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Isolation and Characterization of Polymorphic Microsatellite Markers in *Toxicodendron vernicifluum*

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Abstract

Vu D.-D., Bui T.T.-X., Nguyen T.H.-N., Shah S.N.M., Vu N.-H., Zhu Y.-H., Zhang L., Zhang Y., Huang X.-H. (2018): Isolation and characterization of polymorphic microsatellite markers in *Toxicodendron vernicifluum*. Czech J. Genet. Plant Breed., 54: 17–25.

A total 20 074 230 sequencing reads were generated by Illumina HiSeq™ 2500 from three different *Toxicodendron vernicifluum* tissue samples. In total, 48 693 unigenes with an average length of 703.34 bp were obtained by *de novo* assembly. 3392 potential EST-SSRs (expressed sequence tag-simple sequence repeat) were identified as potential molecular markers from unigenes with lengths exceeding 1 kb. A total of 80 pairs of PCR primers were randomly selected to validate the assembly quality and develop EST-SSR markers from genomic DNA. Of these primer pairs, 14 primer pairs successfully amplified DNA fragments and detected significant amounts of polymorphism within the lacquer tree population in Langao, Shaanxi province, China. There was high genetic diversity (number of alleles per locus (*A*) = 2.93, polymorphic information content (*PIC*) = 0.53, observed heterozygosity (*Ho*) = 0.62 and expected heterozygosity (*He*) = 0.85) in the lacquer tree natural population. The four loci deviated significantly from the Hardy-Weinberg equilibrium. These results suggested high homozygosity in the population and low or missing heterozygosity (inbreeding coefficient (*Fis*) = 0.27). These polymorphic EST-SSR markers will provide the base for further studies of genetic structure and breeding in *T. vernicifluum*.

Keywords: EST-SSRs; Illumina HiSeq™ 2500; genetic diversity; lacquer tree

Toxicodendron vernicifluum (Stokes) F.A. Barkley (syn. *Rhus verniciflua*), commonly known as Chinese lacquer, is a deciduous tree of the family Anacardiaceae, which is native to Korea, Japan and China. It is an important plant species both economically and culturally. The tree is the main source of varnish used in handicraft industry (Hashida *et al.* 2014). It is also used as food additive, natural dye or in herbal medicine to improve the blood circulation and to prevent blood stasis, while the methanolic extract of the leaves has neuroprotective and anti-inflammatory activity (Cho *et al.* 2012). A *T. vernicifluum* extract has antiproliferative and apoptotic activities in various human cancer cell lines via activation of caspase-9 and inhibition of the PI3KAkt/PKB pathway, as well as antioxidant effects (Kim *et al.* 2015), suppresses

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the proliferative capability of B lymphoma cells (Lee *et al*. 2004). The genetic and structural diversity of the wild populations was rarely explored due to the lack of reliable and informative molecular markers of *T. vernicifluum*. Recently the genome of *Toxicodendron radicans* has been sequenced (Hsu *et al*. 2013). There are limited genomic resources available for the lacquer tree. Up to now, only 549 expressed sequence tags (ESTs) of *Toxicodendron* have been deposited in dbEST (http://www.ncbi.nlm.nih.gov/dbEST/) of GenBank (as of 30/9/2016), including 495 ESTs from *T. vernicifluum*, 42 from *T. radicans*, 12 from *Toxicodendron succedaneum*. Therefore, the EST-SSRs of *Toxicodendron* are not yet sufficient to explore the genetic diversity and for breeding.

Simple sequence repeat (SSR) markers are useful tools to study the diversity of allelic sequences as they are abundantly dispersed within the genome and have a high polymorphism level, co-dominant inheritance, high reproducibility, and good genome coverage (Li *et al.* 2004; Kalia *et al.* 2011; Vieira *et al.* 2016). The EST availability increased the possibility of SSR identification in some woody species (Kaur *et al.* 2012). As a functional molecular type of markers, EST-SSR markers have the potential for the functional diversity analysis of germplasm collection and may prove to be more useful for marker-assisted selection (Aggarwal *et al.* 2007). The Illumina sequencing technology has been proven to be an efficient option for the discovery of genome-wide EST-SSR markers (Wei *et al.* 2011; Liu *et al.* 2012, 2013).

In the present study, we have developed microsatellite markers for *T. vernicifluum*. On the basis of EST-SSR information, the EST resources were characterized. Then the genetic diversity of natural populations of *T. vernicifluum* was studied using genetic information derived from the microsatellites. The present study will help in future studies on the genetic diversity and population structure of *T. vernicifluum* and related species.

MATERIAL AND METHODS

Plant material. For Illumina sequencing, plant samples were collected from roots, leaves and stems (Figure 1A) of lacquer tree in a nursery, Northwest A&F University, Yangling, Shaanxi province, China. All sampled tissues were immediately frozen in liquid nitrogen and stored at –80°C till RNA extraction.

To explore the genetic variation in the *T. vernicifluum* population, mature leaves from 120 living individuals were randomly collected in Langao (32018'59''N, 109001'46''E, 946.3 m a.s.l.), Shaanxi province, P.R. China (Figure 1B). The samples were placed in labelled plastic bags containing a silica gel desiccant and then transported to the laboratory for further analysis. The samples were stored at –40°C till DNA extraction.

DNA/RNA extraction, cDNA sequencing and microsatellite loci identification. For Illumina sequencing, the total RNA was isolated from each sample using the OmniPlant RNA Kit (DNase I) (www.cwbiotech.com). Both the quantity and quality of RNA were checked through a Nanodrop ND-2000 spectrophotometer (Thermo Electron Corporation, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, USA). Equal amount of total RNA from each sample was pooled together and sent to Breeding Biotechnologies Co., Ltd., for cDNA library construction and transcriptome sequencing using Illumina HiSeq™ 2500. SSRs were detected in the Simple

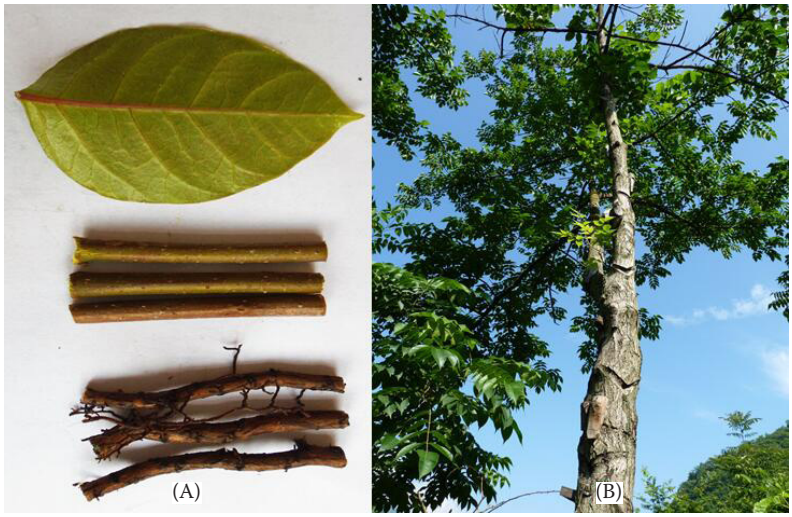

Figure 1. The tissues in nursery (A) and adult tree (B) of *Toxicodendron vernicifluum* in Langao, Shaanxi province, China

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Sequence Repeat Identification Tool program (SSRIT, http://www.gramene.org/db/markers/ssrtool). Only unigenes that were longer than 1 kb were included in the EST-SSR detection. The parameters were set for the detection of perfect mono-, di-, tri-, tetra-, penta- and hexa-nucleotide motifs with a minimum of 6, 5, 5, 5, and 5 repeats, respectively. Microsatellite loci identification showed simple sequence repeats and they were deposited in GenBank. The ETS-SSR primers were designed for using Primer 5.0 (Clarke & Gorley 2001).

For SSR validation, total genomic DNA was extracted using the plant DNA isolation kit (Norgenbiotek, Canada). Both the quantity and quality of the DNA were checked through a Nanodrop ND-2000 spectrophotometer (Thermo Electron Corporation, USA) and 1.2% agarose gel electrophoresis.

EST-SSR amplification. Ten individuals were used to evaluate the ability of 80 randomly selected primer pairs to produce monomorphic or polymorphic PCR products. PCR reactions were carried out in 25 µl vol. containing 1× PCR buffer, 2.5 µl $MgCl_2$, 2 µl dNTP, 0.1 µl of each primer, 1.25U Tag DNA polymerase and 1.5 µl of template DNA. The PCR cycling conditions were as follows: pre-denaturation at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, extension at 72°C for 10 min. The PCR products were separated by a Sequi-Gen® GT DNA electrophoresis system in 8% polyacrylamide gels in TAE buffer and Gelred™ 10000x stain. Banding patterns had been visualized under UV light, and then they were photographed with GenoCapture. The sizes of PCR products were determined and analysed by Gel Analysis software to the GenoSens1850 Gel Doc System (Clinx Science Instruments Co., Ltd., China). A 50 bp DNA ladder (Invitrogen, USA) was used as standard.

Table 1. Overview of *de novo* sequence assembly for *Toxicodendron vernicifluum* (the percentages are shown in brackets)

Length range (bp)	Unigenes	Contigs	Transcripts
200–300	18 891 (38.80)	2 005 195 (98.35)	22 699 (23.81)
300–500	11 115 (22.83)	13 185 (0.65)	16 801 (17.63)
500–1000	7 700 (15.81)	8 976 (0.44)	19 735 (20.70)
1000–2000	7 196 (14.78)	7 530 (0.37)	23 066 (24.20)
2000	3 791 (7.79)	3883 (0.19)	13 015 (13.65)
Total number	48 693	2 038 769	95 316
Total length	35 562 406	132 942 209	97 422 706
N50 length	1 347	63	1 659
Mean length	730.34	65.21	1 022.10

Data analysis. The parameters of genetic diversity such as number of alleles per locus (*A*), observed heterozygosity (*Ho*) and expected heterozygosity (*He*) were calculated by the GenAlex 6.5 programs (Peakall & Smouse 2006). Hardy-Weinberg equilibrium (HWE) was estimated by Arlequin 3.1 (Excoffier *et al.* 2005), FSTAT version 2.9.3 (Goudet 1995) was used to calculate the inbreeding coefficient (*Fis*). The polymorphic information content (*PIC*) value of each locus was estimated based on the following formula:

$$PIC_i = 1 - \Sigma P_{ij}^2$$

where:

P_i frequency of the i^{th} allele in the genotypes

RESULTS AND DISCUSSION

Annotation and functional characterization of lacquer tree unigenes. The clean reads were assembled by Trinity program (Table 1) and 95 316 transcripts were generated with an average length of 1022.1 bp and an N50 length of 1659 bp. A total of 48 693 unigenes was obtained with a mean length of 730.34 bp and an N50 value of 1347 bp. The results show high coverage of transcriptome in the species. The overall functional annotations are shown in Table 2. A total of 28 722 (58.96%) unigenes of Chinese lacquer were identified. Our result is in line

Table 2. Functional annotation of the *Toxicodendron vernicifluum* transcriptome

Annotated database	Annotated No.	300–1000 (bp)	≥ 1000 (bp)
COG_Annotation	8 820	2 691	4 474
GO_Annotation	18 057	6 398	7 242
KEGG_Annotation	6 343	2 146	2 750
KOG_Annotation	16 604	5 895	6 955
Pfam_Annotation	19 104	6 527	9 401
Swissprot_Annotation	17 340	63 99	7 265
Nr_Annotation	28 722	10 898	1 0753
All_Annotated	28 877	10 946	10 754

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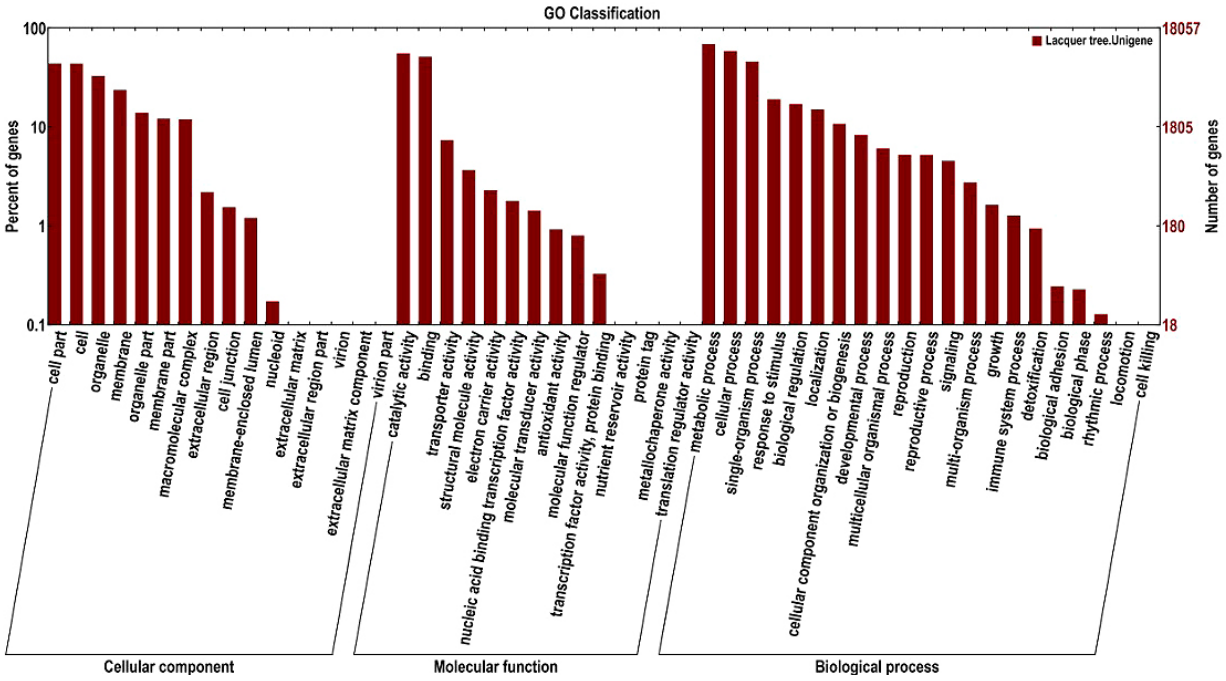

Figure 2. Gene ontology (GO) classification of assembled unigenes in *Toxicodendron vernicifluum*

with previous studies in which sequences deposited in non-redundant protein (Nr) database were homologous 67.5% in bamboo (Liu *et al*. 2012), 71.8% in alfalfa (Liu *et al*. 2013) and 53.91% in sesame (Wei *et al*. 2011)

Based on 28 722 non-redundant protein (Nr) annotations, 18 057 (37.08%) unigenes were assigned to Gene Ontology (GO). The GO-annotated unigenes belonged to the biological process, cellular component, and molecular function clusters and were distributed across 51 categories with the dominant group of 46.53% in biological processes, followed by cellular components of 32% and 21.47% in molecular functions (Figure 2). These results also confirm previous studies in alfalfa (Liu *et al*. 2013) and sesame (Wei *et al*. 2011)

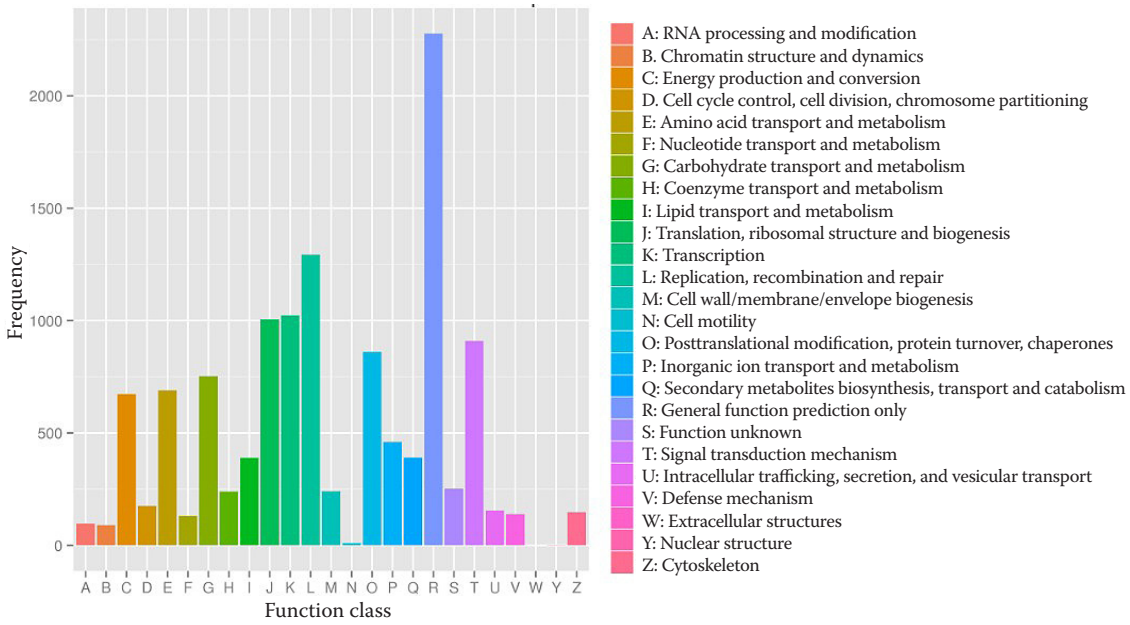

Figure 3. Clusters of orthologous groups (COG) classification in *Toxicodendron vernicifluum*

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Table 3. Summary statistics for the different types of SSRs identified in *Toxicodendron vernicifluum* transcriptome (the percentages are shown in brackets)

Repeat type	Repeat numbers																		Total No.
	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
Mono-	0	0	0	0	0	641	328	184	124	83	65	33	50	50	76	75	56	25	9	1799 (53.04)
Di-	0	223	114	84	55	53	37	3	0	0	0	0	0	0	0	0	0	0	0	569 (16.77)
Tri-	441	199	96	16	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	756 (22.29)
Tetra-	31	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36 (1.06)
Penta-	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11 (0.32)
Hexa-	8	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12 (0.35)
Compound form																				212 (6.24)

In addition, all 48 693 unigenes were subjected to a search against the Cluster of Orthologous Groups (COG) database. The 28 722 unigenes showed significant similarity to non-redundant proteins, 8820 (18.11%) were assigned to COG classifications (Figure 3). Among the 25 COG categories, the cluster for general function prediction represented the largest group, followed by replication, recombination and repair, etc. (Figure 3). To further analyse the lacquer tree transcriptome, all 48 693 unigenes were analysed on Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway tools. This process predicted a total of 117 pathways that were represented by 7301 unigenes (Table S1 in Electronic Supplementary Material (ESM) – for the ESM see the electronic version).

EST-SSR identification. All 48 693 unigenes that were identified in the present study were used to the mining of microsatellites which are defined as di- to hexa-nucleotide SSRs with a minimum of five repeats for all motifs. The 10 987 unigenes with lengths greater than 1 kb were screened. Using the SSRIT tool, a total of 3392 potential EST-SSRs were identified, numbers of SSR containing sequences (2843), of which 599 (21.07%) sequences contained more than one EST-SSR, in which 486 sequences contained simple sequence repeats. The mononucleotide repeats were a rich (53.04%) type of SSR, di- and tri-nucleotide repeats were 16.77% and 22.29%, respectively, while tetra-, penta- and hexa-nucleotide repeats had a small percentage of the total SSR motif with 1.06%, 0.32% and 0.35%, respectively (Table 3). After the most common SSR motif type, i.e. mononucleotide repeats, trinucleotide repeats were the most frequent SSR motif type. While in the Kumpatla and Mukhopadhyay (2005) study mononucleotide repeats and dinucleotide repeats were the most frequent SSR motif type in *Vitis vinifera* (37.5%; 39%), *Arabidopsis thaliana* (7.5%; 42.5%), *Arachis hypogaea* (10%; 49%), *Glycine max* (20%; 41.5%) and *Helianthus annuus* (18%; 40%).

The dominant motif in mononucleotide repeats was A/T (99.22%), AG/TC (64.7%) in dinucleotide repeats, followed by AT/TA (30%) and AC/TG (5.3%). In the 10 types of trinucleotide repeats, the four most frequent

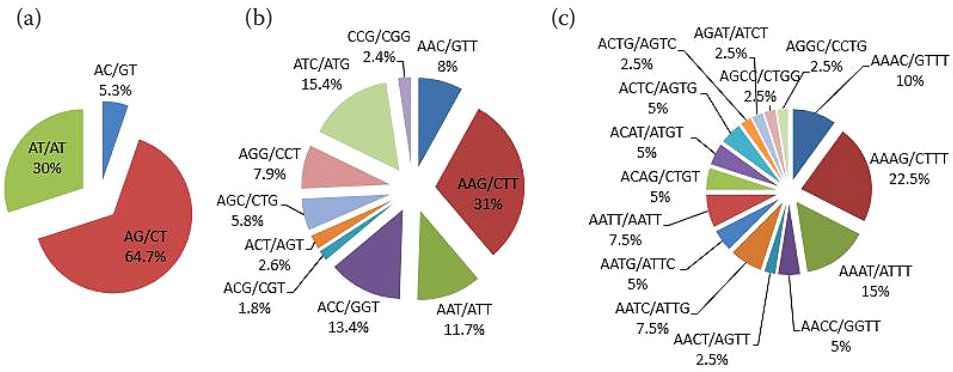

Figure 4. Percentage of different motifs in dinucleotide (a), trinucleotide (b), and tetranucleotide (c) repeats in *Toxicodendron vernicifluum*

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Table 4. Characterization and polymorphism level of 14 microsatellite loci in lacquer tree populations (N = 120)

Primers	Primer sequence (5'–3')	Repeat motif	Fragment size (bp)	Ta (°C)	*A*	*PIC*	*Ho*	*He*	*Fis*	GenBank accession No.
c17310*	F: CAAAGCAAAGACATTGAAACAA R: CTTGATTTTGAAGGACCCGA	(AGCC)5	287–295	55	2	0.38	0.51	0.77	0.34	KU947524
c18564	F: CTCTTGAGGATGGTGGTGGT R: GGGGGACACAGCTTTTTGTA	(CCA)8	116–147	55	4	0.65	0.82	0.89	0.09	KU947556
c20035	F: TGCTGGAGGATTATTAGCCG R: CATTTGGTGGCCAGTTCATA	(TTCT)5	275–289	55	3	0.55	0.66	0.83	0.21	KU947579
c22604	F: ACAAACCCTTCCCTTCGATT R: GTTCTCGAAGACAAGAGGCG	(TAGT)5	262–271	55	3	0.62	0.61	0.88	0.31	KU947632
c22708*	F: TTCTTCCTTCTCTCTTTCACGTC R: GATCGCCTCCATGTTCTCTC	(CT)10	143–172	55	3	0.51	0.76	0.86	0.11	KU947636
c22876	F: AGCAACAGTGGCCATAGCTC R: AACCCACGTGCTCAAAAATC	(AG)10	253–263	55	3	0.48	0.17	0.84	0.79	KU947645
c23254	F: GTGAGGGTTTCATTTGGGAA R: TCCTCCATTTCCATCTCCAC	(GAAA)5	122–138	55	3	0.53	0.81	0.88	0.08	KU947653
c23578	F: TTGTCTCCTCACCACAGCC R: GAAGAGGCGTGCTTTATTGC	(CCA)7	92–111	55	2	0.54	0.63	0.87	0.27	KU947669
c23770	F: TTCTGATCCCAAGAACCCAG R: GGGATTGATGGAAAGGGAAT	(ACA)7	220–236	55	3	0.57	0.79	0.88	0.11	KU947673
c25711	F: TGAGGTTTAGACGCAGAGCC R: CCATCATAACAATGTGCTCAAAA	(TGCC)8	229–256	55	2	0.58	0.47	0.86	0.45	KU947745
c26126*	F: ATTCTCTGGTGGAGGTGGTG R: GCAATCAGCCATCAGAACAA	(GGT)7	150–169	55	3	0.53	0.34	0.80	0.58	KU947758
c26770	F: TCCGCCTCCAATAACTGAAC R: GCTTCTCAAGGGGCTTCTCT	(CCG)8	202–243	55	4	0.58	0.82	0.87	0.06	KU947786
c26938*	F: TACGATGGTTCTGTGGTGGA R: TTCATTTTCACCCTCAACTCAA	(TTTGAG)5	219–231	55	2	0.48	0.51	0.84	0.38	KU947792
c26949	F: TGATGACCCACAACTCCAAA R: TGATGCTCATGGCTTCAGTG	(GAG)7	101–110	55	4	0.48	0.77	0.78	0.01	KU947794
Mean					2.93	0.53	0.62	0.85	0.27	

N – sample size for the lacquer tree population; *Ta* – annealing temperature; *A* – number of alleles; *PIC* – polymorphism information content; *Ho* – observed heterozygosity; *He* – expected heterozygosity; *Fis* – inbreeding coefficient; *indicates significant deviation from Hardy-Weinberg equilibrium ($P < 0.05$)

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motifs were AAG/TTC (31%), ATC/TAG (15.4%), ACC/TGG (13.4%) and AAT/TTA (11.7%). The most common motif type of tetranucleotide repeats was AAAG/TTTC (22.5%), followed by AAAT/TTTA (15%) and AATC/TTAG; AATT/TTAA (7.5%) (Figure 4). The percentages of different motifs in each SSR were similar to previous studies in woody plants such as *Salix*, *Populus*, *Eucalyptus* (He *et al.* 2015).

SSR marker validation and analysis of genetic diversity. The 6429 pairs of EST-SSR primers were designed by Primer 5.0 based on 3392 EST-SSRs. Of the 80 pairs randomly tested (Table S2 in ESM), 14 pairs produced PCR products of the expected size and revealed polymorphisms which were used to investigate genetic variation in the lacquer tree population in Langao, Shaanxi province, China. *PIC* value at each locus ranged from 0.38 to 0.65 with an average of 0.53 (Table 4, Figure 5). Botstein *et al.* (1980) indicated that *PIC* value higher than 0.5 is considered a highly informative marker, while 0.5 > *PIC* > 0.25 is a moderately informative marker. In our study, the mean value of *PIC* (0.53) indicated high polymorphism of loci. In total 41 alleles were identified across the 14 loci, each polymorphic locus possessing 2–4 alleles with average value of 2.93. *Ho* ranged from 0.17 to 0.82 with average value of 0.62. *He* ranged from 0.77 to 0.89 with average value of 0.85. There were 4 loci out of the 14 analysed loci that significantly deviated from Hardy-Weinberg equilibrium ($P < 0.05$) due to an excess of homozygosity (Table 4). This result showed that the natural populations of *T. vernicifluum* maintain a high genetic diversity level. Whereas Hsu *et al.* (2013) reported the lower genetic diversity levels in *Toxicodendron diversilobum* (A = 1 to 10; *He* = 0.1 to 0.87) and *Toxicodendron pubescens* (A = 2 to 8; *He* = 0.26 to 0.83). The inbreeding coef-

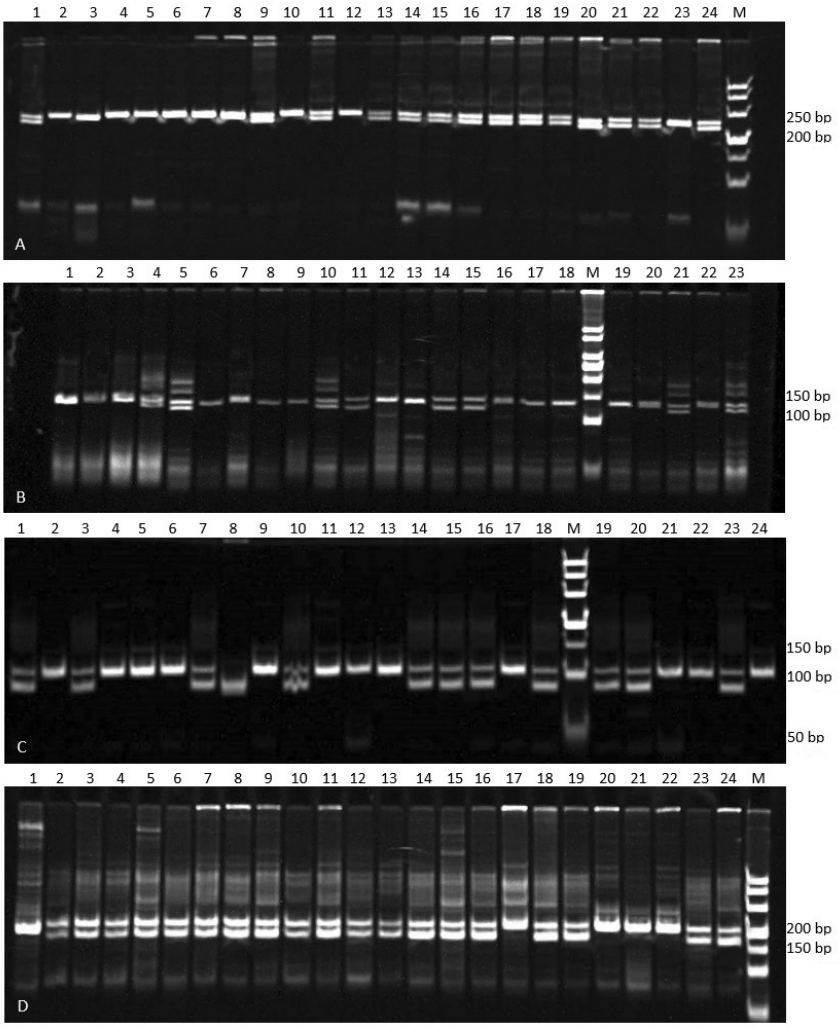

Figure 5. Gel pictures of 120 *Toxicodendron vernicifluum* genotypes produced with some SSR primer pairs (A: c26938, B: c23254, C: c23578 and D: c23770; lane M is a 50 bp ladder and lanes 1 to 24 represent different genotypes)

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ficient ranged from 0.01 (c26949) to 0.79 (c22876), the mean heterozygote deficit (*Fis* = 0.27). In the studied population, there were positive values of the fixation index, indicating an excess of homozygotes and inbreeding. Generally, the heterozygosity excess is caused by small reproductive population size, overdominance, negative associative mating, or asexual reproduction (Stoeckel *et al.* 2006),

CONCLUSIONS

In the present study, the Illumina HiSeq™ 2500 platform was used for the profile of *T. vernicifluum* transcriptome. A total of 48 693 unigenes were identified from a *de novo* assembly and 486 microsatellite loci were isolated (GenBank code: KU947470 to KU947955). There were 80 pairs in the determined SSR loci that were selected to test the primer amplification efficiency. Among them 14 primer pairs yielded polymorphic and single locus amplification products. Using these SSR primers, it was revealed that natural populations of *T. vernicifluum* maintained a high level of genetic diversity. Microsatellite markers for *T. vernicifluum* developed in the present study will provide a platform for the study of genetic diversity, species identification, understanding of the population structure, conservation and breeding of *T. vernicifluum*.

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References

Aggarwal R.K., Hendre P.S., Varshney R.K., Bhat P.R., Krishnakumar V., Singh L. (2007): Identification, characterization and utilization of EST-derived genic microsatellite markers for genome analyses of coffee and related species. Theoretical and Applied Genetics, 114: 359–372.

Botstein D., White R.L., Skolnick M., Davis R.W. (1980): Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Medical Genetics, 32: 314–331.

Cho N., Choi J., Yang H., Jeong E.J., Lee K.Y., Kim Y.C., Sung S.H. (2012): Neuroprotective and anti-inflammatory effects of flavonoids isolated from *Rhus verniciflua* in neuronal HT22 and microglial BV2 cell lines. Food and Chemical Toxicology, 50: 1940–1945.

Clarke K.R., Gorley R.N. (2001): Primer (Plymouth Routines in Multivariate Ecological Research) v5: User Manual. Plymouth, PRIMER-E, Ltd.

Excoffier L., Laval G., Schneider S. (2005): Arlequin v. 3.1. an integrated software package for population genetics data analysis. Evolutionary Bioinformatics online, 1: 47–50.

Goudet J. (1995): FSTAT (version 1.2): A computer program to calculate *F*-statistics. Journal of Heredity, 86: 485–486.

Hashida K., Tabata M., Kuroda K., Otsuka Y., Kubo S., Makio R., Kubojima Y., Tonosaki M., Ohara S. (2014): Phenolic extractives in the trunk of *Toxicodendron vernicifluum*: chemical characteristics, contents and radial distribution. Journal Wood Science, 60: 160–168.

He X.D., Zheng J.W., Zhou J., He K., Shi S.Z., Wang B.S. (2015): Characterization and comparison of EST-SSRs in *Salix*, *Populus*, and *Eucalyptus*. Tree Genetics Genomes, 11: 820.

Hsu T.W., Shih H.C., Kuo C.C., Chiang T.Y., Chiang Y.C. (2013): Characterizatin of 42 microsatellite markers from poison ivy, *Toxicodendron radicans* (Anacardiaceae). International Journal of Molecular Sciences, 14: 20414–20426.

Kalia R.K., Rai M.K., Kalia S., Singh R., Dhawan A.K (2011): Microsatellite markers: an overview of the recent progress in plants. Euphytica, 177: 309–334.

Kaur S., Pembleton L.W., Cogan N.O., Savin W.K., Leonforte T., Paull J., Materne M., Forster J.W. (2012): Transcriptome sequencing of field pea and faba bean for discovery and validation of SSR genetic markers. BMC Genomics, 13: 104.

Kim K.H., Moon E., Choi S.U., Pang C., Yeou K.S., Lee K.R. (2015): Identification of cytotoxic and anti-inflammatory constituents from the bark of *Toxicodendron vernicifluum* (Stokes) F.A. Barkley. Journal of Ethnopharmacology, 162: 231–237.

Kumpatla S.P., Mukhopadhyay S. (2005): Mining and survey of simple sequence repeats in expressed sequence tags of dicotyledonous species. Genome, 48: 985–998.

Lee J.C., Lee K.Y., Kim J., Na C.S., Jung N.C., Chung G.H., Jang Y.S. (2004): Extract from *Rhus verniciflua* stokes is capable of inhibiting the growth of human lymphoma cells. Food and Chemical Toxicology, 42: 1383–1388.

Li Y.C., Korol A.B., Fahima T., Nevo E. (2004): Microsatellites within genes: structure, function, and evolution. Molecular Biology and Evolution, 21: 991–1007.

Liu M., Qiao G., Jiang J., Yang H., Xie L., Xie J., Zhuo R. (2012): Transcriptome sequencing and de novo analysis for ma bamboo (*Dendrocalamus latiflorus* Munro) using the Illumina platform. PLoS ONE, 7: e46766.

Liu Z., Chen T., Ma L., Zhao Z., Zhao P., Nan Z., Wang Y. (2013): Global transcriptome sequencing using the illu-

doi: 10.17221/183/2016-CJGPB

mina platform and the development of ESTSSR markers in autotetraploid alfalfa. PLoS ONE, 8: e83549.

Peakall R., Smouse P.E. (2006): GenAlex 6.5: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, 6: 288–295.

Stoeckel S., Grange J., Manjarres F.J.F., Bilger I., Lacoste F.N., Mariette S. (2006): Heterozygote excess in a self-incompatible and partially clonal forest tree species – *Prunus avium* L. Molecular Ecology, 15: 2109–2118.

Vieira M.L.C., Santini L., Diniz A.L., Munhoz C.F. (2016): Microsatellite markers: what they mean and why they are so useful. Genetics and Molecular Biology, 39: 312–328.

Wei W., Qi X., Wang L., Zhang Y., Hua W., Li D., Lv H., Zhang X. (2011): Characterization of the sesame (*Sesamum indicum* L.) global transcriptome using Illumina paired end sequencing and development of EST-SSR markers. BMC Genomics, 12: 451.

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