www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online; ISSN 1308-5301 Print 9/2 (2016) 24-29

Research article/Araştırma makalesi

ISSR variations of four populations of *Glycyrrhiza glabra* (Fabaceae)

Houshang NOSRATI [*1], MohammadAli HOSSEINPOUR-FEIZI [1], Marjan BAGHERI [1], Ahmad Razban-HAGHIGHI [2]

[1] Department of Plant Science, University of Tabriz, Tabriz, East Azerbaijan, Iran
[2] Research Centre for Agriculture and Natural Resources, Tabriz, East Azerbaijan, Iran

Abstract

Information on patterns and structure of populations' genetics in plants are useful in understanding breeding systems, population dynamics and designing conservation programs. *Glycyrrhiza glabra* L. (liquorice, Fabaceae) is a perennial plant and has high commercial uses for medicinal, flavouring and sweeting purposes. However, there is little information on the genetic structure of liquorice. In the current study the levels of genetic variations were investigated in four eco-geographically different populations of liquorice using Inter Simple Sequence Repeats (ISSRs) by randomly sampling 10 individual plants from each of population in East-Azerbaijan Province, Iran. The population genetic variation were measured based on Nei's and Shannon's information indices using Popgen, and the genetic similarity among the populations was studied by the clustering analysis of Unweighted Pair-Group Method with Arithmetical Averages (UPGMA). Total genetic variation was partitioned into within and among the populations using analysis of molecular variance (AMOVA) by Arlequin. A total of 139 polymorphic reproducible ISSRs loci were obtained. The percentage of polymorphic ISSRs loci ranged from 35.97% to 61.15%. Consequently, the range of within-population Nei's diversity varied from 0.172 to 0.301. Partitioning of total ISSRs variation by AMOVA showed that total genetic variation was equally partitioned to within-population and among-populations, indicating that the species has intermediate breeding systems by carrying out equally self- and outcrossing. Nei's based UPGMA dendrogram showed no ecological correlation among the populations. Moreover, the genetic similarity among populations was not correlated with the geographical distances (Spearman rho correlation, N=6, $P > 0.787$), while as expected, the populations size affected significantly on population genetic variation (Spearman, N=4, $P < 0.05$). This study emphasizes the application of conservation program in the study sites in order to prevent the potential risk of the species extinction.

Key words: conservation programs, genetic diversity, *Glycyrrhiza glabra*, ISSRs, population size

1. Introduction

Understanding the patterns of population genetic structure and the levels of genetic variations in plants are important for estimating the mode of breeding systems and also useful in designing conservation programs (Hamrick and Godt, 1989; 1996; Nybom and Bartish, 2000). Furthermore, studying the patterns of the environmental impact on populations' genetic structure in plants could be used to understand the adaptation patterns of plant populations to the environments (Fahima et al., 1999; Feder and Mitchell-Olds, 2003), the selective forces shaping the population genetic structure (Allnutt et al., 2001; 2003).

Studies have shown that there is relationship between levels of plant populations genetic variations and several factors including breeding systems (Hamrick and Godt, 1996; Nybom and Bartish, 2000), population's size (Frankham, 1996; Reed and Frankham, 2003), environmental stresses e.g. edaphic and climatic (Nevoet al., 1991; 1998) and floral morphology and display (Richards, 1986; Brunet and Echert, 1998). Outcrossing plant species have usually higher levels of within-population genetic variation while selfing species were shown to have the greatest values of between-population genetic variation (Hamrick and Godt, 1989). The population size has a major impact on levels of genetic variation and structure in plants. For instance, there is often pollination failure in small and fragmented plant populations, since small-sized populations are less attractive for pollinators (Jennersten 1988; Wilcock and Neiland, 2002; Andrieu et al., 2009). Habitat fragmentation and destruction and consequently decreasing the plant populations' size are mostly caused by human activities and misuses (Young and Clarke, 2000). This eventually can lead to genetic

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +984133392713; Fax.: +984133356027; E-mail: hnosrati@tabrizu.ac.ir
 BioDiCon. 480-0815

consequences e.g. genetic drift due to loss of genetic diversity, and therefore, can produce challenges and problems in conservation biology (Fahrig, 2003).

This work aimed to study the impact of both population size and geographical distance on the genetic patterns in eco-geographically different populations of *Glycyrrhiza glabra* in East Azerbaijan Province, Iran using ISSRs (Inter Simple Sequence Repeats) markers.

The genus *Glycyrrhiza* (Fabaceae) includes about 30 species, some of which, especially *G. glabra* L. (liquorice) have economic importance and commercial uses due to possessing glycyrrhizic acid, a triterpenoid saponin, in rhizomes and roots (Nomura et al., 2002). *G. glabra* is a hermaphrodite perennial herbaceous plant native to Eurasia, northern Africa and western Asia, and is widely cultivated for its rhizomes. The products of the rhizome are used as sweetening and flavouring agents in tobaccos, chewing gums, candies, and also as pharmaceutical products e.g. antiulcer, antihepatitis medicines and antitussives (Davis and Morris, 1991; Nomura et al., 2002).

Inter-simple sequence repeats (ISSRs) is a PCR-based technique, in which DNA segments between two microsatellite sites oppositely situated on double-strand DNA were amplified by a single 16-25bp primer complementary to microsatellite site (Reddy et al., 2002). The most limitations of the other DNA markers such as low reproducibility of RAPDs, high cost of AFLP, and requirement for preliminary knowledge on flanking primers sites of SSRs were resolved in ISSRs (Zietkiewiczet et al., 1994). The higher evolutionary rate of ISSRs has provided these markers as the best suitable markers for genetic studies at lower taxonomic levels (Reddy et al., 2002). ISSRs have been widely used for investigating of genetic similarities among population and accessions at different taxonomic levels i.e. intra-specific, inter-specific levels and cultivars (Bornet and Branchard, 2001; Rakoczy-Trojanowska and Bolibok, 2004; Li and Chen, 2008; Wang et al., 2009). More recently, ISSRs have been the markers of choice for population genetic studies in plants such as *Ipomoea batatas* (Moulin et al., 2012), *Michelia coriacea* (Zhao et al., 2012), *Allium* (Mukherjee et al., 2013), *Vicia* (Bozkurt et al., 2013), *Magnolia wufengensis* (Chen et al., 2014), *Abelmoschus esculentus* (Yuan et al., 2014) and *Gossypium* (Ashraf et al., 2016).

2. Materials and methods

2.1 Populations' studied

A number of ten individual plants were randomly sampled from each of four eco-geographically different regions in East-Azerbaijan Province, Iran. Sampling in each population was randomly carried out with a minimum distance of 200m between each individual plant. The four regions studied differ in terms of the edaphic and climatic conditions. The geographical distances between population pairs vary from 153 km to 315. One population was included from saline soil (Malekan), while the two other populations were sampled from cold areas (Khalkhal and Saivan), and the fourth population was included from temperate-warm climate and low altitude (Aslandoz).

2.2 DNA extraction, ISSRs-PCR amplification and analysis

Nuclear DNA was extracted from seeds and/or seedlings following Miller (2002) with replacement of silver sand by liquid nitrogen. The DNA concentration was measured by spectrophotometry, and wherever needed, adjusted at 10ng/ml. A number of ten ISSRs primers were examined, of which 5 primers produced the most polymorphic and clear reproducible bands, and therefore, were used in the study (Table 1). The ISSRs amplifications were repeated three times to insure the reproducibility. The ISSRs loci were scored as 1 for present and 0 for absent. Consequently, the obtained dataset were entered in a binary matrix for cluster analysis using the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System, ver. 2.02). The levels of genetic variation were measured for each population based on Nei's (1973) and Shannon's information index using Popgen, version 1.32. To study the genetic similarity among the populations, the UPGMA (Unweighted Pair-Group Method with Arithmetical Averages) dendrogram was generated based on matrix of Nei's distances between populations pairs through the SHAN (sequential, hierarchical, agglomerative and nested clustering of the NTSYS-pc). Total genetic variation was partitioned into within- and among-populations using analysis of molecular variance (AMOVA) in Arlequin. AMOVA (Excoffier et al., 1992) has widely used in ISSRs analysis to estimate between-population variation (Nybom and Bartish, 2000).

Table 1. The ISSRs-primers used for analyzing the population genetic variations in *Glycyrrhiza glabra*

Primer code	Primer sequence	Primer annealing time (s)
H2	5′-$(AC)_8$T-3′	52
H3	5′-$(AC)_8$G-3	52
H4	5′-$(AC)_8$CG-3′	52
E	5′-$(AG)_{10}$C-3′	50
F	5′-$(AG)_8$GC-3′	50

3. Results

Applying 5 ISSRs primers in 40 individual plants randomly sampled from four eco-geographically different populations of *Glycyrrhiza glabra* produced a total of 139 polymorphic reproducible bands (Figure 1). The lowest number and percentage of polymorphic ISSR loci were detected in Khalkhal population (50 and 35.97, respectively), while the highest values were revealed in Aslandoz population (85 and 61.15%). As a result, the Khalkhal population had the smallest within-population Shannon's and Nei's diversity (0.116 and 0.172, respectively), whereas the Aslandoz population possessed the highest values of these diversity (0.195 and 0.301) (Table 2).

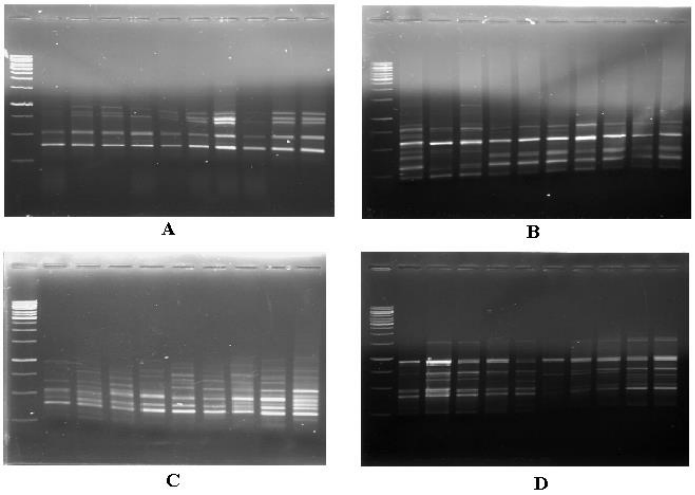

Figure 1. Samples of ISSRs patterns in different populations of *Glycyrrhiza glabra* L. produced by primer H2 (A= Aslandoz, B=Saivan, C=Malekan, D=Khalkhal)

Table 2. Number and percentage of polymorphic ISSRs loci, Nei's genetic diversity and Shannon information index in different populations of *Glycyrrhiza glabra*

Population name	No. polymorphic ISSRs loci	% Polymorphic ISSRs loci	Shannon's index (I)	Nei's gene diversity (h)
Aslandoz	85	61.15%	0.195	0.301
Saivan	69	49.64%	0.176	0.264
Malekan	59	42.45%	0.136	0.208
Khalkhal	50	35.97%	0.116	0.172
Total polymorphic bands=139				

AMOVA analysis showed that total ISSRs variation was equally partitioned to within- and among-populations (Table 3). UPGMA dendrogram conducted for genetic similarity among the populations clustered Malekan, Khalkhal and Saivan populations in one cluster, while Aslandoz population was situated in distinct branch (Figure 2).

Table 3. Analysis of molecular variance (AMOVA) within- and among-populations of *Glycyrrhiza glabra* using ISSRs

Source of variation	*d.f.*	SS	Est. Var.	%Genetic variation	*P* value
Among populations	3	327.425	9.910	50	< 0.01
Within populations	36	361.500	10.042	50	< 0.01

There was no correlation between the ISSRs-based genetic similarity and the geographical distances among populations of *G. glabra* (Spearman rho correlation, N=6, $P > 0.787$, SPSS, ver. 11.2, Table 4 and Figure 3). However, population size in *G. glabra* was found to be significantly correlated with percentage of polymorphic ISSRs loci, Shannon and Nei's diversities, since the larger populations e.g. Aslandoz had higher variations (61.15%, 0.195, 0.301, respectively) compared to small-sized populations e.g. Khalkhal (35.97%, 0.116, 0.172, respectively) (Spearman rho correlation, N=4, $P < 0.05$, SPSS, ver. 11.2).

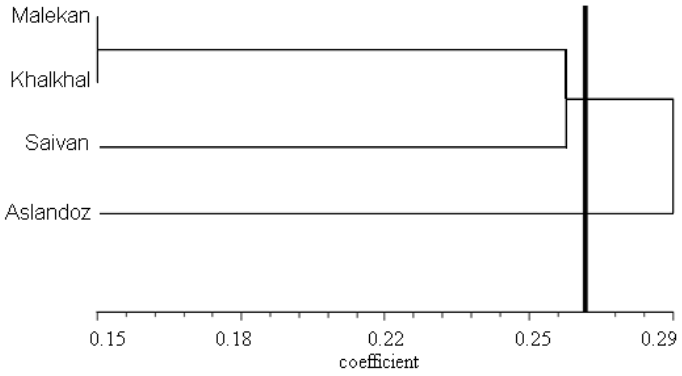

Figure 2. UPGMA dendrogram generated from Nei's genetic distances based on ISSRs loci showing similarity among different populations of *Glycyrrhiza glabra*

Table 4. Geographical and ISSRs-based genetic distances between pairs of populations of *Glycyrrhiza glabra*

Population pairs	Geographical distance (Km)	Genetic distance (Nei's)
Khalkhal- Aslandoz	276	0.252
Khalkhal- Saivan	229	0.236
Khalkhal- Malekan	251	0.147
Saivan- Aslandoz	201	0.289
Aslandoz- Malekan	315	0.318
Malekan- Saivan	148	0.285

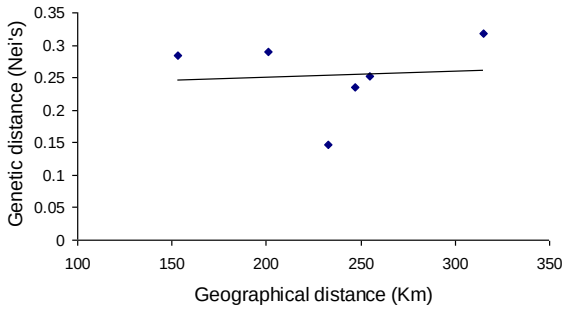

Figure 3. Lack of correlation between geographical and ISSRs-based genetic distances among populations of *Glycyrrhiza glabra* (Pearson rank correlation test, N = 6, $P > 0.877$, correlation coefficient = 0.082)

4. Conclusions and discussion

The current study showed that total ISSRs variation was equally allocated to within- and among-populations in *Glycyrrhiza glabra*. On the UPGMA dendrogram, nesting the three eco-geographically different populations of Saivan, Malekan and Khalkhal within one cluster indicated that there was no eco-geographical based relationship among populations of *G. glabra*, rather this similarity was most likely caused by random. This was further supported by lack of correlation between geographical and genetic distances among populations of *G. glabra*. This finding is incongruent with some empirical studies (e.g. Nevoet al., 1998; Fahima et al., 1999; Nianxi et al., 2006), which have shown that similarities in ISSRs pattern between populations of plant species have been caused due to natural selection, rather than random.

The genetic similarities between two different populations are caused by either gene flow due to geographical closeness or ecological similarities due to adaptive by the natural selection (Hamrick and Godt, 1989; 1996; Nybom and Bartish, 2000). However, since there is wide geographical distances among the populations of *G. glabra* investigated, it is most unlikely for gene flow to occur among the populations. Moreover, the results showed a lack of geographical correlations among populations in genetic similarity. These data indicate that ISSRs patterns are most likely under random genetic causes rather than by selective forces.

The positive correlation between ISSRs variation and population size revealed in the current study in *G. glabra* is consistent with the general long-known rule in the population genetics. The uncontrolled excessive collection of the roots of *G. glabra* for commercial purposes in the study regions resulted in gradual decline in populations' size. That is in turn has caused decreased ISSRs variations in the study sites. The decreased level of ISSRs diversity detected in small populations of *G. glabra* in the current study is most likely caused by genetic drift because in small populations random genetic drift is more strong (Nei et al., 1975; Rich et al., 1979), and consequently reduces genetic diversity of

the population through the generations, and increases the frequency of slightly deleterious recessive mutations (Lienert, 2004). As a result of lower genetic diversity, small populations have low ability to adapt to environmental change and consequently have a higher likelihood of extinction (Lande and Barrowclough, 1987). Moreover, inbreeding depression in such small populations increases (Frankham, 2003). That subsequently gives rise to decreased reproductive fitness and success (Lande, 1988; Reed and Frankham, 2003).

This study indicates the importance of genetic variation studies in the conservation strategies, and suggests the necessity of application the conservation programs to liquorice populations in the study sites.

References

Allnutt, T.R., Courtis, J. R., Gardner, M., Newton A. C. 2001. Genetic variation in wild Chilean and cultivated British populations of *Podocarpus salignus* D. Don (Podocarpaceae). *Edinburgh Journal of Botany*, 58: 459-473.

Andrieu, E., Dornier, A., Rouifed, S., Schatz, B., Cheptou, P. O. 2009. The town *Crepis* and the country *Crepis*: How does fragmentation affect a plant–pollinator interaction? *Acta Oecologia*, 35: 1-7.

Ashraf, J., Malik, W., Iqbal, M.Z., Ali-Khan, A., Qayyum, A., Noor, E., Abid, M.A., Naseer-Cheema, H.M., Ahmad, M.Q. 2016. Comparative analysis of genetic diversity among Bt cotton genotypes using EST-SSR, ISSR and Morphological Markers. *Journal of Agricultural Science and Technology*, 18: 517-531.

Bornet, B., Branchard, M. 2001. Nonanchored Inter Simple Sequence Repeat (ISSR) markers: Reproducible and specific tools for genome fingerprinting. *Plant Molecular Biological Reports*, 19: 209-215.

Bozkurt, M., Ertuğrul, K., Uysal, T. 2013. The determination of genetic relationships among some *Vicia* L. (Vetch) taxa by using ISSR markers. Biolo*gical Diversity and Conservation*, 6/3: 135-139.

Brunet, J., Echert, C.G. 1998. Effects of floral morphology and display on outcrossing in Blue Columbine Aquilegia caerulea (Ranunculaceae). *Functional Ecology*, 12: 596–606.

Davis, E. A., Morris, D. J. 1991. Medicinal uses of liquorice through the millennia the good and plenty of it. *Molecular Cell Endocrinology*, 78: 1-6.

Chen, L., Chen, F., He, S., Ma, L. 2014. High genetic diversity and small genetic variation among populations of *Magnolia wufengensis* (Magnoliaceae), revealed by ISSR and SRAP markers. *Electronical Journal of Biotechnology*, 17/6: 268–274.

Excoffier, L., Smouse, P.E., Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes application to human mitochondrial DNA restriction data. *Genetics* 131: 479-491.

Fahima, T., Sun, G. L., Beharav, A., Krugman, T., Beiles A., Nevo E. 1999. RAPD polymorphism of wild emmer wheat populations *Triticum dicoccoides* in Israel. *Theoretical* and *Applied Genetics*, 98: 434-447.

Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. *Annual Review* of *Ecology, Evolution, and Systematics*, 34: 487-515.

Feder, M. E., Mitchell-Olds, T. 2003. Evolutionary and ecological functional genomics. *Nature Reviews Genetics*, 4: 651-657.

Frankham, R. 1996. Relationship of genetic variation to population size in wildlife. *Conservation Biology*, 10: 1500-1508.

Frankhamn, R. 2003. Genetics and conservation biology. *Comptes rendus Biologies*, 326: S22-S29.

Jennersten, O. 1988. Pollination in *Dianthus deltoids* (Caryophyllaceae). Effects of habitat fragmentation on visitation and seed set. *Conservation Biology*, 2: 359-366.

Hamrick, J.L., Godt M.J.W. 1989. Allozyme diversity in plant species In. Brown A. H. D., Clegg M. T., Kahler A. L., Weir B. S. (Eds.) Plant population genetics breeding and genetic resources. *Sinauer Associates Sunderland MA, USA*.

Hamrick, J.L., Godt, M.J.W. 1996. Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London, Sec. B*, 351: 1292-1298.

Lande, R. 1988. Genetics and demography in biological conservation. *Science*, 241: 1455-1460.

Lande, R., Barrowclough, G.F. 1987. Effective population size genetic variation and their use in population management. In: Soule M. (ed) Viable populations for conservation. *Cambridge University Press, England.*

Li, H., Chen, G. 2008. Genetic relationship among species in the genus *Sonneratia* in China as revealed by inter-simple sequence repeat (ISSR) markers. *Biochemical Systematics and Ecology*, 36: 392-398.

Lienert, J. 2004. Habitat fragmentation effects on fitness of plant populations - a review. *Journal for Nature Conservation*, 12: 53-72.

Moulin, M.M., Rodrigues, R., Gonçalves, L.S.A., Sudré, C.P., Pereira M.G. 2012. A comparison of RAPD and ISSR markers reveals genetic diversity among sweet potato landraces (*Ipomoea batatas* (L.) Lam.). *Acta Scientifica*, 34/2: 139-147.

Mukherjee, A., Sikdar, B., Ghosh, B., Banerjee, A., Ghosh, E., Bhattacharya, M., Roy S.C. 2013. RAPD and ISSR analysis of some economically important species, varieties, and cultivars of the genus *Allium* (Alliaceae). *Turkish Journal of Botany*, 37: 605-618.

Nei, M., Maruyama, T., Chakraborty, R. 1975. The bottleneck effect and genetic variability in populations. *Evolution*, 29: 1-10.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the Academy of Natural Sciences of Philadelphia, USA*, 70: 3321-3323.

Nevo, E., Noy-Meir, I., Beiles A. 1991. Natural selection of allozyme polymorphism.micro-geographical spatial and temporal ecological differentiation in wild emmer wheat. *Israel Journal of Botany*, 40: 419-450.

Nevo, E., Baum, B., Beiles A., Johnson D.A. 1998. Ecological correlates of RAPD DNA diversity of wild barley *Hordeum spontaneum* in the Fertile Crescent. *Genetic Resources and Crop Evolution*, 45: 151-159.

Nianxi, Z., Yubao, G., Jinlong, W., Anzhi, R., Hua X. 2006. RAPD diversity of *Stipa grandis* populations and its relationship with some ecological factors. *Acta Ecological Sinaica*, 26: 1312-1319.

Nomura, T., Fukai, T., Akiyama, T. 2002. Chemistry of phenolic compounds of licorice (*Glycyrrhiza* species) and their estrogenic and cytotoxic activities. *Pure and Applied Chemistry*, 74: 1199-1206.

Nybom, H., Bartish, I.V. 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives in Plant Ecology, Evolution and Systematics*, 3: 293-114.

Rakoczy-Trojanowska, M., Bolibok, H. 2004. Characteristics and a comparison of three classes of microsatellite-based markers and their application in plants. *Cellular and Molecular Biology Letters*, 9: 221-238.

Reddy, P.M., Sarla, N., Siddiq, E.A. 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica*, 128: 9-17.

Reed, D.H., Frankham R. 2003. Correlation between fitness and genetic diversity. *Conservation Biology*, 17: 230-237.

Richards, A.J. 1986. Plant breeding systems. *George Allen and Unwin London, England*.

Rich, S.S., Bell, A.E., Wilson, S.P. 1979. Genetic drift in small populations of *Trifolium*. *Evolution*, 33: 579-584.

Wang, H.Z., Feng, S.G., Lu, J.J., Shi, N.N., Liu, J.J. 2009. Phylogenetic study and molecular identification of 31 *Dendrobium* species using inter-simple sequence repeat (ISSR) markers. *Science Horticulturae*, 122: 440-447.

Wilcock, C., Neiland, M.R.M. 2002. Pollination failure in plants why it happens and when it matters. *Trends in Plant Science*, 7: 270-277.

Young, A.G., Clarke, G.M. 2000. Genetics demography and viability of fragmented populations conservation biology. Cambridge University Press, Cambridge.

Yuan, C.Y., Zhang, C., Wang, P., Hu, S., Chang, H.P., Xiao, W.J., Lu, X.T., Jiang, S.B., Yu, J.Z., Guo, X.H. 2014. Genetic diversity analysis of okra (*Abelmoschus esculentus* L.) by inter-simple sequence repeat (ISSR) markers. *Genetic Molecular Research*, 13/2: 3165 -3175.

Zietkiewitcz, E., Rafalski, A., Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*, 20: 176-183.

Zhao, X., Ma, Y., Sun, W., Wen, X., Milne, R. 2012. High Genetic Diversity and Low Differentiation of Michelia coriacea (Magnoliaceae), a Critically Endangered Endemic in Southeast Yunnan, China. *International Journal of Molecular Science*, 13: 4396-4411.

(Received for publication 04 August 2015; The date of publication 15 August 2016)