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Analysis of the Genetic Relationship among Mulberry (*Morus* spp.) Cultivars Using Inter-Simple Sequence Repeat (ISSR) Markers

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Abstract

Mulberry (*Morus* spp. family: *Moraceae*) has prime importance in the sericulture industry, and its foliage is the only natural feed of the silkworm *Bombyx mori* L. Traditional classification methods using morphological traits were largely unsuccessful in assessing the diversity and relationships among different mulberry species because of environmental influences on the traits of interest. For these reasons, it is difficult to differentiate between the varieties and cultivars of *Morus* spp. In the present study, inter-simple sequence repeat (ISSR) markers were used to investigate the genetic diversity of 48 mulberry samples genotyped using nine ISSR primers. The ISSR markers exhibited polymorphisms (53.2%) among mulberry genotypes. Furthermore, similarity coefficient estimated for these ISSR markers was found to vary between 0.67 and 0.99 for the combined pooled data. The phenogram drawn using the UPGMA cluster method based on combined pooled data of the ISSR markers divided the 48 mulberry genotypes into seven major groups. No genetic association was found in the collection area, and there was a mixed pattern between the mulberry lines. The hybridization between different mulberry species is highly likely to be homogenized due to natural hybridization.

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UPGMA cluster

Introduction

Mulberry (*Morus*) are tall perennial summer-green trees belonging to the *Moraceae* family. Currently, the main mulberry varieties cultivated in Korea are *Morus bombycis* Koidz, *Morus alba* L., and *Morus lhou* Koidz. In addition, 130 other cultivars are grown in Korea, including *Morus nigra* L., *Morus rubra* L., and *Morus indica* L. (Machii *et al*., 1999).

Mulberry trees are widely distributed throughout the world, from tropical to temperate regions, and mulberry leaves are the only feed crops for silkworms (*Bombyx mori*) (Kalpana *et al*., 2012). Moreover, mulberries contain various bioactive substances, including flavones, steroids, and triterpenes (Kondo, 1957). Thus, they are also becoming interesting resources in the raw food and pharmaceutical industries (Lee *et al*., 2003).

In Korea, 587 cultivars (varieties), i.e., genetic resources of mulberry are currently preserved, including the breeding lines and recommended cultivars for silkworm breeding. Most of these cultivars were collected from various regions of Korea, and some strains were also collected from oversea territories, such as

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Japan, India, Iran, Turkey, and France (Kim *et al*., 2013).

In order to analyze the genetic relationships among mulberry trees, it is necessary to use different methods to complement the classification system according to morphological and physiological characteristics (Sung *et al*., 2001). However, to date, most studies have focused on the analysis, extraction, and use of functional ingredients, and only a few studies have focused on the conservation of genetic resources (Vijayan *et al*., 2004, 2006). In particular, genetic studies on molecular biological characteristics, which are important basic data for the preservation of genetic resources, are lacking (Awasthi et al., 2004).

Molecular biological methods are essential for assessing and understanding inter- and intra-species relationships (Gupta *et al*., 2008), and these methods can be used in basic studies on the breeding of mulberry trees because DNA is not affected by the cultivation environment or the plant development stage. Therefore, analysis of species' relationships using nucleotide sequences and polymorphisms is objective and has a higher reliability (Kalpana *et al*., 2012).

Analysis of random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), simple sequence repeats (SSR), restriction fragment length polymorphisms (RFLP), and inter simple sequence repeats (ISSR) (Zietkiewicz *et al*., 1994; Agarwal *et al*., 2008; Grover and Sharma, 2016) are commonly performed to classify cultivars and assess their relationships based on genetics. These methods have been used to assess taxa, phylogenetic relationships, and diversity within species at the molecular genetic level (Iruela *et al*., 2002).

ISSR markers are used to amplify the gap between two identical microsatellites in opposite directions through PCR, after which the differences are analyzed. Complimentary SSRs in the genome are 4–10 times repeatedly amplified to detect polymorphisms. As repetitive sequences are located in the genome, species or groups can be specifically identified, which is useful for studies on population genetics and genetic diversity of related species (Ryu and Bae, 2011). Moreover, ISSR profiling is a simple method, similar to RAPD. However, it uses longer primers (15–30 mers) than those used in RAPD (10 mers), and they can be used at higher temperatures. ISSRs are also evenly spread over the chromosome, and thus, information they contain is less concentrated (Schlötterer, 2004). ISSR analysis can complement the low reproducibility of RAPD analysis (Nagaoka and Ogihara, 1997), high cost of AFLP analysis, and complexity in marker production of SSR analysis (Camacho and Liston, 2001).

We derived the results of analysis with the above ISSR markers, UPGMA (unweighted pair group method with arithmetic mean) by a simple agglomerative (bottom-up) hierarchical clustering method.

In the present study, we analyzed the genetic diversity and relationships among domestically preserved mulberry genetic resources through ISSR analysis and UPGMA clustering. We expected to obtain basic data necessary to discover the materials required for breeding of new mulberry cultivars and for the commercialization of mulberry genetic resources.

Materials and Methods

Study materials

In this study, we analyzed 48 mulberry cultivars that have been cultivated in the same environment using the same methods and were preserved in the genetic resource experimental field by the Rural Development Administration (RDA) of the National Institute of Agricultural Sciences (NIAS) (Located in Wanju-gu, Jeollabuk-do) (http://genebank.rda.go.kr/)(Table 1).

Genomic DNA extraction

Total DNA was extracted from the collected samples (leaves or winter buds) using a modified cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980). The concentration and purity of the extracted DNA were quantified using an Epoch microplate spectrophotometer (Bio-Tek, USA), and the samples were then diluted to a final concentration of 10 ng/μL. PCR was performed according to the method described in Williams *et al*. (1990), and DNA was amplified using Mastercycler Pro (Eppendorf, USA).

Primer selection and PCR

To analyze the relationship between cultivars of mulberry trees, 60 ISSR primers from the University of British Columbia (UBC ISSR Primers) were used in a preliminary analysis. Among them, nine primers that showed polymorphisms, excellent reproducibility, and clear amplification products (#813, #815, #824, #834, #840, #843, #844, #845, and #853) were selected through primer screening (Table 2).

The PCR reaction solution (master mix) included 30 ng of template DNA, 10 μL Prime Taq Premix (2×) (Genetbio, Korea) (Prime Taq DNA Polymerase 1 unit/10 μL), and 1 μL of primer in a total volume of 20 μL. After pretreating the master mix at 94 °C for 5 min, a cycle of 20 s at 94 °C, 1 min at 50 °C, and

Table 1. Information of 48 mulberry (*Morus* spp.) cultivars used in this study. (http://genebank.rda.go.kr/)

No.	Cultivar	Scientific Name	Collection Region	Reference Number
1	*Jeokmoksipyung*	*Morus bombycis*	Japan	IT 238900
2	*Gillim*	*Morus sp.*	China	IT 238692
3	*Turkey yasang*	*Morus sp.*	Turkey	IT 238947
4	*Turkey F*	*Morus sp.*	Turkey	IT 238950
5	*Iran A*	*Morus sp.*	Iran	IT 238863
6	*Iran Q*	*Morus sp.*	Iran	IT 238876
7	*India 2*	*Morus alba*	Indonesia	IT 238879
8	*India 6*	*Morus alba*	Indonesia	IT 238882
9	*Kaeryangppong*	*Morus alba*	Japan	IT 238590
10	*Guksang 15*	*Morus sp.*	Japan	IT 238673
11	*Joseonsang*	*Morus alba*		IT 238918
12	*Suwonsang 3*	*Morus sp.*	Korea, South	IT 238820
13	*Cheongilppong*	*Morus alba*	Japan	IT 238593
14	*Sinilppong*	*Morus alba*	Japan	IT 238561
15	*Suseongppong*	*Morus alba*	Korea, South	IT 238562
16	*Sugeppong*	*Morus alba*	Korea, South	IT 238592
17	*Mujeonsibmunja*	*Morus alba*	Japan	IT 238748
18	*Botongsibmunja*	*Morus alba*	Japan	IT 238764
19	*Baekyeopnosang*	*Morus lhou*		IT 238757
20	*Sangilppong*	*Morus alba*	Japan	IT 238565
21	*Josaenghongpinosang*	*Morus lhou*		IT 238916
22	*Daejosaeng*	*Morus bombycis*	Japan	IT 238729
23	*Daeroukppong*	*Morus lhou*	China	IT 238587
24	*Suwonppong*	*Morus alba*	Korea, South	IT 238591
25	*Yongcheonppong*	*Morus alba*	Korea, South	IT 238589
26	*Gunmajeokmok*	*Morus bombycis*	Japan	IT 238679
27	*Gumsulppong*	*Morus bombycis*	Japan	IT 238594
28	*Dangsang 1*	*Morus lhou*		IT 238713
29	*Chuncheon 1*	*Morus sp.*		IT 238942
30	*Yeongbyeonchu-u*	*Morus sp.*	Korea, South	IT 238846
31	*92/19*	*Morus sp.*	Korea, South	IT 238631
32	*92/10*	*Morus sp.*	Korea, South	IT 238625
33	*Sawonppong 22*	*Morus lhou*	Korea, South	IT 238796
34	*4X Guksang 21*	*Morus lhou*	Korea, South	IT 238599
35	*Shinkwangppong*	*Morus lhou*	Japan	IT 238563
36	*Hwikaseu 4X*	*Morus lhou*		IT 239002
37	*Jamsang 117*	*Morus sp.*	Korea, South	IT 238577
38	*Hasusang*	*Morus alba*		IT 238981
39	*Euncheuksang*	*Morus sp.*		IT 238856
40	*Hwikaseu2X*	*Morus lhou*		IT 239001
41	*Philliphin 1*	*Morus sp.*	Philippines	IT 38980
42	*Hokkaido yasang*	-		
43	*Japangum*	*Morus lhou*	Japan	IT 238884
44	*America13*	-		
45	*Jeokmok*	*Morus bombycis*	Japan	IT 238899
46	*Canada*	*Morus lhou*	Canada	IT 238972
47	*Sangberry(CA4X)*	-		
48	*Bengal*	*Morus lhou*		IT 238763

Table 2. Nucleotide sequences of nine selected primers exhibiting polymorphisms of inter-simple sequence repeat (ISSR) markers.

UBC ISSR Primer Number	Nucleotide Sequence (5'- 3')	Number of Polymorphic Bands	Polymorphism Rate (%)	Size Range (bp)
UBC-813	CTC TCT CTC TCT CTC TT	6	63.4	300–1500
UBC-815	CTC TCT CTC TCT CTC TG	6	61.1	300–1500
UBC-824	TCT CTC TCT CTC TCT CG	12	51.8	300–2000
UBC-834	AGA GAG AGA GAG AGA GYT	7	67.4	200–1500
UBC-840	GAG AGA GAG AGA GAG AYT	8	57.2	300–1000
UBC-843	CTC TCT CTC TCT CTC TRA	7	27.4	300–1500
UBC-844	CTC TCT CTC TCT CTC TRC	11	37.5	300–2000
UBC-845	CTC TCT CTC TCT CTC TRG	8	45.9	100–2000
UBC-853	TCT CTC TCT CTC TCT CRT	8	55.2	300–1500

1 min at 72 °C was repeated 35 times. The samples were then maintained at 72 °C for 10 min.

Electrophoresis was performed with 1.5% agarose gel to separate the amplified DNA. Photographs of the gel were taken using the Gel Image Analysis System Corebio system (I-MAX-2000, Korea), and polymorphisms of the amplified products were assessed. The agarose gel was treated with 0.5 µg/mL ethidium bromide solution, and a 100 bp DNA ladder (Bioneer Co., Korea) was used as a marker to compare molecular weight.

Analysis of the relationships among mulberry cultivars

ISSR polymorphism analysis was performed to select the bands with high reproducibility and polymorphism level as

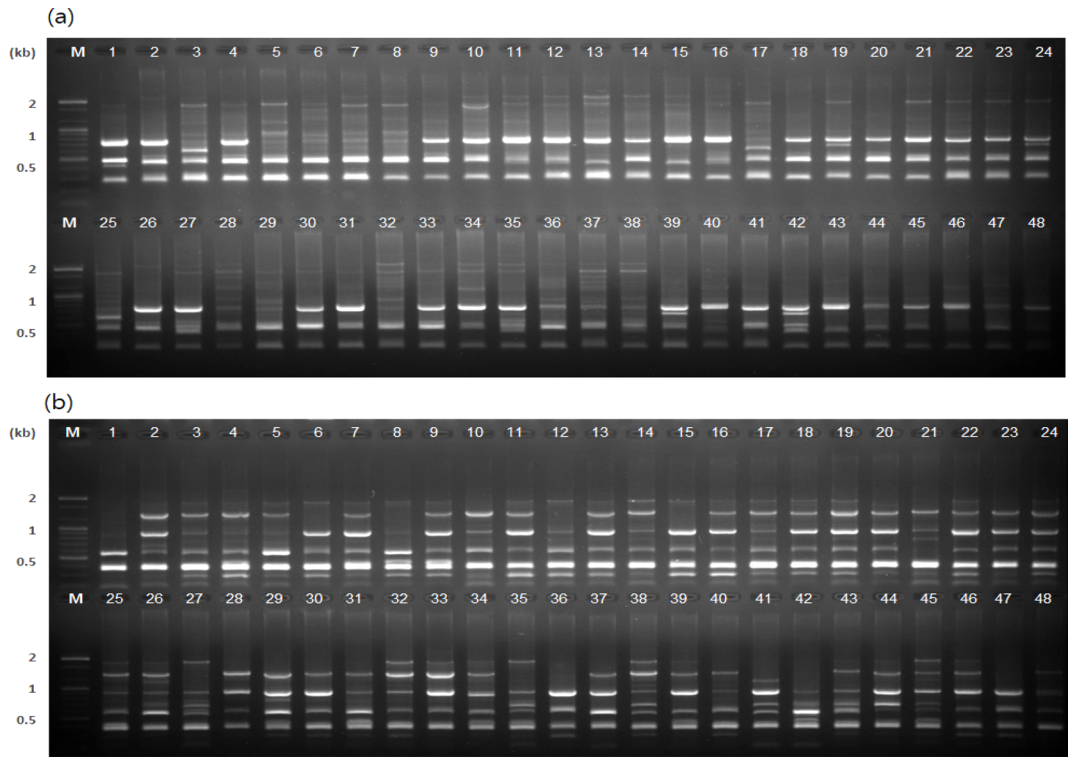

Fig. 1. Polymerase chain reaction (PCR) amplification products obtained with inter simple sequence repeat (ISSR) primers (a) (UBC-843) and (b) (UBC-845). lanes 1–48. (Table 2.)

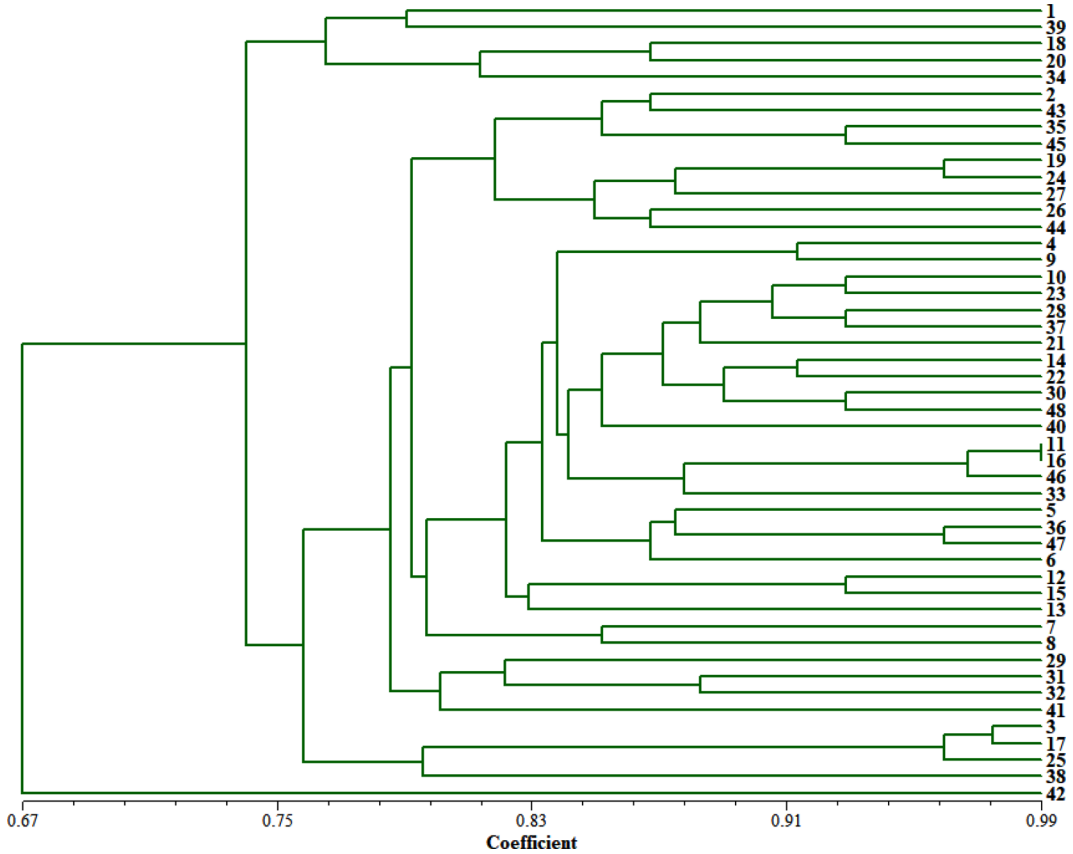

Fig. 2. An UPGMA cluster based on genetic distances obtained using nine polymorphic ISSR markers in 48 mulberry cultivars.

markers. The presence and absence of bands were coded as 1 and 0, respectively, and the Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc2.2) program was used to perform the statistical analysis. The SIMQUAL method in the NTSYS-pc2.2 program was selected, and data derived using the unweighted pair-group method with arithmetic mean (UPGMA) algorithm was analyzed by SHAN clustering. Relationships between the cultivars were presented using UPGMA.

Results and Discussion

ISSR polymorphism analysis

Assessment of ISSR requirements and primer selection

Among the 60 UBC ISSR primers (UBC Primer Set no. 9), nine primers that showed polymorphisms, excellent reproducibility, and clear amplification products (#813, #815, #824, #834, #840, #843, #844, #845, and #853) were selected through primer screening (Fig. 1, Table 2). Most PCR amplification products were found at 100 bp–2 kb, and at least 6–12 bands were amplified per ISSR primer. Primer #843 had the lowest polymorphism rate (27.4%), whereas primers #813, #815, and #834 had polymorphism rates higher than 60%.

UPGMA cluster analysis of relationships according to ISSR polymorphism in 48 varieties of mulberry trees

In this study, we UPGMA cluster analyzed the relationships among 48 representative cultivars, including those with a clear origin, those registered as strains during their development, and those that were brought from overseas (Fig. 2, Table 3). The similarity values for the 48 cultivars ranged from 0.67–0.99, and they were clustered into seven groups. *Jeokmoksipyung* (1) and *Yongcheonppong* (25) had the lowest similarity at 0.577, whereas *Joseonsang* (11) and *Sugeppong* (16) had the highest similarity at 0.984. There was no clear relationship between the species and collection regions, and there was a mixed pattern between cultivars.

Group 1 consisted of five cultivars, including *Jeokmoksipyung* (1), and Group 2 consisted of nine cultivars, including *Gillim* (2). In group 3, 23 cultivars were observed, including *Kaeryangp-*

Table 3. Genetic similarity coefficients among 48 mulberry cultivars obtained using ISSR markers.

GENO TYPE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
1	1																																																	
2	0.789	1																																															
3	0.607	0.718	1																																														
4	0.667	0.767	0.768	1																																													
5	0.676	0.75	0.838	0.849	1																																											
6	0.617	0.761	0.82	0.81	0.852	1																																										
7	0.777	0.825	0.762	0.843	0.825	0.775	1																																										
8	0.717	0.737	0.699	0.756	0.795	0.775	0.844	1																																									
9	0.714	0.789	0.759	0.917	0.817	0.802	0.807	0.777	1																																								
10	0.677	0.812	0.75	0.859	0.87	0.826	0.8	0.8	0.824	1																																							
11	0.729	0.748	0.774	0.827	0.868	0.82	0.794	0.794	0.85	0.875	1																																						
12	0.677	0.783	0.719	0.773	0.783	0.795	0.769	0.769	0.794	0.848	0.813	1																																					
13	0.676	0.778	0.748	0.822	0.778	0.791	0.795	0.766	0.817	0.87	0.838	0.841	1																																				
14	0.725	0.829	0.77	0.846	0.829	0.783	0.849	0.788	0.87	0.896	0.862	0.896	0.857	1																																			
15	0.687	0.737	0.699	0.785	0.795	0.807	0.75	0.781	0.837	0.831	0.857	0.923	0.825	0.91	1																																		
16	0.71	0.761	0.787	0.84	0.882	0.833	0.807	0.807	0.864	0.89	0.984	0.826	0.852	0.877	0.871	1																																		
17	0.647	0.754	0.969	0.744	0.841	0.795	0.769	0.708	0.765	0.758	0.782	0.758	0.783	0.776	0.708	0.795	1																																	
18	0.765	0.754	0.688	0.801	0.783	0.699	0.831	0.769	0.824	0.788	0.813	0.818	0.812	0.866	0.831	0.826	0.727	1																																
19	0.768	0.811	0.758	0.8	0.838	0.77	0.832	0.746	0.795	0.847	0.874	0.847	0.865	0.89	0.832	0.889	0.791	0.819	1																																
20	0.777	0.707	0.699	0.756	0.737	0.678	0.75	0.688	0.807	0.769	0.762	0.739	0.795	0.788	0.75	0.775	0.739	0.862	0.774	1																														
21	0.714	0.789	0.729	0.834	0.789	0.772	0.837	0.717	0.8	0.883	0.82	0.794	0.873	0.87	0.777	0.833	0.736	0.824	0.85	0.807	1																													
22	0.717	0.825	0.794	0.872	0.795	0.807	0.875	0.75	0.837	0.831	0.826	0.862	0.854	0.91	0.844	0.839	0.8	0.862	0.86	0.781	0.867	1																												
23	0.717	0.795	0.762	0.843	0.854	0.807	0.781	0.781	0.867	0.923	0.889	0.831	0.854	0.879	0.844	0.904	0.769	0.862	0.832	0.844	0.896	0.844	1																											
24	0.723	0.795	0.797	0.784	0.822	0.75	0.814	0.727	0.778	0.83	0.856	0.801	0.849	0.846	0.785	0.87	0.83	0.773	0.96	0.756	0.834	0.814	0.814	1																										
25	0.577	0.688	0.968	0.768	0.808	0.787	0.73	0.699	0.759	0.719	0.742	0.688	0.718	0.739	0.667	0.754	0.938	0.657	0.728	0.667	0.698	0.762	0.73	0.768	1																									
26	0.714	0.817	0.759	0.778	0.817	0.741	0.777	0.717	0.771	0.824	0.85	0.736	0.817	0.841	0.747	0.864	0.765	0.765	0.877	0.717	0.8	0.777	0.807	0.861	0.729	1																								
27	0.754	0.829	0.708	0.789	0.8	0.751	0.819	0.728	0.783	0.806	0.832	0.776	0.8	0.824	0.758	0.845	0.746	0.746	0.89	0.697	0.812	0.788	0.788	0.874	0.708	0.87	1																						
28	0.657	0.795	0.794	0.872	0.884	0.871	0.844	0.75	0.837	0.892	0.826	0.8	0.795	0.849	0.813	0.839	0.769	0.769	0.803	0.75	0.867	0.844	0.906	0.785	0.762	0.777	0.788	1																				
29	0.657	0.789	0.759	0.834	0.761	0.802	0.777	0.717	0.829	0.824	0.729	0.706	0.761	0.783	0.687	0.741	0.736	0.677	0.768	0.687	0.857	0.777	0.807	0.75	0.759	0.743	0.754	0.867	1																			
30	0.706	0.754	0.75	0.887	0.87	0.763	0.8	0.739	0.853	0.909	0.844	0.818	0.841	0.896	0.831	0.858	0.758	0.848	0.847	0.862	0.853	0.862	0.892	0.801	0.719	0.824	0.776	0.862	0.765	1																		
31	0.713	0.811	0.758	0.853	0.811	0.8	0.746	0.746	0.85	0.875	0.816	0.791	0.838	0.835	0.774	0.829	0.762	0.791	0.816	0.774	0.877	0.832	0.918	0.8	0.758	0.823	0.779	0.86	0.877	0.847	1																
32	0.695	0.791	0.767	0.806	0.817	0.808	0.755	0.727	0.829	0.826	0.824	0.826	0.843	0.841	0.811	0.808	0.771	0.771	0.821	0.727	0.802	0.811	0.866	0.806	0.738	0.802	0.759	0.839	0.802	0.798	0.898	1															
33	0.687	0.766	0.826	0.785	0.825	0.775	0.781	0.813	0.807	0.8	0.857	0.769	0.825	0.819	0.781	0.871	0.831	0.769	0.803	0.75	0.747	0.844	0.844	0.785	0.794	0.807	0.758	0.781	0.717	0.8	0.803	0.783	1														
34	0.74	0.757	0.699	0.8	0.703	0.681	0.774	0.746	0.795	0.734	0.728	0.734	0.811	0.807	0.746	0.74	0.734	0.847	0.763	0.803	0.768	0.803	0.774	0.747	0.699	0.74	0.696	0.688	0.686	0.791	0.789	0.744	0.746	1													
35	0.75	0.877	0.738	0.865	0.767	0.78	0.843	0.785	0.861	0.83	0.797	0.83	0.849	0.874	0.814	0.81	0.773	0.801	0.853	0.727	0.834	0.872	0.814	0.838	0.738	0.834	0.902	0.814	0.834	0.801	0.853	0.806	0.785	0.8	1											
36	0.628	0.681	0.767	0.824	0.867	0.848	0.788	0.722	0.753	0.84	0.8	0.743	0.774	0.796	0.788	0.814	0.743	0.743	0.783	0.722	0.816	0.788	0.821	0.763	0.734	0.816	0.764	0.886	0.753	0.873	0.783	0.763	0.755	0.663	0.763	1									
37	0.691	0.805	0.8	0.824	0.836	0.814	0.853	0.788	0.785	0.873	0.8	0.808	0.805	0.86	0.788	0.814	0.776	0.808	0.783	0.755	0.879	0.886	0.919	0.763	0.767	0.753	0.732	0.919	0.816	0.84	0.874	0.851	0.821	0.723	0.794	0.828	1								
38	0.704	0.778	0.808	0.767	0.75	0.761	0.795	0.678	0.789	0.725	0.778	0.667	0.75	0.743	0.678	0.791	0.812	0.696	0.784	0.737	0.761	0.766	0.737	0.822	0.778	0.817	0.8	0.766	0.761	0.696	0.73	0.738	0.766	0.73	0.795	0.712	0.712	1							
39	0.818	0.755	0.729	0.822	0.781	0.713	0.828	0.773	0.818	0.761	0.785	0.761	0.807	0.83	0.773	0.798	0.734	0.816	0.861	0.773	0.766	0.801	0.773	0.822	0.729	0.792	0.777	0.745	0.739	0.788	0.811	0.79	0.773	0.836	0.822	0.696	0.754	0.755	1						
40	0.71	0.761	0.82	0.81	0.791	0.767	0.742	0.71	0.864	0.826	0.82	0.795	0.791	0.877	0.839	0.833	0.826	0.826	0.8	0.839	0.802	0.871	0.871	0.78	0.787	0.741	0.72	0.807	0.772	0.858	0.829	0.779	0.807	0.77	0.81	0.746	0.814	0.761	0.77	1					
41	0.686	0.761	0.698	0.834	0.732	0.772	0.747	0.687	0.8	0.794	0.759	0.736	0.817	0.783	0.747	0.772	0.677	0.736	0.768	0.717	0.829	0.807	0.837	0.75	0.688	0.8	0.841	0.837	0.8	0.794	0.85	0.802	0.747	0.686	0.861	0.785	0.816	0.761	0.739	0.772	1				
42	0.74	0.757	0.612	0.667	0.649	0.622	0.688	0.688	0.713	0.678	0.67	0.706	0.703	0.696	0.688	0.681	0.649	0.678	0.763	0.602	0.686	0.688	0.717	0.747	0.812	0.74	0.751	0.66	0.686	0.621	0.763	0.744	0.717	0.684	0.8	0.633	0.693	0.703	0.785	0.652	0.713	1			
43	0.806	0.877	0.797	0.811	0.822	0.78	0.843	0.785	0.834	0.801	0.797	0.744	0.767	0.818	0.727	0.81	0.801	0.773	0.827	0.727	0.75	0.785	0.785	0.811	0.768	0.861	0.846	0.785	0.806	0.773	0.827	0.806	0.814	0.773	0.865	0.702	0.763	0.822	0.873	0.78	0.778	0.773	1		
44	0.667	0.743	0.832	0.789	0.8	0.783	0.758	0.697	0.783	0.776	0.832	0.687	0.8	0.794	0.758	0.845	0.806	0.716	0.835	0.728	0.783	0.788	0.788	0.874	0.801	0.87	0.824	0.788	0.754	0.776	0.779	0.759	0.819	0.751	0.818	0.796	0.732	0.857	0.75	0.783	0.783	0.696	0.818	1	
45	0.713	0.838	0.758	0.88	0.811	0.77	0.832	0.774	0.85	0.847	0.816	0.762	0.811	0.862	0.774	0.829	0.762	0.791	0.842	0.688	0.795	0.832	0.803	0.827	0.758	0.877	0.862	0.803	0.823	0.819	0.842	0.795	0.803	0.763	0.933	0.813	0.783	0.784	0.836	0.8	0.823	0.769	0.88	0.835	1
46	0.687	0.737	0.826	0.843	0.854	0.839	0.781	0.781	0.867	0.862	0.953	0.8	0.854	0.849	0.844	0.968	0.831	0.8	0.86	0.75	0.807	0.844	0.875	0.872	0.794	0.837	0.819	0.813	0.747	0.831	0.832	0.811	0.875	0.774	0.814	0.788	0.788	0.825	0.801	0.839	0.777	0.688	0.814	0.879	0.832	1
47	0.687	0.737	0.762	0.872	0.884	0.871	0.813	0.75	0.807	0.892	0.857	0.8	0.795	0.849	0.844	0.871	0.739	0.769	0.832	0.75	0.867	0.844	0.875	0.814	0.73	0.807	0.819	0.906	0.777	0.892	0.832	0.783	0.781	0.688	0.814	0.952	0.853	0.737	0.745	0.807	0.837	0.66	0.756	0.819	0.832	0.844	1
48	0.777	0.795	0.699	0.843	0.825	0.742	0.875	0.813	0.837	0.892	0.826	0.8	0.825	0.91	0.813	0.839	0.708	0.862	0.86	0.844	0.867	0.875	0.875	0.814	0.667	0.807	0.788	0.844	0.777	0.923	0.832	0.783	0.781	0.803	0.814	0.788	0.853	0.707	0.828	0.839	0.777	0.66	0.814	0.758	0.803	0.813	0.844	1

pong (9), whereas group 4 included two Indian mulberries (7, 8). Group 5 consisted of four cultivars, including *Philliphin 1* (41). Additionally, groups 6 and 7 consisted of four cultivars, including *Turkey yasang* (3) and one cultivar of *Hokkaido yasang* (42), respectively.

The *Hokkaido yasang* (42) formed an individual group and showed the greatest genetic difference from other groups, suggesting that it could be used as a breeding material. However, information such as its scientific name and collection region are not registered in the genetic resource data. Thus, more studies are necessary for describing and assessing this cultivar in detail.

The collection region of *Euncheuksang* (39) was not indicated in the genetic resource data. However, its genetic similarity was close to that of *Jeokmok* (45) at 0.818, suggesting that its collection region may be close to Japan, where *Jeokmoksipyung* (1) was collected. Additionally, *Joseonsang* (11) and *Sugeppong* (16) had a high similarity of 0.984, suggesting that both were collected near the Korean Peninsula.

The cultivars collected from overseas showed low mutual genetic relationships, and patterns between them were mixed *Gillim* (2) was collected from China; however, it showed a high similarity of 0.877 with *Japangum* (43) collected from Japan. *Turkey yasang* (3) and *Mujeonsibmunja* (17) also had a high genetic similarity of 0.969, even though they were collected from different regions. In contrast, *India 2* (7) and *India 6* (8) mulberries showed a high degree of similarity and were collected from the same region.

From our results, we concluded that more ISSR primers are needed, as well as the comparison of ISSR and RAPD, to study the relationships between the studied cultivars more clearly. Furthermore, natural hybridization has made it difficult to maintain the genetic uniformity of mulberry species and cultivars, and our opinion is that genetic variation analysis of mulberry trees would be necessary for the preservation of their genetic resources.

In summary, in this study, we generated a phylogenetic tree of 48 mulberry cultivars present in Korea using ISSR molecular markers to evaluate their relationships. The findings of this study can be used for molecular breeding of mulberry trees and as a basis for the discovery of breeding materials required for future breeding efforts and the development of practical mulberry genetic resources.

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