Pak. J. Bot., 48(4): 1637-1643, 2016.

GENETIC DIVERSITY OF FOXTAIL MILLET (*SETARIA ITALICA* L.) FROM MAIN ASIAN HABITATS BASED ON THE NRDNA ITS REGION

YAN-LIN SUN[1]*, SHI-LIN ZHENG[1], JU-KYONG LEE[2] AND SOON-KWAN HONG[3, 4]*

[1]*School of Life Sciences, Ludong University, Yantai, Shandong, 264-025, China*
[2]*Deaprtment of Applied Plant Sciences, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon, 200-701, Korea*
[3]*Deaprtment of Bio-Health Technology, Kangwon National University, Chuncheon, 200-701, Korea*
[4]*Institute of Bioscience and Biotechnology, College of Biomedical Science, Kangwon National University, Chuncheon, 200-701, Korea*
[*]*Corresponding author's e-mail: laddiya@hotmail.com (Y.L. Sun), soonkwan@kangwon.ac.kr (S.K. Hong); Tel.: 86-535-6685003 (Y.L. Sun), 82-33-250-6476 (S.K. Hong); Fax: 82-33-250-6470 (S.K Hong)*

Abstract

Foxtail millet [*Setaria italica* (L.) P. Beauv.] is a crop of historical importance in some Asian and European countries. In this study, we selected the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) as the DNA marker to analyze genetic diversity and relationships of 20 foxtail millet strains collected from three representative Asian countries, including China, Korea, and Pakistan. Due to the length limitation of the nrDNA ITS region, 17 typical variable nucleotide sites were only found, of which 4 sites belonged to insertion, 3 sites deletion, and 10 sites substitution. According to the result of sequence alignment, strains were grouped clearly with the relevant of collected geographical region. Based on the sequence similarity and nucleotide variation, one Main China Group (MCG) and one Main Korea Group (MKG) occurred, and the strains from Pakistan were found to be close to MKG, considered to be originally transmitted from Korea and spread to Pakistan. Certain genetic diversity between strains from Pakistan and Korea were recognized as long-time environment evolution and adaptation. Among strains from Korea, K2, K3, K4, and K5 showed nearer phylogenetic relationship to MCG, considered as Chinese populations. All strains from China showed relatively near phylogenetic relationship with each other, supporting the statement that China is one of origin areas. The result also suggested that there was no introduced strain found in the Chinese strains investigated in this study. This work would provide more sequence sources and help clearer strain distinguishing, genetic diversity and phylogenetic relationship of foxtail millet.

Key words: Foxtail millet; nrDNA ITS region; Genetic diversity; Phylogenetic relationship; Strain distinguishing.

Introduction

Foxtail millet [*Setaria italica* (L.) P. Beauv.] is one of the oldest cereals and has played an important role in early agriculture of Asia and Europe (Kawase and Sakamoto, 1984; Sakamoto, 1987; Li and Wu, 1996). In China, one of main centers of origin (Kawase, *et al*., 2005), foxtail millet has been cultivated since Neolithic times (about 8,700 years ago, Li and Wu, 1996; Sun *et al*., 2012). Due to showing stress tolerance of drought, barren soil, salt, and diseases and pests, foxtail millet has more advantages and prospects under structural readjustment of crop farming and global environmental change at present. Based on this situation, the study of phylogenetics of foxtail millet seems extremely important. However, studies about the variety discrimination and population distribution of foxtail millet were very limited. As the wild ancestor of foxtail millet is green foxtail (*S. viridis*), their variety discrimination and population distribution were considered to reflect that of foxtail millet (*S. italica*). However, unexpectedly, the current distribution of green foxtail and variety discrimination dose not accord with the geographical origin of domesticated *S. italica* (Kihara and Kishimoto, 1942; Li *et al*., 1945). The reason might be that little information is known and reported about where, when and how the green foxtail was selected, transited and gathered to domesticate (Lu, 1999; Barton *et al*., 2009; Bettinger *et al*., 2010). Thus, under the present circumstances, studies about genetic diversity and strain/variety discrimination of foxtail millet are urgently needed.

Concerning the genetic diversity of foxtail millet, some researchers have presented their opinions. For example, Vavilov (1926) believed that East Asia is the principal center of genetic diversity of foxtail millet, while Jusuf and Pernes (1985) considered that strains of foxtail millet grown naturally in China, Korea, and Japan were divided into one group by biochemical analysis of five isoenzymes. Among the countries in East Asia, Korea is especially considered as a center of genetic diversity, because Korea is the pathway when foxtail millet was spread from China to Japan (Murai and Ohnishi, 1996; Lee and Kim, 2007). However, Kim *et al*. (2012) reported the accessions of foxtail millet from China showed higher genetic diversity than those from Korea. In view of the vague conclusions, we selected 10 strains of foxtail millet from Korea, the considered center of genetic diversity, 7 strains from China, the ancient origin, and 3 strains from Pakistan, a West Asian country as control, in order to analyze the genetic diversity and relationships. This work would help and promote the understanding of strain/variety identification and genetic diversity of foxtail millet, especially for strains/varieties in Asia.

Nowadays, molecular identification of PCR-based methods sharing more advantages compared to traditional identification is believed to be a reliable alternative tool for accurate authentication (Joshi *et al*., 2004). DNA-based molecular markers could provide useful information regarding genetic diversity, relationships, and population structure between cultivated species and its wild relatives. To

understand the phylogenetic relationships of foxtail millet, the effectiveness of PCR-based DNA markers has also been evaluated based on the polymorphism, such as randomly amplified polymorphic DNA (RAPD, Schontz and Reather, 1999), restriction fragment length polymorphism (RFLP, Fukunaga *et al*., 1997, 2002, 2011), amplified fragment length polymorphism (AFLP, Le Thierry d'Ennequin *et al*., 2000; Kim *et al*., 2011), and simple sequence repeat (SSR, Jia *et al*., 2009; Gupta *et al*., 2011). However, the evidences are still limited to clearly group strains/accessions of foxtail millet even grown naturally in Asian countries. In the present study, we selected the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) comprising the ITS1 intergenic spacer, 5.8S ribosomal RNA (rRNA), and the ITS2 intergenic spacers (ITS1-5.8S-ITS2) as the DNA marker to analyze genetic diversity and relationships of foxtail millet. The nrDNA ITS is one of the most commonly used regions for species identification according to the Barcode of Life data systems (http://www.boldsystems.org) and NCBI GenBank (http://www.ncbi.nlm.nih.gov) database (Coleman, 2003). In this study, we used the nrDNA ITS region as a more efficient and more stable approach for strain/variety identification of foxtail millet collected from in main Asian habitats and examined their level of variation. This work would provide more evidences for further understanding of the genetic diversity and relationships of foxtail millet, especially that of stains/varieties in Asia, and help the efficient breeding and variety conservation of foxtail millet.

Materials and Methods

Plant materials: Twenty foxtail millet accessions investigated in this study were grown naturally in three main Asian habitats, including China, Korea, and Pakistan. Fresh leaf tissues of these 20 foxtail millet accessions were collected from healthy-growing plants, and provided by Department of Applied Plant Sciences, College of Agriculture and Life Sciences, Kangwon National University, Korea. The leaf tissues were sampled and immediately stored in liquid nitrogen condition for DNAs extraction. The specimen vouchers and other detailed information about collection regions and countries were shown in Table 1.

DNA extraction, PCR amplification and sequencing: DNA extractions were performed by using the modified cetyltrimethylammonium bromide (CTAB) method described by Doyle and Doyle (1987). The ITS1-5.8S-ITS2 region was amplified using universal primers ITS1 and ITS4 (White *et al*., 1990) in 20 µl PCR reaction. PCR amplification was conducted using this set of primers with the following program: 35 cycles of denaturation at 95^{o}C for 1 min, annealing 54^{o}C for 1 min, and a final extension step at 72^{o}C for 1 min. The amplification products were checked by electrophoresis through 1.0% agarose gel, and then purified before DNA sequence analysis using a QIAquick PCR Purification Kit (QIAGEN, Korea) or Gel Purification Kit (QIAGEN, Korea) according to the manufacturer's instructions. Purified PCR products were then sequenced at BGI in Beijing, China (http://www.genomics.cn/index).

Sequence editing and alignment: For editing and assembly of the complementary strands, the software program DNAMAN version 6.0 (Lynnon Biosoft Corporation, USA, http://www.lynon.com/) was used. Analogue of our sequences and nucleotide sequence comparisons were detected with Basic Local Alignment Search Tool (BLAST) network services against databases (http://www.ncbi.nlm.nih.gov/). The multiple sequence alignment of ITS1-5.8S-ITS2 region was also performed using DNAMAN version 6.0 software, to detect single nucleotide polymorphisms.

Table 1. The detailed collection information of foxtail millet (*Setaria italica* L.) materials investigated in this study and the accession numbers (Acc. No.) in NCBI database of ITS region.

Sample No.	Specimen voucher	Collection region	Collection country	NCBI Acc. No.
1.	K1	In Jie, Gangwon-do	South Korea	KF012832
2.	K2	In Jie, Gangwon-do	South Korea	KF012833
3.	K3	In Jie, Gangwon-do	South Korea	KF012834
4.	K4	HwaCheon, Gangwon-do	South Korea	KF012835
5.	K5	HwaCheon, Gangwon-do	South Korea	KF012836
6.	K6	Yeongwol, Gangwon-do	South Korea	KF012837
7.	K7	Hong cheon, Gangwon-do	South Korea	KF012838
8.	K8	Hong cheon, Gangwon-do	South Korea	KF012839
9.	K9	Sam cheok, Gangwon-do	South Korea	KF012840
10.	K10	Yang yang, Gangwon-do	South Korea	KF012841
11.	K11	Gwali	Pakistan	KF012842
12.	K12	Reshun	Pakistan	KF012843
13.	K13	Golghmali	Pakistan	KF012844
14.	K14	Kunyang, Yunnan	China	KF012845
15.	K15	Longxi, Shanxi	China	KF012846
16.	K16	Wuwei, Gansu	China	KF012847
17.	K17	Wuwei, Gansu	China	KF012848
18.	K18	Lanzhou, Gansu	China	KF012849
19.	K19	Qinling, Shaanxi	China	KF012850
20.	K20	Donhuan, Gansu	China	KF012851

Phylogenetic analysis: We assessed intraspecific genetic divergences by using pairwise distance calculations (Meyer and Paulay, 2005). Jaccard coefficients used to represent identity among the ecotypes were calculated by similarity coefficient [Sj = a/(a+u)]. In the total ITS region, ITS1 and ITS2 region, '1' was used for base variation and '0' was used for no variation; 'a' represents the number of the same bases and 'u' represents the number of different bases between two accessions. The phylogenetic relationship among 20 foxtail millet accessions was estimated after the construction of a phylogram based on multiple sequence alignment of various DNA sequences with the DNAMAN version 6.0 software. Based on the typical variable nucleotide sites in the total ITS region sequence, the phylogenetic relationship among our materials was estimated again. Genetic distance (GD) was obtained with the help of MEGA software and mean GD of the intraspecific distance was calculated by sum of individual GD divide by number of samples.

Results and Discussion

PCR amplification of the nrDNA ITS region: To amplify the ITS1-5.8S-ITS2 region of *S. italica*, the universal ITS primer set, ITS1 and ITS4 (White *et al*., 1990) were used in this study. All 20 foxtail millet materials were successfully amplified the nrDNA ITS region, with about 650 bp and submitted in NCBI GenBank database (with accession numbers of KF012832-KF012851). The analogue of the PCR products was detected through BLAST on NCBI server (http://www.ncbi.nlm.nih.gov/). The analogue suggested that our sequencing results were 99% similar to *S. italica* ITS1-5.8S-ITS2 region sequence (NCBI GenBank accession number: HQ600502), 90% similar to *S. faberi* ITS1-5.8S-ITS2 region sequence (NCBI GenBank accession number: KF163685), and 90% similar to *S. viridis* ITS1-5.8S-ITS2 region sequence (NCBI GenBank accession number: KF163678). This result forcefully proved the validity of our amplification.

Sequence analysis of the nrDNA ITS region: Among 20 foxtail millet strains, they shared 98.08% similarity according to alignment result using multiple sequence alignment of DNAMAN 6.0 software. The highest dissimilarity rate appeared between K2 and K19, and K14 and K16, with 2.1% dissimilarity rate (Table 2), while some foxtail millet strains showed nearly 100% identity with each other, such as between K7 and K9, K7 and K10, K15 and K18, and K16 and K17. In this study, the sequence variation could be owned to insertion, deletion, or substitution. In the total detected length of 640 bp, there were 17 typical variable nucleotide sites, of which 4 sites belonged to insertion, 3 sites deletion, and 10 sites substitution.

To be noticeable, there were three insertions on sites playing an important role in grouping among these 20 foxtail millet strains, in 71-73 bp site (Table 3). K1, and K6-K13 showed C-T-T in these three nucleotide sites, while other strains showed indel in these sites. In the non-indel group, not all strains were identical in other typical variable nucleotide sites, e.g. K1 showed indels in the 10 bp and 11 bp sites; K11 showed a indel in the 10 bp site and A, T, T substitutions in the 181 bp, 279 bp, and 396 bp, respectively; K12 showed T substitution in the 396 bp and one indel in the 626 bp; K13 showed A insertion in the 4 bp site and T substitution in the 396 bp site (Table 3). In addition, K4, and K15-K20 showed high sequence similarity among 20 foxtail millet strains (Table 3), except K4 showed T substitution in the 168 bp site and a nucleotide indel in the 626 bp site; K16 and K17 both showed T substitution in the 512 bp site; K19 showed T substitution in the 168 bp site; K20 showed a indel in the 4 bp site.

Phylogenetic relationship based on the nrDNA ITS region: Based on the nucleotide similarity of K1, and K6-K13 and these sequence variations, K1, and K6-K13 were near with each other in the phylogenetic tree (Fig. 1). Especially, K1, and K6-K10 showed relatively high similarity rate, formed one group called Main Korea Group (MKG) in the phylogenetic tree. By the analysis from collection geographical regions, strains from Korea and Pakistan showed nearer phylogenetic relationship compared to strains from China, however, K2, K3, K4, and K5 strains from Korea showed higher sequence siminlarity rate with MCG. This result made us hypothesize that these four strains were introduced strains, transmitted from China and spread to In Jie and HwaCheon, Gangwon-do, Korea. Strains from Pakistan were introduced from Korea, and the genetic diversity could be explained by long-time environment evolution and adaptation.

Moreover, based on the nucleotide similarity of K4, and K15-K20 and these sequence variations, K4, and K15-K20 showed near phylogenetic relationship in the tree (Fig. 1). Especially, K15-K18, and K20 showed relatively high similarity rate, formed one group called Main China Group (MCG) in the phylogenetic tree. Seen from the analysis according to geographical regions, strains collected from China were divided into one group, and no introduced strain was found.

To construct a phylogenetic tree showing more accurate phylogenetic relationship, we only collected the 17 typical variable nucleotide sites only, as the analysis object for alignment (Fig. 2). A greater sequence divergence was found according to the alignment analysis, sharing only 50% similarity with each other. The highest dissimilarity rate appeared between K2 and other foxtail millet strains, and among other foxtail millet strains, two groups were clearly divided with 70% similarity. No surprisingly, K1, and K6-K13 formed one group, and K4, and K15-K20 with K3, K5, and K14 formed the other group (Fig. 2). K2 was very different in the nrDNA ITS region sequence from other strains, thus, divided as an independent and individual group. This result supported our previous hypothesis about K3, K4, K5 collected from Korea were originally transmitted from China, because these three strains showed nearer phylogenetic relationship with strains collected from China. Moreover, strains collected from Pakistan were grouped into MKG, supporting that Pakistan strains were originally from Korea. Long-time environment evolution and adaptation made some sequence variations occurring in the nrDNA ITS region, thus, Pakistan strain group showed only 88% similarity with the MKG (Fig. 2).

In conclusion, based on the sequence analysis of nrDNA ITS region, the phylogenetic relationship of foxtail millet strains showed clear grouping and had close relations with collected geographical region. Strains collected from Korea, Pakistan were very different from that collected from China, though there were some introduced strains originally transmitted from China among Korean strains. This work not only provided more sequence sources of ITS region of *Setaria* species but the basis for clearer discrimination of Asian foxtail millet strains.

Table 2. Symmetric matrix of Jaccard coefficients (% similarity) in total ITS1-5.8S-ITS2 region between 20 foxtail millet strains.

Strain	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	K12	K13	K14	K15	K16	K17	K18	K19	K20
K1	100																			
K2	98.9	100																		
K3	98.7	98.7	100																	
K4	98.9	98.3	99.2	100																
K5	99.5	98.7	99.0	99.4	100															
K6	99.8	98.7	99.1	98.7	99.7	100														
K7	99.1	98.1	99.4	99.1	99.0	99.4	100													
K8	99.2	98.3	99.2	98.9	99.2	99.5	99.8	100												
K9	99.2	98.2	99.5	99.4	99.2	99.5	100	99.8	100											
K10	99.1	98.1	99.4	99.1	99.0	99.4	100	99.8	100	100										
K11	98.9	98.1	98.1	98.6	99.2	98.7	98.1	98.3	98.3	98.1	100									
K12	98.9	98.3	98.6	98.4	99.2	99.1	98.4	98.6	98.7	98.4	98.4	100								
K13	99.2	98.6	98.9	99.1	99.7	99.4	98.7	98.9	99.1	98.7	99.2	99.2	100							
K14	98.7	98.1	98.4	98.4	99.5	98.9	98.3	98.4	98.9	98.3	98.6	99.0	99.1	100						
K15	99.4	98.6	99.2	99.5	99.5	99.2	99.2	99.2	99.4	99.2	98.9	99.2	99.5	99.0	100					
K16	98.4	98.1	99.1	99.2	98.6	98.3	98.9	98.7	99.0	98.9	98.1	98.1	98.6	97.9	99.8	100				
K17	99.2	98.7	99.4	99.4	99.4	99.0	99.0	99.0	99.2	99.0	98.7	99.0	99.4	98.9	99.8	100	100			
K18	99.4	98.6	98.6	99.1	99.4	99.1	98.4	98.6	98.7	98.4	98.9	98.9	99.4	98.7	100	99.2	99.8	100		
K19	98.6	97.9	98.9	99.7	99.0	98.4	98.7	98.6	99.0	98.7	98.3	98.3	98.7	98.1	99.8	99.5	99.7	99.4	100	
K20	99.5	98.7	99.0	99.0	99.8	99.7	99.0	99.2	99.2	99.0	99.2	99.0	99.7	99.4	99.5	98.6	99.4	99.4	98.7	100

Table 3. Typical variable nucleotide sites in the ITS region of 20 *Setaria italica* L. materials.

Strain	Nucleotide sequence variation															
	4 bp	10 bp	11 bp	51 bp	71-73 bp	165 bp	168 bp	181 bp	243 bp	250 bp	279 bp	296 bp	396 bp	450 bp	512 bp	626 bp
K1	-	-	-	C	CTT	G	G	G	G	G	G	G	C	T	C	T
K2	-	-	-	T	---	C	G	G	C	C	G	T	C	C	T	-
K3	-	C	A	T	---	G	G	G	G	G	G	G	C	C	T	-
K4	A	C	A	C	---	G	T	G	G	G	G	G	C	C	C	-
K5	-	-	-	C	---	G	T	G	G	G	G	G	C	C	C	-
K6	-	C	A	C	CTT	G	G	G	G	G	G	G	C	T	C	T
K7	-	C	A	C	CTT	G	G	G	G	G	G	G	C	T	C	T
K8	-	C	A	C	CTT	G	G	G	G	G	G	G	C	T	C	T
K9	-	C	A	C	CTT	G	G	G	G	G	G	G	C	T	C	T
K10	-	C	A	C	CTT	G	G	G	G	G	G	G	C	T	C	T
K11	-	-	A	C	CTT	G	G	A	G	G	T	G	T	C	C	T
K12	-	C	A	C	CTT	G	G	G	G	G	G	G	T	C	C	-
K13	A	C	A	C	CTT	G	G	G	G	G	G	G	T	C	C	T
K14	-	C	A	C	---	G	G	G	G	G	G	G	C	C	C	-
K15	A	C	A	C	---	G	G	G	G	G	G	G	C	C	C	T
K16	A	C	A	C	---	G	G	G	G	G	G	G	C	C	T	T
K17	A	C	A	C	---	G	G	G	G	G	G	G	C	C	T	T
K18	A	C	A	C	---	G	G	G	G	G	G	G	C	C	C	T
K19	A	C	A	C	---	G	T	G	G	G	G	G	C	C	C	T
K20	-	C	A	C	---	G	G	G	G	G	G	G	C	C	C	T

- means nucleotide indels or deletions compared to other non-deletion nucleotide sites

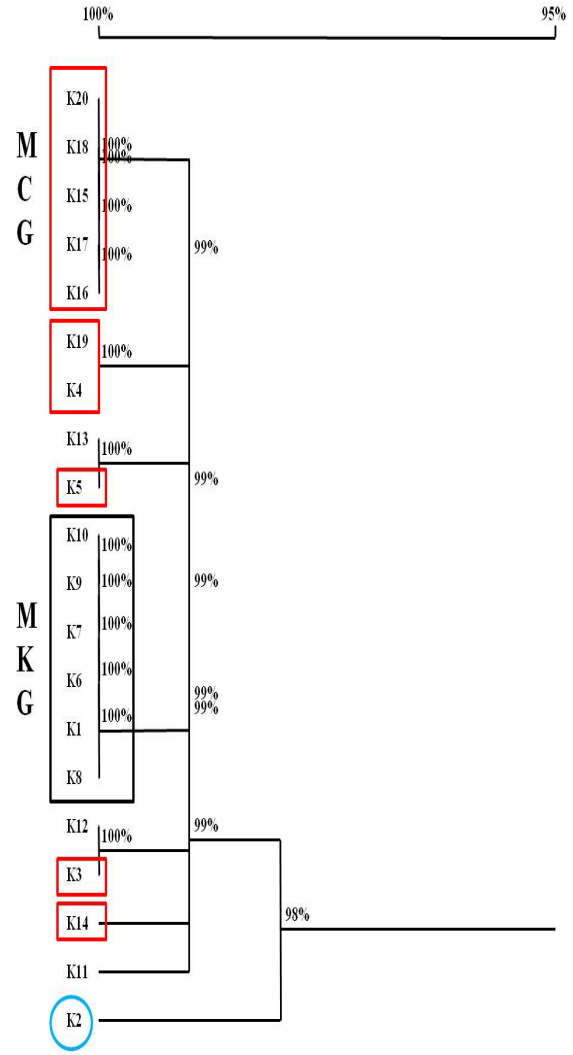

Fig. 1. Phylogenetic tree constructed by the total ITS region sequences of 20 *Setaria italica* L. materials. MCG: Main China Group; MKG: Main Korea Group.

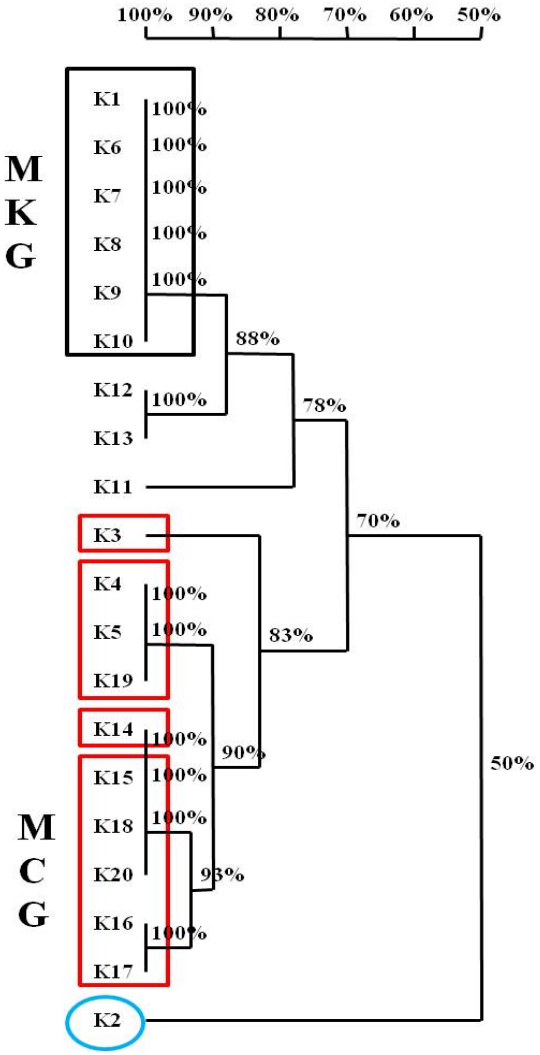

Fig. 2. Phylogenetic tree constructed by the typical variable nucleotide sites in ITS region sequences of 20 *Setaria italica* L. materials. MKG: Main Korea Group; MCG: Main China Group.

Acknowledgement

This study was supported by "Cooperative Research Program for Agriculture Science & Technology Development (Project title #PJ01177601, Project #PJ011776)" Rural Development Administration, Republic of Korea.

References

Barton, L., S.D. Newsome, F.H. Chen, H. Wang, T.P. Guilderson and R.L. Bettinger. 2009. Agricultural origins and the isotopic identity of domestication in northern China. *Proc. Natl. Acad. Sci. U. S. A.*, 106: 5523-5528.

Bettinger, R.L., L. Barton and C. Morgan. 2010. The origins of food production in north China: A different kind of agricultural revolution. *Evol. Anthropol.*, 19: 9-21.

Coleman, A.W. 2003. ITS2 is a double-edged tool for eukaryote evolutionary comparisons. *Trends Genet*., 19: 370-375.

Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull*., 19: 11-15.

Fukunaga, K., E. Domon and M. Kawase. 1997. Ribosomal DNA variation in foxtail millet, *Setaria italica* (L.) P. Beauv., and a survey of variation from Europe and Asia. *Theor. Appl. Genet.*, 95: 751-756.

Fukunaga, K., K. Ichitani and M. Kawase. 2011. rDNA polymorphism of foxtail millet (*Setaria italica* spp. *italica*) landraces in northern Parkistan and Afghanistan and in its wild ancestor (*S. italica* spp. *viridis*). *Genet. Resour. Crop Ev.*, 58: 825-830.

Fukunaga, K., Z. Wang, K. Kato and M. Kawase. 2002. Geographical variation of nuclear genome RFLPs and genetic differentiation in foxtail millet, *Setaria italica* (L.) P. Beauv. *Genet. Resour. Crop Ev.*, 49: 95-101.

Gupta, S., K. Kumari, P.P. Sahu, S. Vidapu and M. Prasad. 2011. Sequence-based novel genomic SSR markers for robust genotyping purposes in foxtail millet [*Setaria italica* (L.) P. Beauv.]. *Plant Cell Rep.*, 31: 323-337.

Jia, X., Z. Zhang, Y. Liu, C. Zhang, Y. Shi, Y. Song, T. Wang and Y. Li. 2009. Development and genetic mapping of SSR markers in foxtail millet (*Setaria italica* (L.) P. Beauv.). *Theor. Appl. Genet.*, 118: 821-829.

Joshi, K., P. Chavan, D. Warude and B. Patwardhan. 2004. Molecular markers in herbal drug technology. *Curr. Sci.*, 87:

159-165.

Jusuf, M. and J. Pernes. 1985. Genetic variability of foxtail millet (*Setaria italica* P. Beauv.). *Theor. Appl. Genet.*, 71: 385-391.

Kawase, M., K. Fukunaga and K. Kato. 2005. Diverse origins of waxy foxtail millet crops in East and Southeast Asia mediated by multiple transposable element insertions. *Mol. Genet. Genomics*, 274: 131-140.

Kawase, M. and S. Sakamoto. 1984. Variation geographical distribution and genetic of esterase isozymes in foxtail millet, *Setaria italica* (L.) P. Beauv. *Theor. Appl. Genet.*, 67: 529-533.

Kihara, H. and E. Kishimoto. 1942. Bastarde zwischen *Setaria italica* und *S. viridis* (in Japanese with German summary). *Bot. Mag.*, 20: 63-67.

Kim, E.J., K.J. Sa and J.K. Lee. 2011. Genetic variation of foxtail millet [*Setaria italica* (L.) P. Beauv.] among accessions collected from Korea revealed by AFLP markers. *Kor. J. Crop Sci.*, 56: 322-328.

Kim, E.J., K.J. Sa, K.C. Park and J.K. Lee. 2012. Study of genetic diversity and relationships among accessions of foxtail millet [*Setaria italica* (L.) P. Beauv.] in Korea, China, and Pakistan using SSR markers. *Genes Genom.*, 34: 529-538.

Le Thierry d'Ennequin, M., O. Panaud, B. Toupance and A. Sarr. 2000. Assessment of genetic relationships between Setaria italica and its wild relative S. viridis using AFLP markers. *Theor. Appl. Genet.*, 100: 1061-1066.

Lee, J.K. and N.S. Kim. 2007. Genetic diversity and relationships of cultivated and weedy types of *Perilla frutescens* collected from East Asia revealed by SSR markers. *Kor. J. Breed. Sci.*, 39: 491-499.

Li, H., C. Li and W. Pao. 1945. Cytological and genetic studies of the interspecific cross of the cultivated foxtail illet, *Setaria italica* P. Beauv., and the green foxtal millet, *S. viridis* L. *J. Am. Soc. Agron.*, 9: 32-54.

Li, Y. and S. Wu. 1996. Traditional maintenance and multiplication of foxtail millet (*Setaria italica* (L.) P. Beauv,) landraces in China. *Euphytica*, 87: 33-38.

Lu, T.L.D. 1999. The transition from foraging to farming and the origin of agriculture in China. Oxford: John and Erica Hedges. Archaeopress in Oxford. England, p. 233.

Meyer, C.P. and G. Paulay. 2005. DNA barcoding: error rates based on comprehensive sampling. *PLoS Biol.*, 3: e422.

Murai, M. and O. Ohnishi. 1996. Population genetics of cultivated common buckwheat, *Fagopyrum esculentum* Moench. X. diffusion routes revealed by RAPD markers. *Genes Genet. Syst.*, 71: 211-218.

Sakamoto, S. 1987. Origin and dispersal of common millet and foxtail millet. *Jpn. Agr. Res. Q.*, 21: 84-89.

Schontz, D. and B. Reather. 1999. Genetic variability in foxtail millet, *Setaria italica* P. Beauv.: Identification and classification of lines with RAPD markers. *Plant Breed.*, 118: 190-192.

Sun, J.M., X.M. Wang, D.J. Wang, Y.B. Yang, L.L. Duan, H. Li and H. Zhang. 2012. Research Progresses on SSR markers in foxtail millet. *Biotechnol.*, 22: 86-89 (in Chinese with English abstract).

Vavilov, N.I. 1926. Studies on the origin of cultivated plants. *Institute of Applied Botany and Plant Breeding, Leningrad*.

White, T.J., T. Bruns, S. Lee and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols-a guide to methods and applications*. (Eds.): Innis, M.A., D.H. Gelfand, J.J. Sninsky and T.J. White. Academic Press, San Diego, Calif, pp. 315-322.

(Received for publication 15 July 2015)