeria japonica* (Ueno *et al*. 2012), and AAG was most common for *Arachis hypogaea* (Bosamia *et al*. 2015).

The high PIC values (0.095 to 0.795) indicated that most loci were highly polymorphic and informative. Flower color is one of the most important characters for ornamental plant breeding. The colors of the 10 varieties in this study can be classified into four categories: pink to rose red (1-6), green (7), pure white (8), and light pink or light purple (9, 10). The two molecular markers P23 and P25 can distinguish the 10 varieties by color and thus will be beneficial for molecular marker-assisted breeding related to flower color. Molecular markers based molecular linkage maps have been constructed in many ornamental plants, such as *Anthurium andraeanum* (Venkat *et al*. 2014), rose (Spiller *et al*. 2011) and *Dendranthema morifolium* (Zhang *et al*. 2011). The large number of primers we designed could be used to construct a molecular linkage map.

The identification of polymorphic EST-SSR markers can not only enhance our understanding of SSRs in the *Curcuma alismatifolia* transcriptome but also provide resources for genetic and genomic studies aimed at improving this ornamental flower.

There are more than fifty species in the *Curcuma* genus worldwide. Many members of the genus, such as *Curcuma longa*, *C. viridiflora* and *C. zedoaria*, have medicinal value due to their high contents of curcumin, which has blood-lipid lowering, antitumor, anti-inflammatory and antioxidant effects (Yang *et al*. 2020). The many SSR molecular markers detected in this work, included 35 identified as polymorphic, are potentially transferable to other *Curcuma* species.

Acknowledgments

The authors gratefully acknowledge the financial supports by the youth talent project of Zhejiang academy of agricultural sciences (number: 10102000319CC2301G/005/028), KeyResearch and development plan of Zhejiang Province (2019c02025), as well as the Science and technology plan guidance project of Hangzhou (number: 20171226Y101).

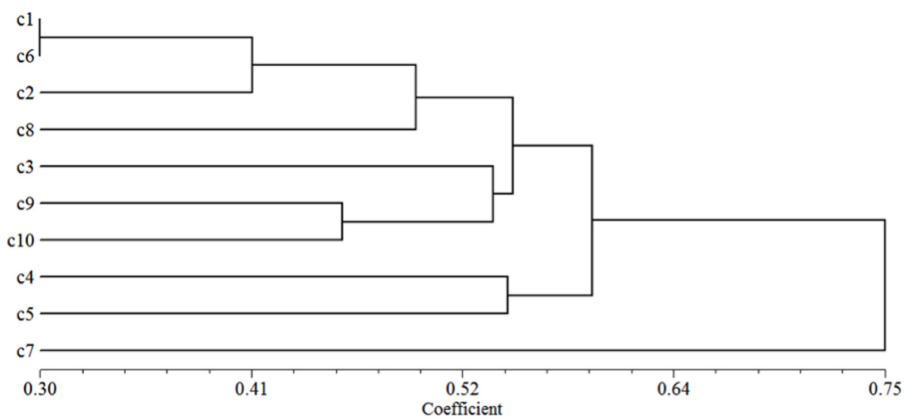

Figure 3. UPGMA cluster of 10 varieties based on SSR.

Literature Cited

Bindler G, Plieske J, Bakaher N, Gunduz I, Ivanov N, Van der Hoeven R, Ganal M, Donini P. 2011. A high density map of tobacco (*Nicotiana tabacum* L.) obtained from large scale microsatellite marker development. *Theoretical and Applied Genetics* **123**: 219-230. DOI: https://doi.org/10.1007/s00122-011-1578-8

Bosamia TC, Mishra GP, Thankappan R, Dobaria JR. 2015. Novel and stresss relevant EST derived SSR markers developed and validated in peanut. *Plos One* **10**: e0129127. DOI: https://doi.org/10.1371/journal.pone.0129127

Chen J, Tang XH, Ren CX, Wei B, Wu YY, Wu QH, Pei J. 2018. Full-length transcriptome sequences and the identification of putative genes for flavonoid biosynthesis in safflower. *BMC Genomics* **19**: 548. DOI: https://doi.org/10.1186/s12864-018-4946-9

Das A, Kesari V, Satyanarayana VM, Parida A, Rangan L. 2011. Genetic relationship of *Curcuma* species from northeast India using pcr-based markers. *Molecular Biotechnology* **49**: 65-76. DOI: https://doi.org/10.1007/s12033-011-9379-5

Doyle JJ, Doyle JL. 1987. A rapid isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11-15.

Gajjar KN, Mishra GP, Radhakrishnan T, Dodia SM, Kumar A. 2014. Validation of ssr markers linked to the rust and late leaf spot diseases resistance in diverse peanut genotypes. *Australian Journal of Crop Science*, **8**: 927.

Hodel RGJ, Gitzendanner MA, Germain-Aubrey CC, Liu X, Crowl AA, Sun M, Landis JB, Segovia-Salcedo MC, Douglas NA, Chen SC, Soltis DE, Soltis PS. 2016. A new resource for the development of SSR markers: millions of loci from a thousand plant transcriptomes1. *Applications in Plant Sciences* **4**: 1600024. DOI: https://doi.org/10.3732/apps.1600024

Kuleung C, Baenziger PS, Dweikat I. 2004. Transferability of SSR markers among wheat, rye, and triticale. *Theoretical and Applied Genetics* **108**: 1147-1150. DOI: https://doi.org/10.1007/s00122-003-1532-5

Li DJ, Deng Z, Qin B, Liu XH, Men ZH. 2012. De novo assembly and characterization of bark transcriptome using Illumina sequencing and development of EST-SSR markers in ruber tree (*Hevea brasiliensis* Muell. Arg.). *BMC Genomics* **13**: 192. DOI: https://doi.org/10.1186/1471-2164-13-192

Li JJ, Guo HL, Wang Y, Zong JQ, Chen JB, Li DD, Li L, Wang JJ, Liu JX, Chiang TY. 2018. High-throughput SSR marker development and its application in a centipedegrass (Eremochloa ophiuroides (Munro) Hack.) genetic diversity analysis. *Plos One* **13**: e0202605. DOI: https://doi.org/10.1371/journal.pone.0202605

Liu JX, Ding HQ, Zou QC, Wang WY, Cai LY. 2013. Study on dwarfing effect and mechanism of paclobutrazol on *Curcuma alismatifolia*. *Northern Horticulture* **20**: 79-82.

Liu JX, Xu XH, Ding HQ. 2017. Physiological and biochemical characteristics of *Curcuma alismatifolia* bulbs and effects of some chemicals on its cold resistance, *Acta Agriculturae Zhejiangensis* **29**: 575-582. DOI: https://doi.org/10.3969/j.issn.1004-1524.2017.04.09

López Y, Kurashev A, Chase C, Gallo M, Sollenberger L, Aptperter F, Wang JP. 2018. Development and validating microsatellite markers in elephant grass (*Pennisetum purpureum* S.). *Euphytica* **214**: 185. DOI: https://doi.org/10.1007/s10681-018-2256-6

Rohlf JF. 2004. NTSYS-pc:2.11 Numerical Taxonomy and Multivariate Analysis System. New York: Exeter Software.

Saha D, Rana RS, Chakraborty S, Datta S, Kumar AA, Chakraborty AA, Karmakar PG. 2017. Development of a set of SSR markers for genetic polymorphism detection and interspecific hybrid jute breeding. *The Crop Journals* **5**: 416-429. DOI: https://doi.org/10.1016/j.cj.2017.02.006

Souza DCL, Rossini BC, de Souza FB, Sebbenn AM, Marino CL, de Moraes MLT. 2018. Development of microsatellite markers for *Myracrodruon urundeuva* (F.F&Allemã), a highly endangered species from tropical forest based on next-generation sequencing. *Molecular Biology Reports* **45**: 71-75. DOI: https://doi.org/10.1007/s11033-017-4142-z

Spiller M, Linde M, Oyant HS, Tsai CJ, Byrne DH, Smulders MJM, Foucher F, Debener T. 2011. Towards a unified genetic map for diploid roses. *Theoretical and Applied Genetics* **122**: 489-500. DOI: https://doi.org/10.1007/s00122-010-1463-x

Syamkumar S, Sasikumar B. 2007. Molecular marker based genetic diversity analysis of Curcuma species from India. *Scientia Horticulturae* **112**: 235-241. DOI: https://doi.org/10.1016/j.scienta.2006.12.021

Ueno S, Moriguchi Y, Uchiyama K, Ujino-ihara T, Futamura N, Sakurai T, Shinohara K, Tsumura Y. 2012. A second generation framework for the analysis of microsatellites in expressed sequence tags and the development of EST-SSR markers for a conifer, *Crypomeria japonica*. *BMC Genomics* **13** : 136. DOI: https://doi.org/10.1186/1471-2164-13-136

Venkat SK, Bommisetty P, Patil MS, Reddy L, Chennareddy A. 2014. The genetic linkage maps of anthurium species based on RAPD, ISSR and SRAP markers. *Scientia Horticulturae* **178**: 132-137. DOI: https://doi.org/10.1016/j.scienta.2014.08.017

Yang QQ, Cheng LZ, Zhang TZ, Yaron S, Jiang HX, Sui ZQ. 2020. Corke H. Phenolic profiles, antioxidant, and antiproliferative activities of turmeric (*Curcuma longa*). *Industrial Crops and Products* **152**: 112561. DOI:https://doi.org/10.1016/j.indcrop.2020.112561

Yeh FC, Yang R, Boyle T. 1999. POPGENE Version 1.31. The User-Friendly Shareware for Population Genetic Analysis. Edmonton, Canada: University of Alberta. DOI: https://sites.ualberta.ca/~fyeh/popgene.html

Yi SK, Zhou XY, Li J, Zhang MM Luo SS. 2018. Full-length transcriptome of *Misgurnus anguillicaudatus* provides insights into evolution of genus *Misgurnus*. *Scientific Reports* **8**: 11699. DOI: https://doi.org/10.1038/s41598-018-29991-6

Zeng J, Chen J, Kou Y, Wang YJ. 2018. Application of EST-SSR markers developed from the transcriptome of *Torreya grandis* (Taxaceae), a threatened nut-yielding conifer tree. *Peer J* **6**: e5606. DOI: https://doi.org/10.7717/peerj.5606

Zhang F, Chen SM, Chen FD, Fang WM, Chen Y, Li FT. 2011. SRAP-based maping and QTL detection for inflorescence-related traits in *Chrysanthemum* (*Dendranthema morifolium*). *Molecular Breeding* **27**: 11-23. DOI: https://doi.org/10.1007/s11032-010-9409-1

Associate editor: Alejandro Casas

Author contributions: LHM writing, data analysis, data collection; JXL, writing, data collection; HQD, data collection, manuscript revision; QCZ, writing, data collection; DQT, data analysis, data collection