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Genetic Diversity in Mazandaranian Native Cattle: A Comparison with Holstein Cattle, using ISSR Marker

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Abstract: This study was carried out to investigate genetic diversity in Mazandaranian native cattle population comparised to the Holstein breed, using Inter Simple Sequence Repeats (ISSR) marker. A total of 175 animals, including 71 native and 104 cattle of Holstein breed were screened. The extraction of DNA samples were carried out, using modified salting out method. A 19-mer oligonucleotide, $(GA)_9C$, was used as primer in PCR reactions. The PCR products showed 15 different fragments with length ranged from 120 to1600 bp in the two breeds.. Genetic variation indexes, including effective number of alleles, Shannon index, Nei's gene diversity and standard genetic distance were estimated, using POPGene software. Generally, the estimated genetic variation indexes showed low levels of diversity in the two breeds. However, Nei's gene diversity and Shannon index estimation was observed almost two folds in native cattle compared to Holstein breed. Less levels of diversity in Holstein cattle may be because of applying intensive selection programs. Conversely, native cattle have been less affected by selection. Therefore, it seems that Mazandaranian native cattle probably are better for breeding programs than Holstein cattle. Results showed that ISSR Markers are reliable and can be used in genetic diversity investigations.

Key words: Biodiversity, indigenous cattle, ISSR marker

INTRODUCTION

Breeds are commonly classified as indigenous and exotic, where indigenous breeds are mainly kept in low input low output production systems while exotic breeds are usually adapted to intensive, high-output systems and do not flourish in unimproved local production environments (Hoffman and Scherf, 2005). A period of traditional selection over the last few centuries has resulted in the establishment of numerous breeds. Then, in the quit recent past, more and more effective breeding programs have been implemented and led to an emphasis on a few specialized stocks. Consequently, breeds that are less suited to current needs tend to see their numbers reduced and to be eventually lost (Ollivier and Foulley, 2005). Today, Holstein cattle have become the pre-dominant dairy breed worldwide. There are about 7.5 million cattle in Iran and Holstein cattle in more than 90% of industrial dairy farms are used. Extensive use of artificial insemination has reduced the number of breeding sires and effective population size. Likewise, it has resulted the high level of inbreeding or homozygosity in herds. Replacement of local breeds with more productive ones (Holstein) has increased the number of endangered breeds globally. Many traditional European breeds have disappeared because of farmers focus on the new cattle breeds. Around 16% of them have become extinct and 15% - are rare or endangered. Losing genetic diversity is considered as the main reason of high level of breeds uniformity and will increase frequency of genetical defects and negatively affects fertility (FAO, 2000). The most famous Iranian cattle breeds are Sarabi, Golpayegani, Mazandarani and Sistani.The Mazandarani is a zebu type breed found in Northern Iran. They are kept for meat and milk production and are see in all colors (Mason, 1996). However, these native cattle breeds are being interbred or replaced by improved Western breeds, so seems all are endangered. The conservation of genetic variation is an essential component of many species management programs. Ultimately, genetic variation allows species to adapt the change of environmental conditions and respond to selection/breeding programs. To manage any

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biological resource effectively, researchers must identify the level of genetic variation within and among livestock populations. Since, the mid 1980s, genome identification and selection has progressed rapidly with the help of PCR technology. The large numbers of marker protocols that are rapid and require only small quantities of DNA have been developed. Each marker technique has its own advantages and disadvantages (Bornet and Branchard, 2001). Since 1994, new molecular marker techniques called Inter Simple Sequence Repeats (ISSR) has been available. ISSRs are semi arbitrary markers amplified by PCR in the present of one primer complementary to a target microsatellite. In such amplification period genome sequence information isn't required and leads to multi locus and highly polymorphous patterns (Bornet and Branchard, 2001). ISSR primers could be successfully used for the detection of new genomic loci and applied in a new way for genomic mapping, fingerprinting, gene tagging (Ye *et al*., 2005) and genetic diversity studies. Different studies have been performed on animals, using these markers (Triapitsyna and Glazko, 2005; Bannikova, 2004; Vaulin and Zakharov, 2008; Glazko *et al*., 1999; Lovenko, 2002; Gorodnaya and Glazko, 2006; Chatterjee and Mohandas, 2003). Furthermore, many studies have shown that, the approach can be used as a useful tool for the genetic diversity monitoring in different populations (or breeds) of animals (Ahani Azari *et al*., 2007; Kol and Lazebny, 2006) . In current study, GA-ISSR marker based on the Polymerase Chain Reaction (PCR), have been developed to establish the genetic diversity within and between Mazandaranian native and Holstein cattle.

MATERIALS AND METHODS

The blood samples from two different cattle breeds, Mazandaranian native cattle (n = 71) and Holstein (n = 104) were collected randomly. The used Holstein cattle were sampled from an industrial dairy cattle farm (Kabiri) on the north of Gorgan (Golestan Provience) and the native cattle belonged to Sabegh Mahaleh Village at the same region. The experiments was performed in Molecular Genetics Laboratory of Animal Science, Department of Gorgan University of Agricultural Sciences and Natural Resources from July 2008 to 20 January 2009. DNA was extracted, using modified salting out as described by Miller *et al.* (1988). Extracted DNA was dissolved in Tris-EDTA (TE) buffer. Extracted DNA was quantified by gel electrophoresis and its quality was verified by spectrophotometer. DNA samples stored at -20°C. In PCR reaction, a 19-mer oligonucleotide GAGAGAGAGAGAGAGAGAC (Glazko *et al*., 1999), was used. PCR was performed in a final volume of 25 μL, using PCR Master Mix kit (Sinagene™. Iran). Amplification was performed in a DNA thermal cycler (Biometra Thermo-Cycler (Personal™) with the following parameters: 94°C for 2 min; 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 2 min followed by final extension at 72°C for 10 min. PCR products were electrophoresed at 120 V in 2% agarose gel containing 1X Tris-Boric acid-EDTA (TBE) buffer. Twelve microliter of PCR products were loaded with 2 μL of tracking dye and run until the dye was scatted 10 cm from the wells. The profiles were stained with ethidium bromide (0.5 μg mL^{-1}) and documented by the gel documentation under UV and then were photographed. The amplicons later were used for constructing of binary file. Allele sizing for different DNA fragments was carried out by Dscan-ONE (1994-1997) software package (V1.31). Molecular data analyses were carried out using POPGene V1.31 software (Yeh *et al*., 1997). The following parameters were computed at the reference population level: expected heterozygosity (Nei, 1978) as:

$$H = 1 - \sum_{i} P_i^2 \quad (1)$$

where, p_i is the frequency of the ith allele.

Effective alleles number (Hartl and Clark, 2007) as:

$$ne = 1/\sum_{i} P_i^2 \quad (2)$$

Shannon index (Shannon and Weaver, 1949) as:

$$I = -\sum_{i} P_i \ln(P_i) \quad (3)$$

Standard genetic distance (Nei, 1978) as:

$$D = -\ln\left[\left(\sum_{j}^{r}\sum_{h}^{mj} x_{ij}\, y_{ij}\right) \Big/ \sqrt{\left(\sum_{j}^{r}\sum_{i}^{mj} x^2_{ij}\right)\left(\sum_{j}^{r}\sum_{i}^{mj} y^2_{ij}\right)}\right] \quad (4)$$

where, x_{ij} and y_{ij} are frequency of the ith allele in jth locus in x and y populations, mj refers to number of alleles in j locus and r is the number of loci.

All the locus-population combinations were tested for deviations from Hardy-Weinberg equilibrium (HWE), using Chi-square (χ^2) goodness of fit test.

RESULTS

An ISSR-PCR pattern obtained by GA-ISSR marker is shown in Fig. 1. The observed amplicon patterns were complete and clear by contrasting. In the patterns of PCR

products totally 15 different fragments were found, showing different length between 120 to 1600 bp. The fragments size (bp) and frequency, accompanied with Chi-square and G^2 (likelihood ratio) tests for evaluating gene frequencies homogeneity between two breeds (Yeh *et al*., 1997) are presented in Table 1.

The number of polymorphic loci in native and Holstein herds were 6 and 3, respectively. The monomorphic fragments (frequency= 1) were 9 fragments in native cattle, while it was 11 fragments in Holstein breed. The monomorphic fragments between the two breeds were the same in 9 fragments and differ in 5 fragments. Gene frequency differences between two breeds were significant ($p<0.05$) for fragments number 2, 4, 6, 11 and 14 (Table 1). Fragment number 9 (525-590 bp) was observed only in native cattle. The proportion of polymorphic fragments in Mazandaranian and Holstein breeds were 0.46 and 0.20, respectively. To analysis inter population diversity four parameters, including number of observed alleles, effective number of the alleles, Nei's gene diversity and Shannon index were estimated using POPGene software (Table 2).

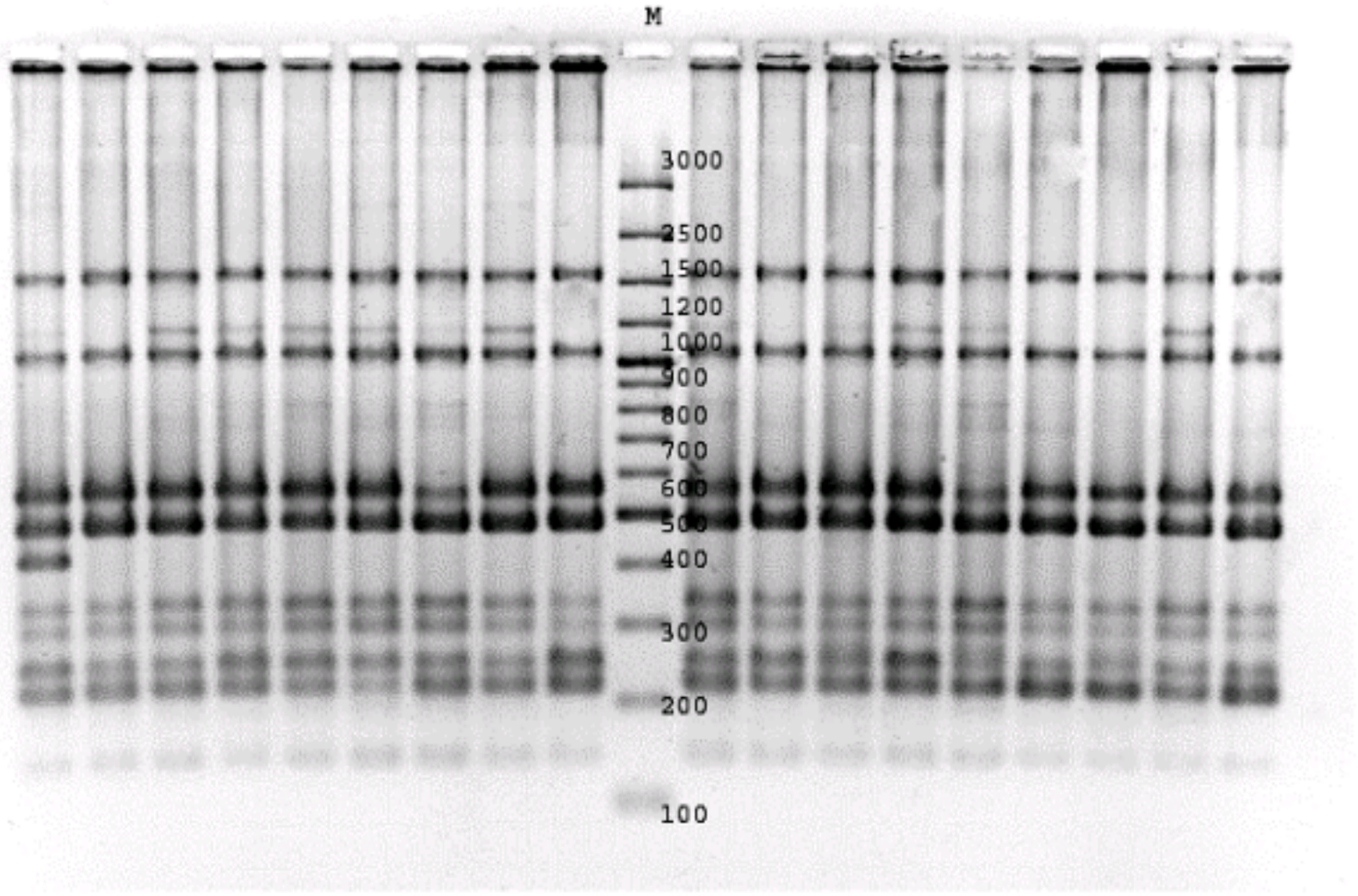

Fig 1: ISSR-PCR pattern, obtained by GA-ISSR marker, M: GeneRuler™ bp DNA Ladder standard marker

Table 1: Fragment length, gene frequencies, G2 and chi-square test results for evaluating gene frequencies homogeneity in two cattle breeds

		Fragment frequency		Tests	
Fragments (locus)	Band size range (bp)	Mazandaranian	Holstein	G^2	Chi-square
1	120-160	1.000	1.000	0(1.000)	0 (1.000)
2	175-205	0.556	1.000	67.53 (0.000)	56.33 (0.000)
3	207-230	1.000	1.000	0 (0.000)	0 (1.000)
4	235-280	0.606	1.000	58.52 (0.000)	48.71 (0.000)
5	285-315	0.606	0.741	3.49 (0.061)	3.53 (0.06)
6	320-370	0.152	0.675	47.98 (0.000)	44.79 (0.000)
7	390-440	1.000	1.000	0 (1.000)	0 (1.000)
8	460-520	1.000	1.000	0 (1.000)	0 (1.000)
9	525-590	0.029	0.000	3.69 (0.055)	3 (0.083)
10	600-650	1.000	1.000	0 (1.000)	0 (1.000)
11	700-760	1.000	0.861	15.89 (0.000)	10.73 (0.001)
12	765-850	1.000	1.000	0 (1.000)	0 (1.000)
13	980-1080	1.000	1.000	0 (1.000)	0 (1.000)
14	1100-1200	0.161	1.000	161.84 (0.000)	132.33 (0.000)
15	1400-1600	1.000	1.000	0(1.000)	0 (1.000)

Values in the brackets are the probabilities. Values less than 0.05 are significant.

Table 2: The mean of genetic diversity indexes in two breeds

Breed	N	Na	Ne	H	I
Mazandaranian	71	1.4±0.50	1.24 ±0.38	0.14±0.20	0.21 ±0.28
Holstein	104	1.2 ±0.41	1.11 ±0.25	0.07±0.15	0.11 ±0.22

N: Number of animals, Na: Effective allele number, Ne: Observed allele number, H: Nei's gene diversity, I: Shannon index. Data are expressed as Mean±SD

Based on Table 2, all of the mentioned parameters in native cattle are more than Holstein breed. High standard deviation estimates are mainly related to low number of detected fragments and number of the used animals (Nei, 1978).

DISCUSSION

This is the first attempt to specifically quantify the genetic diversity of the Mazandaranian native cattle with ISSR markers. Based on the results Mazandaranian native and Holstein were different in the fragment size and frequency. Some fragments were found in both breeds but didn't show the same frequency. The fragment number 9 was not detected in Holstein but since, it's frequency was very low (0.029) can't be used as an important breed-specific marker. Based on allelic frequencies (Table 1) the two investigated breeds did not show high polymorphic bands and lower polymorphism rate in Holstein than Mazandaranian native cattle could be due to higher genome uniformity. Low levels of polymorphic fragments in other breeds investigated by the same marker, was reported by Ahani Azari *et al*. (2007). In the present study, most of the loci-population combinations deviated from HWE were in agreement with some prior studies (Rachagani *et al*., 2006; Elbeltagy *et al*., 2008; Santos-silva *et al*., 2008). Means of heterozygosity and effective number of alleles in indigenous cattle (Table 2) were more than Holstein breed. Furthermore, Shannon genetic diversity in Mazandaranian and Holstein cattle were 0.21 and 0.11, respectively (Table 2). On the other hand, genetic diversity estimation was high in Mazandaranian cattle compared to Holstein breed (0.21 and 0.11, respectively (Table 2)). As well as, Nei's gene diversity estimation in Mazandaranian cattle was almost two folds of Holstein cattle. Consequently, as expected, all genetic diversity indexes in native cattle were higher than Holstein. Findings by Ahani Azari *et al*. (2007) reported more genetic diversity indexes in the breeds of *Bos taurus* and *Bos indicus* than Holstein. Furthermore, similarity indices of animals in these breeds were high. Present results indicated that because of intensive selection inbreeding programs in Holstein, it's genetic diversity has been lost. Generally, reduction of the number and genetic diversity of other breeds have been happened in the past decades. In the year 2000, over 6300 breeds of domesticated livestock were identified. Of these, over 1300 are now extinct or considered to be in danger of extinction. Many others have not been formally identified and may disappear, before they are recorded or widely known. Europe records the highest percentage of extinct breeds or breeds at risk (55% for mammalian and 69% for avian breeds). Asia and Africa record only 14 and 18%, respectively, but the data for developing countries are much less fully documented in the World Watch List for Domestic Animal Diversity than those of developed countries (Hoffman and Scherf, 2005). The biological unit for conservation in domesticated animals is usually the breed. Obtaining information from molecular markers in different breeds made it possible to create a hypothetical scenario for assessing different methods of analyzing diversity for conservation (Solis *et al*., 2005). Genetic distance of the two breeds was estimated 0.105 and no considerable. Although, estimated genetic diversity indexes in Mazandaranian cattle were lower than expected values, but were higher than Holstein breed. These results may be because of sampling of the native animals in a limited area and undesirable inbreeding. With the relatively small total population size and small individual flock sizes, genetic drift is an important factor affecting within-breed genetic diversity, so it is expected that random gene frequency changes would be cumulative over generations (Maiwashe and Blackburn, 2004). The balance between drift, natural and artificial selection and mutation needs further evaluation for the indigenous breed. Since, designing any selection program at the first step needs knowledge of the genetic diversity of the herds, specially pedigree-less ones, conducting of such researches seems very essential. Meanwhile, it should be emphasized that genetic distances between breeds provide an initial guide for conservation decisions, which need to be completed by more detailed characterization. In conclusion, the marker (ISSR-GA) indicated genetic diversity indexes successfully and will be useful in other researches that, screening more number of animals. Since, the use of ISSR-PCR markers on biodiversity of the animals are rare and usually other markers are used, it is suggested to increase the number of ISSR primers and investigated animals and perform comparative analysis with other molecular markers to obtain reliable results. In this way, may test the potential of this marker in monitoring genetic variability of animals.

CONCLUSION

Results of this study clearly showed that genetic diversity in both investigated cattle breeds, especially in Holestein breed, has been lost. Although, sampling of the studied native cattle was conducted in a limited region and animals undergo close matings and inbreeding at some rate, but all the genetic diversity indexes in native

cattle were better than Hostein breed. Furthermore, earlier studies were in agreement with our findings. Consequently, for avoiding negative effects of genome uniformity, extinction of breeds and inbreeding defects, it is suggested permanent investigating genetic variability in all domestic species with more reliable molecular markers. Conducting such researches certainly helps specialists to design selection breeding programs more carefully in the future and prevents or limits the trend of diminishing genetic variability in breeds of livestock.

REFERENCES

Ahani Azari, M., O.E. Lazebny and G.E. Sulimova, 2007. Determination of heterozygosity level in fifteen various cattle breeds using ISSR-PCR method. Proceedings of the 5th National Biotechnology Congress of Iran. Summit Metting Conference Hall, Nov. 24-26, Tehran, Iran, pp: 1-1.

Bannikova, A.A., 2004. Molecular markers and modern phylogenetics of mammals. Zh Obshch Biol., 65: 278-305.

Bornet, B. and M. Branchard, 2001. Nonanchored inter simple sequence repeat (ISSR) Markers: Reproducible and specific tools for genome fingerprinting. Plant Mol. Biol. Rep., 19: 209-215.

Chatterjee, S.N. and T.P. Mohandas, 2003. Identification of ISSR markers associated with productivity traits in silkworm, Bombyxmoril. Genome, 46: 438-447.

Elbeltagy, A.R., S. Galal, A.Z. Abdelsalam, F.E. El Keraby, M. Blasi and M.M. Mohamed, 2008. Biodiversity in Mediterranean buffalo using two microsatellite multiplexes. Lives. Sci., 114: 341-346.

FAO, 2000. Global project for the maintenance of domestic animal genetic diversity (MoDAD). http://www.fao.org/dad-is/.

Glazko, V.I., T.N. Dyman, S.I. Tarasiuk and A.V. Dubin, 1999. The polymorphism of proteins, RAPD-PCR and ISSR-PCR markers in European and American bison and cattle. Tsitol Genet., 33: 30-39.

Gorodnaya, A.V. and V.I. Glazko, 2006. Population-genetic study of the polymorphism of structural genes and ISSR-PCR markers in some cattle breeds. Cytol. Genet., 40: 49-57.

Hartl, D.L. and A.G. Clark, 2007. Principles of Population Genetics. 4th Edn., Sinauer Associates, Sunderland, MA.

Hoffman, L. and B. Scherf, 2005. Animal genetic resources-time to worry? Lives. Rep., 1: 57-74.

Kol, N.V. and O.E. Lazebny, 2006. Polymorphism of ISSR-PCR markers in Tuvinian population of Reindeer Rangifer tarandusl. Rus. J. Genet., 42: 1469-1466.

Lovenko, V.N., 2002. Genetic diversity of protein markers in sheep population from Ukraine. Genetika, 38: 1669-1676.

Maiwashe, A.N. and H.D. Blackburn, 2004. Genetic diversity in and conservation strategy considerations for Navajo Churro sheep. Anim. Sci., 82: 2900-2905.

Mason, I.L., 1996. A World Dictionary of Livestock Breeds, Types and Varieties. 4th Edn., CAB International Wallingford, UK., pp: 273.

Miller, S.A., D.D. Dykes and H.F. Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleate cells. Nucl. Acids Res., 16: 1215-1215.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89: 583-590.

ONE-Dscan, 1994-1997. One-Dimensional gel analysis Scanalytics deviation of CSP. Scanalyticas Attach processor System.

Ollivier, L. and J.L. Foulley, 2005. Aggregate diversity new approach combining within-and between-breed genetic diversity. Lives. Prod. Sci., 95: 247-254.

Rachagani, S., I.D. Gupta, N. Gupta and S.C. Gupta, 2006. Genotyping of β-lactoglobulin gene by PCR-RFLP in Sahiwal and Tharparkar cattle breeds. BMC Genet., 7: 31-37.

Santos-silva, F., F.S. Ivo, M.C.O. Sousa, M.I. Carolino, C. Ginja and L.T. Gama, 2008. Assessing genetic diversity and differentiation in Portuguese coarse-wool sheep breeds with microsatellite markers. Small Rum. Res., 78: 32-40.

Shannon, C.E. and W. Weaver, 1949. The Mathematical Theory of Communication. 1st Edn., University of Illinois Press, Urbana, IL., ISBN-10: 0252725484.

Solis, A., B.M. Jugo, J.C. MeRiaux, M. Iriondo, N.L.I. Mazo, A.I. Aguirre, A. Vicario and A. Estomba, 2005. Genetic diversity within and among four South European native horse breeds based on microsatellite DNA analysis: Implications for conservation. Heredity, 96: 670-678.

Triapitsyna, N.V.I. and V. Glazko, 2005. Polymorphism of DNA fragments flanked by microsatellite loci (ISSR-PCR) in cattle reproduced under low-dose irradiation conditions. Tsitol Genet., 39: 41-50.

Vaulin, O.V. and I.K. Zakharov, 2008. Temporal dynamics and variation of multi locus ISSR-PCR DNA markers in the Uman population of Drosophila melanogaster over two decades. Genetika, 44: 359-365.

Ye, C., Z. Yu, F. Kong, S. Wu and B. Wang, 2005. R-ISSR as a new tool for genomic fingerprinting, mapping and gene tagging. Plant Mol. Biol. Rep., 23: 167-177.

Yeh, F.C., R.C. Yang, T. Boiley, Z.H. Ye and J.X. Mao, 1997. POPGENE, the User-Friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Center, University of Alberta, Canada.