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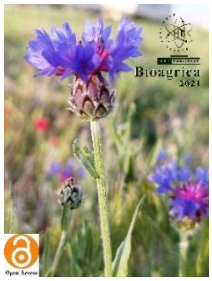

Genetic diversity and a germplasm survey of some selected tea genotypes from west of Mazandadaran, Iran

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Abstract

One of the most important crops in the north of Iran is Tea (*Camellia sinensis* (L.) O. Kuntze), Today, many of these plants are at risk of disappearing therefore having information about genetics can help in designing breeding programs. In this investigation, the genetic diversity of 23 tea plants from west of Mazandaran Province, one of the important regions of producing tea in Iran, and two Iranian cultivars were studied using 21 quantitative and qualitative morphological traits and 10 ISSR markers. In morphological analyses, eigenvalues of the first eight PCs were greater than one indicating that those eight PCs significantly contributed to the variation in the genotypes studied. According to the data, the samples' similarity ranged from 0.121 to 0.543 with an average of 0.367. In cluster analyses, samples were placed in four groups at 0.28 of coefficient dissimilarity. In ISSR analyses polymorphism percent and PIC analysis ranged between 54.55-100 and 0.35-0.49 respectively. From these results, it can be seen that these primers can detect genetic differences very well. The similarity matrix showed range of similarity was from 0.344 to 0.920 with an average of 0.640. The 25 tea genotypes were grouped into four groups by UPGMA cluster analysis based on ISSR data. In a two-dimensional plot (2D plot) generated from PCoA that was generated based on ISSR data of studied samples, four groups were obtained. From the results of this study, two points can be concluded: (1) Morphological and ISSR markers are useful instruments for the Identification of differences between tea samples, and (2) There is high genetic diversity between tea plants due to the sexual reproduction of tea plants in the past in Iran.

1. Introduction

One of the oldest caffeinated soft drinks in the world is the tea plant with the scientific name *Camellia sinensis* (L.) O. Kuntze from the Theaceace family. Tea plants were originated from southwestern China, Yunnan province (Hashimoto and Simura 1978; Fulian 1986). people's economic life in several Asian and African countries, including China, India, Sri Lanka, Kenya, Iran, etc. directly linked to Tea cultivation and industry. Three jats including Betjan, Dhonjan, and Rajghur are made the basis of Iran tea germplasm (Ahmadishad *et al*., 2009). Initially, the tea

plant was imported to Iran, this plant was propagated by seeds and it should be noted that almost all existing tea gardens are the result of this type of propagation. As stated in the sources, the tea plant has self-incompatibility, which made this plant show outcrossing behavior (Bali *et al*., 2013). Therefore, all the plants in a garden can be genetically different from each other. Due to these cases, the tea plant has a large germplasm that needs to be identified by different methods. Since the identification of germplasms is generally done by markers, it is more desirable to use markers that have shown their effectiveness. Since molecular markers are less affected by environmental factors and are also unlimited in number, their use in identifying and studying the genetic relationships of plants is very common.

Several molecular markers such as RFLP (Devarumath *et al*., 2002), RAPD (Falakro and Khiavi, 2020, Roy and Chakraborty, 2009 and Falakro *et al*., 2020), SRAP (Khiavi *et al*., 2020a), SCot (Chaeikar *et al*., 2020), AFLP (Paul *et al*., 1997), and PCR-RFLP genome Chloroplasts (Khiavi *et al*., 2020b) have been used to study the genetic diversity of tea. ISSS molecular marker has been widely used in tea plants and all these studies have shown the high efficiency of this marker in studying the genetic diversity of tea plants (Khiavi *et al*., 2020a, Devarumath *et al*., 2002, Roy & Chakraborty, 2009 and Falakro and Khiavi, 2020). The use of morphological markers to study germplasms along with molecular markers is also common. The use of morphological markers to study germplasms along with molecular markers is common and this marker has been used in many studies and many different plants (Khiavi *et al*., 2016; Parvathaneni *et al*., 2011; Sedaghatfar *et al*., 2012; Guliyev *et al*., 2018; Chen *et al*., 2020). In the tea plants, the morphological markers have been used well (Piyasundara *et al*., 2008; Khiavi *et al*., 2020a,b; Rajanna *et al*., 2011; Karthigeyan & Sud 2010; Mahmood *et al*., 2010). The overall purpose of this study is to identify the genetic diversity of some tea genotypes from the western region of Mazandaran using the ISSR and morphological markers to help protect the existing germplasm of tea and be a step towards designing future breeding programs.

2. Materials and methods

2.1. Plant material

Twenty-three selected tea plants from the western region of Mazandaran (Ramsar & Tonekabon Counties) that were planted in the collection of Shahid Chamran Tea Research Station in Neshtarood and two newly introduced cultivars (Kashef & Lahij) were selected for this study. Samples were coded from G1 to G23, S1 for Lahij, and S2 for Kashef. Figure 1 shows the study areas from which samples were collected.

2.2. Morphological Traits Analyses

To investigate genetic diversity based on morphological data, 21 traits including five quantitative and 16 qualitative traits were studied. The studied traits were selected based on the description letter submitted by the International Institute of Plant Genetic Reservoirs (IPGRI) (IPGRI, 2000). The studied traits are given in Table 1.

The characters were recorded and then they were analyzed. Past-PC, v. 4.03 software was used for cluster analyses by the Gower coefficient and UPGMA Algorithm. To depict non-hierarchical relationships among the samples, Principal components analysis (PCA) was used.

Eigenvalues and eigenvectors were calculated by SAS-ver 8.1 and the Bi-plot cluster was designed by MultiVariate Statistical Package (MVSP) software.

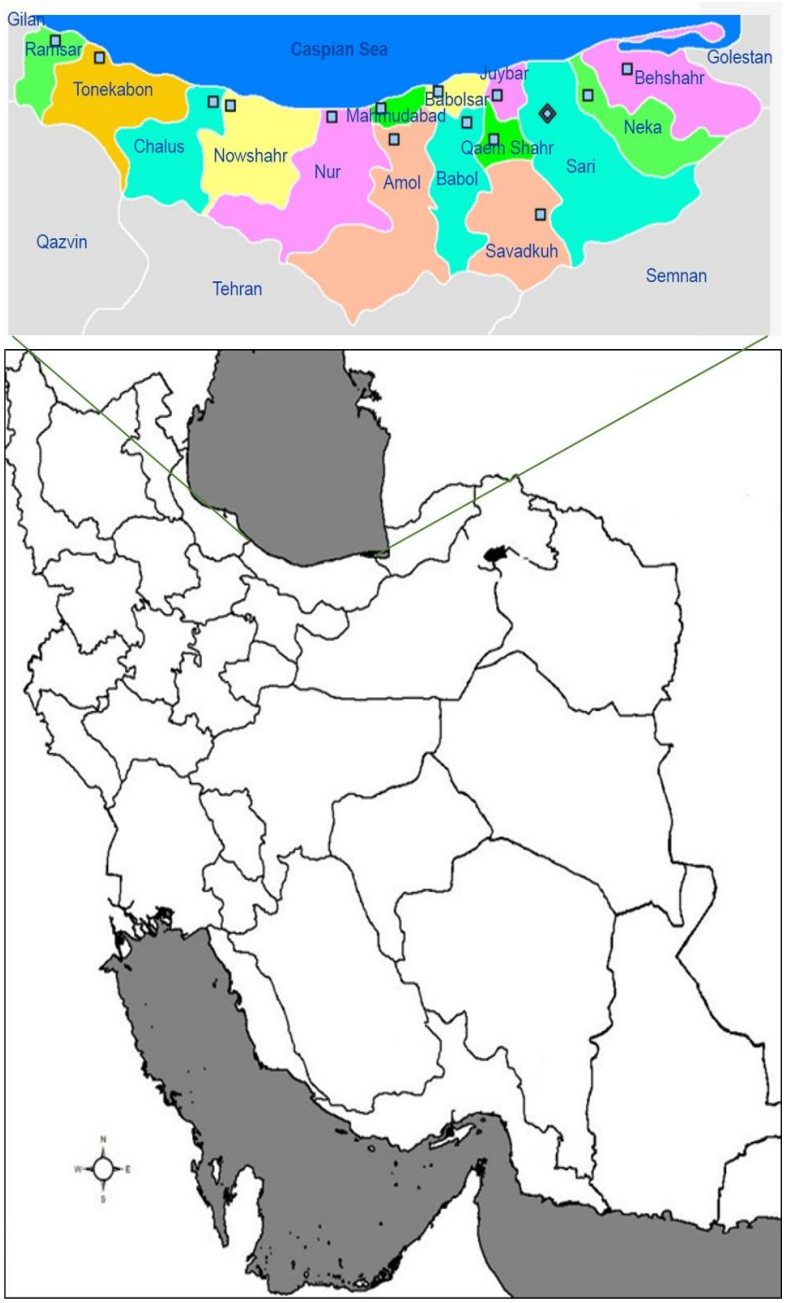

Figure 1. Collection areas of samples cultivated in the collection of Shahid Chamran Tea Research Station in Neshtarood (Tonekabon and Ramsar Counties).

2.3. ISSR Analyses

Young and fully expanded leaves were selected and stored at -80℃ until they were used. genomic DNA was extracted by using the Dellaporta method (Dellaporta *et al*. 1983) with minor modifications. The spectrophotometric method and agarose gel electrophoresis were used to investigate the quantity and quality of extracted DNA. For ISSR amplification, 20 primers were screened using four DNA samples, and 10 better primers were selected for use in the present investigation. DNA template (40ng) was used for PCR amplification by adding 0.2 mM of each of

four dNTPs, 1 µM of primer, 2.5µl of 10X Taq buffer (containing $MgCl_2$), and 1 unit of Taq DNA polymerase, in a final volume of 25µl. The PCR was carried out with a Bio-Rad thermocycler in a condition that initial denaturation at 94℃ for 4 min, 35 cycles of 1 minute at 94℃, 45 seconds at 54℃, 2 minutes at 72℃ and a final extension for 7 minutes at 72℃ then brought down to 4℃.

Table 1. Investigated morphological characters.

characters	Row	characters	Row
Internode length	*1*	*Length/ Width of mature leaf*	*12*
Pigmentation in young leaves (In growth season)	*2*	*Length of mature leaf*	*13*
Pigmentation in young leaves (In dormant season)	*3*	*Width of mature leaf*	*14*
Immature leaf color	*4*	*Leaf angle*	*15*
Mature leaf color	*5*	*Leaf venation*	*16*
Leaf shape	*6*	*Leaf pose (angle)*	*17*
Leaf upper surface	*7*	*Leaf waxiness*	*18*
Leaf apex shape	*8*	*Petiole color*	*19*
Leaf apex habit	*9*	*Length of mature leaf petiole*	*20*
Leaf base shape	*10*	*Young shoot color*	*21*
Leaf margin	*11*		

The amplification fragments were fractionated in 1.5% agarose gel electrophoresis in 1X TBE buffer at a constant voltage (70V), for 2.5 hours. Fragments were visualized under UV light by using a safe stain. The DNA size marker of 1 kb was used for analyzing the size of polymorphic bands. Fragments resolved on gels were scored as 1 or 0 for presence or absence, respectively. The similarity matrix was calculated by using Dice's coefficient with the UPGMA algorithm. The dendrogram was drawn using the SAHN module in NTSYSpc v.2.2 software (Rohlf, 2008; Nargesy Dehdasht *et al*., 2019).

3. Results and discussion

3.1. Morphological Analysis

Characterization of selected tea samples was done based on IUPGRI's recommended descriptor for tea (IPGRI, 2000). Multivariate statistical techniques such as Principal component analysis (PCA) and dendrogram analysis are generally used for characterization and genetic diversity evaluation of germplasm and can increase the resolution of explanation of information generated in characterization studies (Piyasundara *et al*., 2008).

Eigenvalues of the first eight PCs were greater than one (see Table 2), indicating that those eight PCs significantly contributed to the variation in the genotypes studied. The mean values of 20 morphological descriptors of 25 germplasm accessions were subjected to PCA and the eigenvalues showed that the first eight principle components accounted for about 79.24% of the total variation (Table 2). The Pc1 has been calculated as about 16.96% of the total variation, it was mainly associated with the length of the mature leaf, Length of mature leaf petiole, Leaf angle, Leaf venation, and Leaf pose (angle). The PC2 which was calculated for 16.18% of the total variation was related to the length/ Width of the mature leaf, Leaf upper surface, Leaf base shape, Leaf venation, and Young shoot color. The PC3 which was calculated for 12.61% of the total variation was shown related to the length of the mature leaf, pigmentation in young leaves (In growth season), immature leaf color, and mature leaf color.

As can be seen, leaf traits were highly effective in showing diversity. Erxu *et al*. (2009), also, were reported that a morphometrical analysis of leaf morphology is a useful and rapid method for the identification of species. Shah *et al*., 2008 and Hu, 2004 noted that leaf characters are a powerful tool in separating and identifying species in morphologically unstable plant groups like Taxus and *Camellia*.

Table 2. Eigenvalues of the correlation matrix based on the PCA of the 20 morphological characters.

PC	Eigenvalues	Percentage	Cum. Percentage
1	*3.39*	*16.96*	*16.96*
2	*3.24*	*16.18*	*33.14*
3	*2.52*	*12.61*	*45.75*
4	*1.79*	*8.97*	*54.71*
5	*1.36*	*6.80*	*61.51*
6	*1.33*	*6.67*	*68.17*
7	*1.17*	*5.83*	*74.00*
8	*1.05*	*5.24*	*79.24*

The results of the principal component analysis of the present study were similar to the results of the above studies. In other studies, Rajanna *et al*., (2011) and Piyasundara *et al*., (2008) PC1 calculated 29.84% and 14.93% and PC2 accounted for 19.83 and 14.36 respectively. The most important traits in the investigations of Rajanna *et al*., (2011) and Piyasundara *et al*., (2008) that had the most roles in creating differences in the PC1 and PC2 were leaf-related traits, which are similar in our results. No specific groupings were obtained from a two-dimensional plot (2D plot) generated from PCA. The distribution of samples based on the PC1 and PC2 is almost similar; it can be seen in Figure 2. But three samples (N3, N6, and N16) were placed at a distance from other samples. This mode of distribution can be justified according to the main origin and source of cultivated tea in Iran (Khiavi *et al*., 2020b).

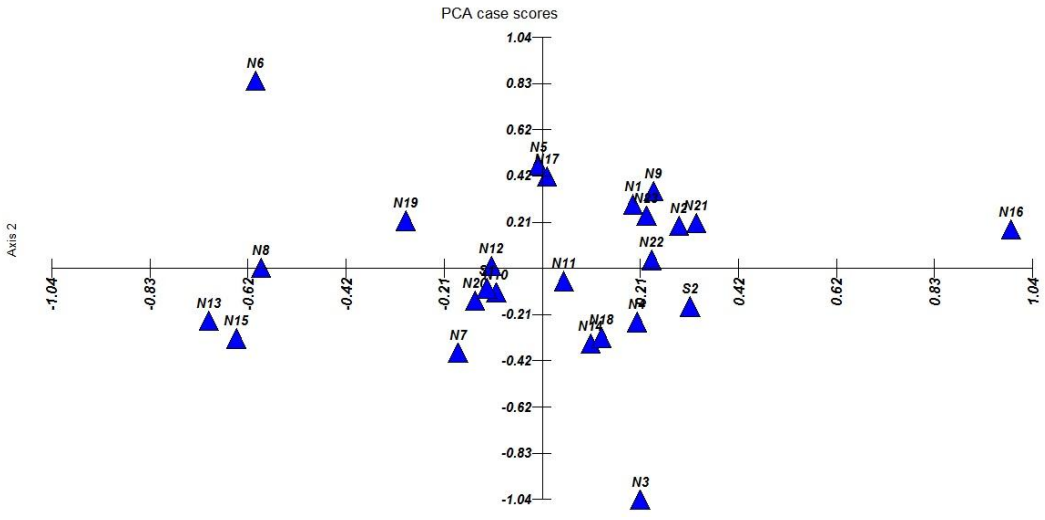

Figure 2. D-plot that generated based morphological characters of 25 of Tea samples.

For cluster analyses, the morphological data was analyzed by Past-pc, v. 4.03 software, and cophenetic coefficients showed that the Gower coefficient and UPGMA Algorithm were the best coefficient and Algorithm for this analysis. A pair-wise dissimilarity among the samples ranged

from 0.121 to 0.543 with an average of 0.367 based on morpho-metric data. The highest similarity (0.543) was calculated between"N15 and N16", while the lowest (0.121) was found among "N9 and N23". The average difference between samples S1 and S2 with the rest of the samples was 0.285 and 0.298, respectively. These calculated numbers, when compared to the total average, show that these two samples, now known as Kashef and Lahij cultivars, are almost at the center of the calculated difference. Based on the morphological parameter, our obtained similarity/dissimilarity was supported by the result of Khiavi *et al*., (2020), Rajanna *et al*., (2011), Piyasundara *et al*., (2008), and Chen *et al*., (2005). Considering these results, it could be understood that morphological characters can distinguish samples.

The cluster that was generated according to the morphological parameters grouped samples into four main groups at 0.28 of coefficient dissimilarity (approximately) (Figure 4). Group one just has three individual members, N6, N17, and N19; this group was the smallest group that created by cluster analyses. The second, third, and fourth groups had eight, ten, and four members, respectively. The largest group was the third group, which comprised about 40 percent of the total sample. Two samples, Kashef and Lahij cultivars, are also in this group (see Figure 3)

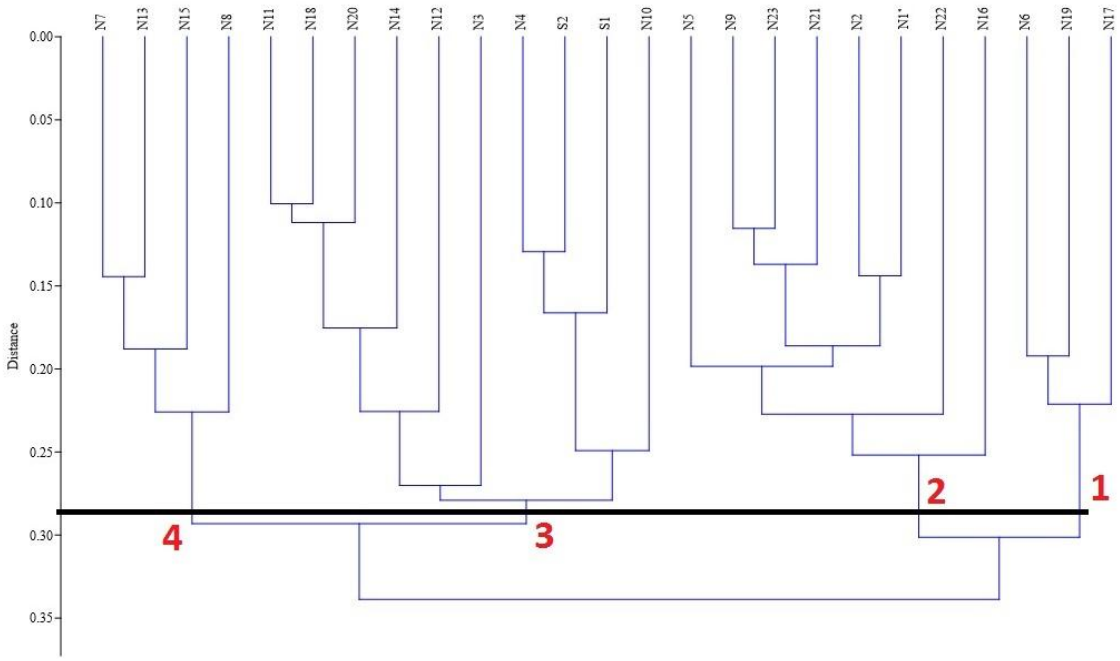

Figure 3. Dendrogram of 25 tea samples using morphological markers based on UPGMA.

3.2. ISSR Analysis

To find the genetic similarity of 23 tea genotypes grown in the collection of Shahid Chamran Tea Research Station in Neshtarood and two newly introduced cultivars (Kashef & Lahij) from Iran, we tested 25 ISSR primers. From these, 10 primers were selected according to those that gave the most polymorphic bands, based on reproducibility and produced scorable PCR fragments. The range of polymorphic bands that were scored, was from 300 to 3000 bp in size. Weak and unclear bands were not scored for data analysis. These di-nucleotide repeats, (AG), (GA), (CT), (TC), (AC), and (CA), showed higher polymorphism in plants than other repeats (Yao *et al*., 2008). Herein, we used the ISSR primer combinations (AC)8C, (AG)8YC, (GA)8C, (TC)8C, (AG)8YT, (AC)8YC, and (AC)8YG. In addition, primers (CAG)6T, (TG)6G and (CAA)6G were used,

which have shown high efficiency based on sources (Roy and Chakraborty, 2009; Yao *et al*., 2008; Ben-Ying *et al*., 2010). The number of fragments per primer ranged from 8 (P8) to 17 (P9) with an average of 10.90 per primer. The number of polymorphic fragments scored ranged from 6 (P1 and P8) to 12 (P9) with an average of 8.30 per primer. Of the total 109 scorable fragments, 76.15 percent of them were polymorphic among the samples (Table 3). Thomas *et al*. (2006) reported a polymorphic percentage of about 53% in the study of tea plant diversity resulting from somaclonal diversity, and in the study of genetic diversity between tea cultivars by Roy and Chakraborty (2009), this rate was 88.54%. Due to the percentage of polymorphism obtained in this range, it can be said that this amount of polymorphism concerning the genotypes of cultivated tea in Iran is acceptable. Yao *et al*. (2008) in the study of the genetic diversity of tea plants in China, Japan, and Korea reported a very high percentage of polymorphism (99.7%), in first this percentage of polymorphism is high but when looking at the results from each country alone, the polymorphic percentages decrease and approach the range obtained in the present study, so that for China, where four tea-growing regions have been sampled, the polymorphic percentage range is wide. It is from 54.2 to 88.2 and considering that Iranian tea plants are also of Chinese origin, it can be seen that the percentage of polymorphs obtained in this study is very close to the maximum obtained in China.

To determine the potential of primers used in the present study, the amount of polymorphic information content (PIC) for primers was calculated and the maximum PIC for primers P8 and P10 was 0.49, and the minimum for primers P16 was 0.35. The total polymorphic information content (PIC) was also calculated to be 0.46. A noteworthy point about the amount of polymorphic information content for dominant markers such as the ISSR is that the maximum PIC is 0.50, and when the calculated PIC is closer to this value, this indicates the higher power of the markers used to distinguish and identify sample differences (Roldain-Ruiz *et al*., 2000) therefore, it can be said that the used markers have a very good ability to distinguished and identified tea samples.

Table 3. Details of amplified bands generated in 25 tea studied genotypes based on ten ISSR primers.

No.	Primer	Sequences (50–30)	Total no. of amplified bands	No. of poly morphic bands	% of polymorphism	PIC value
1	*P1*	*(AC)8C*	*8*	*6*	*75.00*	*0.47*
2	*P3*	*(AG)8YC*	*8*	*8*	*100.00*	*0.48*
3	*P5*	*(CAG)6T*	*12*	*10*	*83.33*	*0.42*
4	*P8*	*(TG)6G*	*11*	*6*	*54.55*	*0.49*
5	*P9*	*(CAA)6G*	*17*	*12*	*70.59*	*0.46*
6	*P10*	*(GA)8C*	*9*	*9*	*100.00*	*0.49*
7	*P13*	*(TC)8C*	*11*	*7*	*63.64*	*0.48*
8	*P14*	*(AG)8YT*	*13*	*11*	*84.62*	*0.42*
9	*P15*	*(AC)8YC*	*9*	*7*	*77.78*	*0.45*
10	*P16*	*(AC)8YG*	*11*	*7*	*63.64*	*0.35*
total	-	-	*109*	*83*	*76.15*	*0.46*
Av.	-	-	*10.90*	*8.3*	-	-

A similarity coefficient matrix based on simple matching (SM) similarity coefficient (data not given) showed that the highest similarity was determined between N1 and N18 (0.920) while the lowest similarity index was between N8 and N12 (0.344) and the average similarity was calculated 0.640. This amount of similarity is acceptable according to the results of the morphology section, and these two markers confirm each other, which can be deduced from the

fact that the moth methods morphological and ISSR primer are suitable for identification and differentiation within the genus *Camellia*.

The high degree of similarity (0.920) is consistent with the studies of Kafkas *et al*. (2009) in the study of the genetic diversity of tea plant in Turkey using the AFLP marker. They calculated the maximum similarity of 92% that due to the import of tea plant to these two countries (Iran and Turkey), it is acceptable that a high degree of similarity between the samples due to the method of propagation of this plant in the past. As Falakro and Khiavi (2020) report when comparing the calculated similarity of Kafka *et al*. (2009) with the similarity of the present report, these two points should be considered: first, the potency and accuracy of used markers in both studies, and second, the way of tea introduction in these countries. Ben-Ying *et al*., (2010) also used ISSR markers in the study of a tea plant in the Yunnan region of China and obtained similarity in the same range (the average similarity of their study was 0.51), considering that the tea plant cultivated in Iran originates from china types, these results indicate the high relationship between these two groups of plants.

Cluster analyses were done by using simple matching (SM) similarity coefficient and UPGMA algorithm. The results of cluster analyses of 25 studied tea samples are shown in Figure 4. Each of groups 1 and 2 have three members, Lahij, Kashef and N16 were members of the first group and three samples N11, N12 and N23 were in the second group. Group 3 has five members (N8, N9, N10, N15 and N21). The biggest created group (group 4) has 13 members (56 percent of total samples).

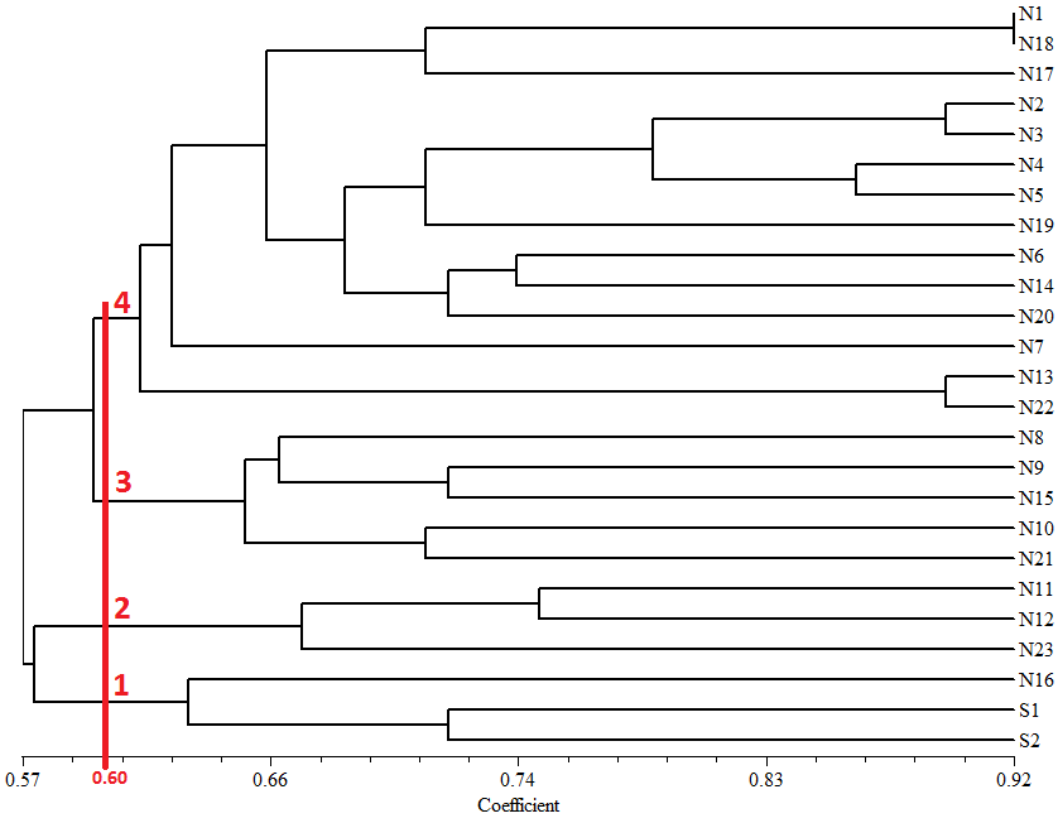

Figure 4. Dendrogram of 25 tea samples using ISSR markers based on UPGMA.

Principal coordinates Analyses (PCoA) were done to design the 2-D plot based on ISSR data. The result of this analysis showed that the first ten principal components accounted for about 76.61% of the total variation (Table 4). In a two-dimensional plot (2D plot) generated from PCoA that was generated based on ISSR data of studied samples, four groups were obtained.

Table 4. Eigen values of the correlation matrix based on the PCoA of the ISSR data.

PCo	Eigenvalues	Percentage	Cum. Percentage
1	*1.359*	*14.52*	*14.52*
2	*1.066*	*11.39*	*25.91*
3	*0.854*	*9.13*	*35.04*
4	*0.79*	*8.44*	*43.48*
5	*0.68*	*7.27*	*50.75*
6	*0.63*	*6.74*	*57.49*
7	*0.537*	*5.73*	*63.22*
8	*0.477*	*5.10*	*68.32*
9	*0.414*	*4.42*	*72.74*
10	*0.362*	*3.87*	*76.61*

The distribution of samples based on the PCo1 and PCo2 is shown in Figure 2. Group one consists of three members (N8, N9, and N21); group two has seven members (N1, N2, N3, N4, N5, N18, and N19); group three was the biggest achieved group and it had 16 members (64 present of all samples) and the last group or group 4 consists of three members (N12, N23, and S1).

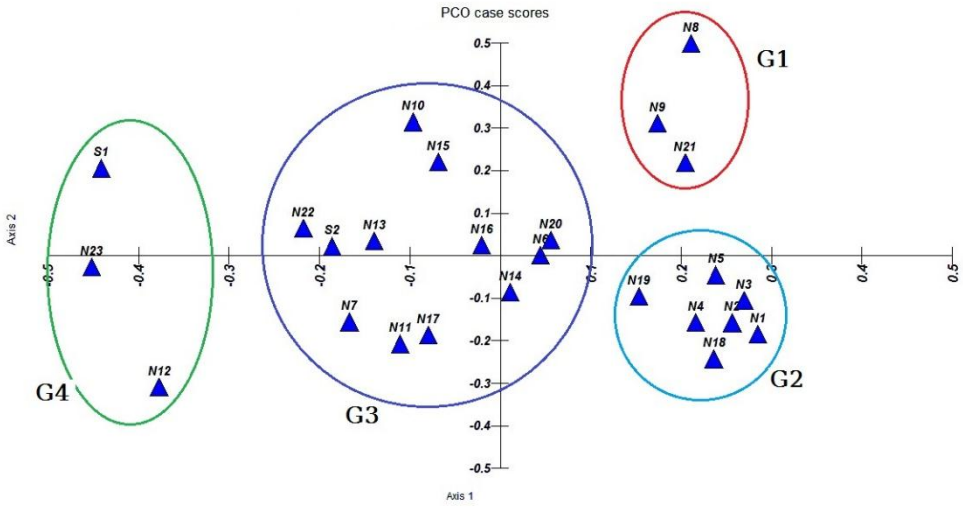

Figure 5. D-plot that generated based ISSR data of 25 of Tea samples.

References

Ahmadishad, M.A., Kazemitabar, S.K., Babaeian Jelodar, N.A., Gholami, M. and Kazemi Poshtmasari, H. 2009. An Assessment of genetic diversity in cultivated tea (*Camellia sinensis* L.) clones in Iran using RAPD markers, J. Crop Breed. 1: 4. 65-76. (In Persian)

Bali, S., Raina, S. N., Bhat, V., Aggarwal, R. K., & Goel, S. (2013). Development of a set of genomic microsatellite markers in tea (*Camellia* L.)(*Camellia*ceae). Molecular breeding, 32(3), 735-741.

Ben-Ying, L.I.U., You-Yong, L.I., Yi-Chun, T.A.N.G., Li-Yuan, W.A.N.G., Cheng, H. and Ping-Sheng, W.A.N.G., 2010. Assessment of genetic diversity and relationship of tea germplasm in Yunnan as revealed by ISSR markers. Acta Agro. Sinic 36(3), pp.391-400.

Chaeikar, S.S. Falakro, K., Rahimi, M., Khiavi, S.J. and Ashourpour, M. (2020). The investigation of genetic diversity based on SCoT markers, morphological, and chemical characters in tea (*Camellia sinensis* L.) clones. Journal of Horticulture and Postharvest Research. 3(2), 269-284

Chen, H., Guo, A., Wang, J., Gao, J., Zhang, S., Zheng, J., ... & Yi, K. (2020). Evaluation of genetic diversity within asparagus germplasm based on morphological traits and ISSR markers. Physiology and Molecular Biology of Plants, 26(2), 305-315.

Chen, J., Wang, P., Xia, Y., Xu, M., & Pei, S. (2005). Genetic diversity and differentiation of *Camellia sinensis* L. (cultivated tea) and its wild relatives in Yunnan province of China, revealed by morphology, biochemistry and allozyme studies. Genetic Resources and Crop Evolution, 52(1), 41-52.

Dellaporta, S.L., Wood, J. and Hicks, J.B. (1983). A plant DNA minipreparation: version II. Plant molecular biology reporter, 1(4), pp.19-21.

Devarumath, R., Nandy, S., Rani, V., Marimuthu, S., Muraleedharan, N., and Raina, S. (2002). RAPD, ISSR and RFLP fingerprints as useful markers to evaluate genetic integrity of micropropagated plants of three diploid and triploid elite tea clones representing *Camellia sinensis* (China type) and C. assamica ssp. assamica (Assam-India type). Plant Cell Reports. 21(2), 166-173.

Devarumath, R.M., S. Nandy, V. Rani, S.Marimuthu, N.Muraleedharan, and S.N. Raina. (2002). RAPD, ISSR and RFLP fingerprints as useful markers to evaluate genetic integrity of micropropagated plants of three diploid and triploid elite clones representing *Camellia sinensis* (China type) and C. assamica ssp. assamica (Assam-India type). Plant Cell Rep. 21,166–173.

Erxu, P. I., Qiufa, P., Hongfei, L., Jingbo, S., Yueqiang, D., Feilai, H., & Hui, H. (2009). Leaf morphology and anatomy of *Camellia* section *Camellia* (Theaceae). Botanical Journal of the Linnean Society, 159(3), 456-476.

Falakro, K. and Khiavi, S.J. (2020). Assessment of genetic diversity and relationships among tea genotypes in Iran based on RAPD and ISSR markers. Journal of Horticulture and Postharvest Research. 3(2), pp.209-220.

Fulian, Y. (1986). Discussion on the originating place and the originating center of tea plant. Journal of Tea Science, 6(1), 1–8.

Guliyev, N., Sharifova, S., Ojaghi, J., Abbasov, M., & Akparov, Z. (2018). Genetic diversity among melon (Cucumis melo L.) accessions revealed by morphological traits and ISSR markers. Turkish Journal of Agriculture and Forestry, 42(6), 393-401.

Hasimoto, M., & Simura, T. (1978). Morphological studies on the origin of the tea plant. Japanese Journal of Tropical Agriculture, 21(2), 93-101. https://doi.org/10.11248/jsta1957.24.1

Hu, C. Y. (2004). Studies on the variations in leaf characters and DNA sequences of tea germplasm in Taiwan, MS Thesis, National Taiwan University, Taiwan.

IPGRI. (2000). Descriptors for tea. International Plant Genetic Resources Institute, Rome, Italy, Available at: http://www.cgiar.org/ipgri./

Kafkas, S., Ercisxli, S., Dogan, Y., Erturk, Y., Haznedar, A., and Sekban, R. (2009). Polymorphism and genetic relationships among tea genotypes from turkey revealed by amplified fragment length polymorphism markers. J Amer Soc Hort Sci 134, 428–434

Karthigeyan, S., & Sud, R. K. (2010). Genetic diversity of indian tea (*Camellia sinensis* (L.) Kuntze) germplasm detected using morphological characteristics. Journal Cell and Plant Science, 1(1), 13-22.

Khiavi, S. J., Hamidoghli, Y. O. U. S. E. F., Golein, B. E. H. R. O. Z., & Sabouri, A. T. E. F. E. H. (2016). Assessment of lime genetic diversity in three regions of Iran using morphological and ISSR markers. Agricultural Communications, 4(3), 18-29.

Khiavi, S.J., Azadi Gonbad, R. and Falakro, K. (2020a). Identification of genetic diversity and relationships of some Iranian tea genotypes using SRAP markers. Journal of Horticulture and Postharvest Research, 3(1), pp.25-34.

Khiavi, S.J., Falakro, K., & Chaeikar, S.S. (2021). PCR-RFLP analyses of chloroplast DNA in some cultivated tea (*Camellia* sp.) genotypes. Journal of Horticulture and Postharvest Research, 4(1), 25-36.

Khiavi, S.J., Falakro, K., Chaeikar, S.S., Ramzi, S., Kahneh, E. (2020b). Usage of morphological and ISSR markers for investigation of some Tea genotypes, Plant Production Research Journal, 26 (4), 131-147. (In Persian)

Mahmood, T., Akhtar, N., & Khan, B. A. (2010). The morphology, characteristics, and medicinal properties of *Camellia sinensis* tea. Journal of Medicinal Plants Research, 4(19), 2028-2033.

Nargesy Dehdasht, A., Sedaghatfar, E., Hejazi, R. (2019). Exploring multiple housekeeping genes to identify the most effective reference genes for gene expression analysis in rice through qPCR. 5th International Congress on Technology – Engineering & Science (ICONTES), KL, Malaysia.

Parvathaneni, R. K., Natesan, S., Devaraj, A. A., Muthuraja, R., Venkatachalam, R., Subramani, A. P., & Laxmanan, P. (2011). Fingerprinting in cucumber and melon (Cucumis spp.) genotypes using morphological and ISSR markers. Journal of Crop Science and Biotechnology, 14(1), 39-43.

Paul, S., Wachira, F.N., Powell, W. and Waugh, R. (1997). Diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia sinensis* (L.) O. Kuntze) revealed by AFLP markers. Theoretical and Applied Genetics, 94(2), pp.255-263.

Piyasundara, J. H. N., Gunasekare, M. T. K., & Wickramasinghe, I. P. (2008). Identification of discriminating morphological descriptors for characterization of tea (*Camellia sinensis* L.) germplasm in Sri Lanka. Tropical agricultural research, 20, 193-199.

Rajanna, L., Ramakrishnan, M., & Simon, L. (2011). Evaluation of morphological diversity in south Indian tea clones using statistical methods. Maejo International Journal of Science and Technology, 5(1), 1.

Roldain-Ruiz, I.,E. Calsyn, T.J. Gilliand, R. Coll, M.J.T. Van- Eijk and De-Loose, M. (2000). Estimating genetic conformity between related ryegrass (Lolium) varieties, 2 AFLP characterizations. Molec. Breed. 6, 593-602.

Roy, S.C., and Chakraborty, B.N. (2009). Genetic diversity and relationships among tea (*Camellia* sinensis) cultivars as revealed by RAPD and ISSR based fingerprinting. Indian Journal of Biotechnology. 8(4): 370-376.

Sedaghatfar, E., Zamanizadeh, H.R., Roohparvar, R., Farsad, L. K., Fazeli, A., Rezaee, S., & Mardi, M. (2012). Gene expression profiling of defense-related genes resistant to Septoria tritici blotch in wheat. African Journal of Biotechnology, 11(72), 13633-13644.

Shah, A., Li, D. Z., Möller, M., Gao, L. M., Hollingsworth, M. L., & Gibby, M. (2008). Delimitation of Taxus fuana Nan Li & RR Mill (Taxaceae) based on morphological and molecular data. Taxon, 57(1), 211-222.

Thomas, J., Vijayan, D., Joshi, S.D., Lopez, S.J. and Kumar, R.R. (2006). Genetic integrity of somaclonal variants in tea (*Camellia sinensis* (L.) O Kuntze) as revealed by inter simple sequence repeats. J. Biotech., 123(2), 149-154.

Yao, M.Z., Chen, L., Liang, Y.R. (2008). Genetic diversity among tea cultivars from China, Japan and Kenya revealed by ISSR markers and its implication for parental selection in tea breeding prog. Plant Breed 127, 166–172.