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The investigation of genetic diversity based on SCoT markers, morphological, and chemical characters in tea (*Camellia sinensis* L.) clones

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ABSTRACT

Purpose: Tea is one of the earliest caffeinated non-alcoholic drinks produced from the tip of young shoots. Evaluation of genetic diversity of clones that existed in tea germplasm can be a help to improve the tea breeding program. **Research Method**: The genetic diversity of 9 tea clones based on morphological, chemical and molecular markers were evaluated at Shahid Eftekhari Fashalam Experimental Station, Tea Research Center, Shaft, Guilan, Iran. **Findings**: Analysis of variance showed a significant difference between the clones for the studied traits. Descriptive statistics showed that green leaf yield had the highest phenotypic variation with CV of 56.47% and water extract showed the least phenotypic variation (4.40%). Clones 399, 285 and 100 had a significantly higher content of the number of plucking shoots, fresh and dry weight of plucking shoot and green leaf yield than other clones. Regarding the water extract, clones 272 and 100 have a significantly higher value than the other clones. Contents of polyphenols in all of clones were high except clones 276 and 278. The cluster analysis classified tea clones into three groups based on morphological and chemical traits as well as SCoT markers. **Research limitations**: Not using other molecular markers and biochemical traits. **Originality/Value**: Great variation of morphological characters was apparent among the selected clones. Based on the Mantel test, the grouping of clones with molecular data was partially corresponding with morphological and chemical traits.

INTRODUCTION

Tea (*Camellia sinensis* (L.) O. Kuntze) belongs to the family Theaceae. It is the oldest non-alcoholic caffeine-containing beverage in the world. Tea is an evergreen, perennial, cross-pollinated plant and grows naturally as tall as 15 m. However, under cultivated condition, the bush height of 60-100 cm is maintained for harvesting the tender leaves to be processed for making the beverages (Mondal, 2014). Tea occupies about 2.7 million ha of cultivable land of the world with an annual production of about 2.2 million t (Mondal, 2014). Southeast Asia is the original home for tea. According to Wight (1959), the primary centre of origin of tea was considered around the point of intersection of latitude 29°N and longitude 98°E near the source of the river, Irrawaddy, the point of confluence where lands of Assam, North Burma, Southwest China and Tibet met. Secondary centers of origin were considered to be located in Southeast China, Indochina, Mizoram and Meghalaya (Kingdom-Ward, 1950). The above areas were, therefore, considered to be the zone of origin and dispersion of the genus *Camellia* as a whole (Sealy, 1958). However, presently tea is grown within the latitudinal range of 45°N to 34°S. Tea cultivation was extended to Japan, Indonesia, Sri Lanka, USSR, Turkey, Europe and African countries (Mondal, 2014). In Iran, tea is cultivated in two provinces (Guilan and Mazandaran) with an area under cultivation of 25000 ha.

Since many tea plants are currently being destroyed for many reasons, having information about tea genetics for designing suitable breeding programs is very helpful in obtaining appropriate plants for specific purposes (M Perez-de-Castro et al., 2012). The first and most relevant program for plant breeding is the study of diversity, which can be used to select suitable varieties. The rise of genetic diversity in a population extends the range of natural and artificial selection. Therefore, recognizing genetic diversity of varieties and wild cultivars of plants is essential to facilitate the production and creation of new lines through genetic hybridization and prevent the genetic erosion (Richards, 2011).

Classical methods of estimating the genetic variation among plants are based on the morphological traits, but these traits are influenced by the environmental factors (Govindaraj et al., 2015). Nowadays, methods of the identification and diagnosis of genetic diversity have become more recent and accurate with the use of molecular markers. Identification of the species in recent decades is carried out with confidence and ease using molecular markers. These markers are used to identify species and cultivars, estimate biodiversity and improve the breeding cultivars (Bandyopadhyay, 2011; Govindaraj et al., 2015). The advent of DNA markers technology has helped specialists and plant breeders to overcome many of these problems (Nybom et al., 2014). Diverse researches to assess genetic diversity have also been carried out implementing diverse methods, such as morphology (Wickramaratne, 1981; Toyao & Takenda, 1999; Chen et al., 2005; Rajkumar et al., 2010; Kim et al., 2012), biochemistry (Takeda, 1994; Magoma et al., 2000), using genetic markers, e.g., $RFLP_s$ (Matsumoto et al., 1994), $RAPD_s$ (Lee et al., 1995; Wachira et al., 1997; Kaundun et al., 2000; Kaundun & Park, 2002), $AFLP_s$ (Paul et al., 1997; Raina et al., 2012), SSR_s (Kaundun & Matsumoto, 2011; Fang et al., 2012; Bali et al., 2013), $ISSR_s$ (Lin et al., 2012; Liu et al., 2012; Wang & Ruan, 2012; Rahimi et al., 2019) and SRAP (Khiavi et al., 2020).

Gaining knowledge about the genetic distance between the individuals or populations and knowing the kinship relationships of the species in the breeding programs allows for the organization of germplasms and effective sampling of genotypes (Govindaraj et al., 2015; Nybom et al., 2014). The first step in improving plant characteristics is the identification of the

genetic features of germplasm specimens. So considering this issue, a systematic sampling of the germplasm is possible for conservation and breeding purposes (Govindaraj et al., 2015; Upadhyaya et al., 2008; Van Hintum et al., 2000). Genetic diversity refers to the fact that variety and variability between organisms can be found at different levels among individuals of a population, species of the same sex, and so on. Therefore, it is a unique resource for genetic improving and breeding of plant traits as well as increasing the variety of these traits (Govindaraj et al., 2015; Van Bueren et al., 2011; Xu et al., 2017).
Genetic diversity based on morphological and chemical traits and molecular marker was investigated in tea clones with the aim of breeding in the tea improvement program.

MATERIALS AND METHODS

Plant material and experimental conditions

During 2001-2006, Genotypes selected based on clonal selection method (Gholami et al., 2019) from different gardens in the west of Guilan province and were propagated by cutting and cultivated in Tea Plant Germplasm Collection situated at the Shahid Eftekhari Fashalam Research Station (latitude 37°15′54″N, longitude 38°45′49″E and height of -10 meters above sea level) in a Randomized Complete Block Design with three replications. The length and width of each plot were 5 and 4 meters, respectively (including four rows and six plants per row). In each row, the distance between the plants was 70 cm and the gap between the two rows was 100 cm. Physical and chemical tests of the soil were carried out and the soil texture was found to be sandy loam. All plants were held using similar agricultural management practices. Nine clones were chosen (Table 1) for this study from the Fashalam Tea Plant Germplasm Collection.

Morphology

Nine morphological traits were measured among the summer shoots during July 2018, including: number of plucking shoots, fresh and dry weight of plucking shoot, length of plucking shoot, 5^{th} leaf length, 5^{th} leaf width, leaf area, internodes distance and green leaf yield (IPGRI, 1997). For measuring the number and fresh weight of plucking shoot per unit area, 25×25 cm frame was randomly located in 3 locations per plot and the number and fresh weight of plucking shoots containing two leaves and a bud calculating and then converted to unit area (IPGRI, 1997), for dry weight of shoots, plucking shoots dried in 103°C (IPGRI, 1997), length of plucking shoot was obtained by measuring the length from beginning of shoot growth to the terminal bud (IPGRI, 1997). To determine the green leaf yield per unit area, tea shoots were harvested in standard form (two leaves and a bud) from the experimental plots and their weight was measured by a precision scale (IPGRI, 1997). As for the leaf's length and width, the longest and widest part of 5^{th} mature leave was determined. The leaf area was assessed using the following formula (1):

$$\text{Leaf area } (c.m^{-2}) = \text{leaf length} \times \text{leaf width} \times K \text{ (Ng'etich \& Wachira, 1992)} \quad (1)$$

Also, we measured internodes distance between 5^{th} and 6^{th} mature leaf. We have ten replicates for those length measurements and three for the weighted data.

Table 1. The name, type and origin of tea clones studied at Fashalam station

Row	Clone	Varietal type	Origin
1	272	Chinese type of local selection	West of Guilan
2	277	Chinese type of local selection	West of Guilan
3	100	Chinese type of local selection	West of Guilan
4	285	Chinese type of local selection	West of Guilan
5	74	Chinese type of local selection	West of Guilan
6	399	Chinese type of local selection	West of Guilan
7	276	Chinese type of local selection	West of Guilan
8	278	Chinese type of local selection	West of Guilan
9	269	Chinese type of local selection	West of Guilan

Chemistry

Fresh plant materials (including two leaves and a bud) were collected during July in 2018; and they were then dried at 103°C. Samples were analyzed in order to determine their total polyphenols, caffeine, water extract, and total ash at Tea Research Center in Iran. In each of the experimental units, approximately 100 g of the fresh shoots (with one bud and two leaves) were plucked. Then, the samples were placed inside the labeled paper bags and dried at 70°C for 24 hours. The dried samples were blended, placed inside the paper bags in dry and dark conditions until laboratory analysis.

The ISO procedure (2005) was used for the analysis of the total polyphenols. Ground shoot samples (0.2 g) were weighed into 10-ml extraction tubes. 5 ml, 70% v/v methanol (hot methanol/water extraction mixture) was added to every extraction tube. Then, a vortex mixer was used to stopper and shake the tubes. The tubes were placed in a water bath for 10 minutes. Then, the tubes were allowed to be cool at room temperature. Thereafter, the extracts were centrifuged (3500 rpm, 10 minutes). The supernatant was poured into 10-ml tubes. Then, a cold ethanol/water mixture was added to reach 10 ml volume. 1 ml of the extract was poured into a 100-ml flask to more dilute, and then water was added to reach the mark. Standard solutions of gallic acid (1 ml) corresponding to 10, 20, 30, 40 and 50 µg of anhydrous gallic acid and a similar quantity of water for the reagent blanks were poured in duplicate into the different tubes. 1 ml of the diluted sample extract was poured into the separate tubes and 5 ml of the reagent of Folin-Ciocalteu phenol were added to each of the tubes and mixed. 4 ml of sodium carbonate solution, about 5 minutes after adding the Folin-Ciocalteu phenol reagent, were added to each of the tubes and allowed to remain for 60 minutes at room temperature. By using a 10-mm cell on a spectrophotometer set, optical densities were calculated at a wavelength of 765 nm. Polyphenol contents in the tested sample were measured by a standard curve made by gallic acid, and defined as the contents of gallic acid equivalent. By using the mass of the standards of anhydrous gallic acid, the graph of best-fit linear calibration was drawn in comparison with the standard optical densities of gallic acid, and the content of the total polyphenol, expressed as a percent by the mass based on the sample dry matter, was measured by the ISO procedure (ISO Standards, 2005).

To determine the water extract content, the soluble matter from 2 g of the ground shoots of tea (one bud and two leaves) was mixed with boiling water under refluxing, filtering, washing, drying, and weighing the insoluble residue in hot water, and calculating water extract (ISO Standards, 1994).

To measure the total ash, the organic matter of 2 g of ground shoots of tea (one bud and two leaves) was destructed and heated at 525 ± 25°C to a constant mass with a furnace (ISO Standards, 1987).

Genetic relationship through SCoT analysis

100 mg of the leaf tissues were ground in liquid nitrogen and DNA extracted according to Dellaporta et al. (1983) method. Extracted DNA was diluted with distilled water to 25 ng.μl^{-1} and stored at -20°C.

Polymerase chain reaction was carried out in 10 µl mixtures of reaction containing 2µl from each of template DNA (50 ng), 1µl PCR buffer, 0.3 µl $MgCl_2$, 0.1µl each of dNTP, 0.6 µl of each primer, and 0.2 µl of *Taq* DNA polymerase (Sinaclon Co, Iran) and adjusted value by adding double-distilled water. PCR was performed in a Biometra PCR thermal as mentioned: initial denaturation at 94°C for 4 min followed by 35 cycles of 94°C for 40 sec, 51-57°C (depending on the primers) for 40 sec, and 72°C for 2 min, and a final 5 min extension at 72°C. The 1.5% agarose (1×TAE buffer) gel was used to observe the band's pattern, and detection was done using UV transilluminator, stained in Ethidium bromide. Eventually, 13 primers (Table 6) producing clear and proliferous fragments patterns were selected for our final analysis.

To score polymorphism for every clone, the absence and presence of a band were scored as 0 and 1, respectively. The binary data of the SCoT marker were scored as: the presence (1) or absence (0) of a band, providing a genetic dissimilarity matrix to use in genetic diversity. Genetic diversity was examined by several indices such as: the number of observed alleles (Na), the number of effective alleles (Ne) (Kimura & Crow, 1964), Neis gene diversity (Nei, 1972), Shannon diversity index (Shannon, 2001) and the polymorphism information content (PIC) (Anderson et al., 1993).

The screening of the entire set of samples was performed three times to assess the repeatability of the SCoT profiles and identical SCoT patterns were obtained.

Statistical analysis

After performing the data normality test (Kolmogorov-Smirnov), the analysis of variance (ANOVA) was performed to test for the differences of clones on the various morphological and chemical parameters measured. Duncan Multiple Range Test was used to compare means at a significance of $P \leq 0.05$. The data analysis was carried out using the statistical package SAS 9.4 (SAS Institute, 1985). Relationships between clones based on morphological, chemical and molecular data were studied using cluster analysis. The PAST software (Hammer et al., 2001) was used for the cluster analysis.

RESULTS AND DISCUSSION

Morphological and chemical traits in selected tea clones

Morphological traits

Variation of morphological and chemical traits in all clones was presented (Table 2). Six of nine morphological traits exhibited a coefficient of variation >20% across clones, which were identified as follows: the number of plucking shoot, fresh weight of plucking shoot, dry weight of plucking shoot, leaf area, internode distance and green leaf yield. Among the above-mentioned traits, green leaf yield showed the highest coefficient of variation among the studied traits and thus had the highest diversity in comparison with other traits. The lowest diversity among the studied clones related to water extract was 4.40%. The trait including number of plucking shoots in tea clones also had a high variation of 40.40%, so there is also a choice between clones for this trait, and suitable clones can be selected. Due to the high diversity of

these traits, these traits can be of interest to the breeders, and selection of clones based on these traits leads to the improvement of these traits. Still, other traits with a lower coefficient of variation have less chance of selection (Table 2).

The results of the analysis of variance (ANOVA) for all the studied traits except the length of plucking shoot showed significant differences between the tea clones (Table 3). The coefficient of variation of the randomized complete block design was between 2.66% for the water extract to 26.29% for the fresh weight of plucking shoot, which indicated the appropriate accuracy of the test. A significant difference between studied traits indicating the difference between the tea clones and the acceptable genetic variation for most studied traits. The diversity between genotypes can improve the traits, and in particular, the amount of genetic diversity is effective in determining the usefulness of selection (Balasaravanan et al., 2003).

The number of plucking shoots, fresh and dry weight of the plucking shoot of clones 399 and 285 were significantly higher than the other clones (Table 4). The 5th leaf width and leaf area of clone 278 measured were measured to be 3.48 cm and 29.71 c.m^{-2}, respectively, resulting in the largest value. Clone 285 showed greater value for green leaf yield, followed by clones 100 and 399 (Table 4).

Chemistry

Different clones of tea may have various chemical contents and these are important factors that contribute to tea quality (Wright & Gilchrist, 1961). Comparing the four chemical traits, all of them showed the low variation with a coefficient of variations (CV) of 4.40 to 8.96 (Table 2), but based on variance analyses, there are significant differences among clones for four chemical traits (Table 3). Regarding the content of water extract, clones 272 and 100 have a significantly higher content of water extract value than the other clones. Clones of 272 and 100 showed a high content of polyphenols compared to other clones (Table 4).

To select the best parents in each cross and achieve maximum heterosis, researchers select genotypes which are genetically heterogeneous. This can be achieved by examining genetic distance among genotypes based on phenotypic traits using the clustering method. While using morphological and chemical traits, clones sorted together in distant groups are used as parents in crossings to acquire greater variety in hybridization and breeding programs.

Different cluster analysis methods were used to determine the variation among the tea clones based on morphological and chemical traits. The results showed that the Wards' method had the highest amount of cophenetic correlation coefficient (0.90). Therefore, cluster analysis was done with this method and tea clones were divided into three groups (Fig. 1). The first group consisted of three clones (100, 285 and 399), the second group contains two clones (272, 276) and the third group consisted of clones of 277, 269, 74 and 278. As stated, clones due to different genetic bases or other environmental factors are placed in completely separate groups. They can justify the ability of morphological and chemical traits to determine this distinction. The results of cluster analysis showed the differences between clones of each group with other groups and the similarity of clones within each group. The reason for differences in clones of groups can be due to differences in the genetic structure or the effect of other environmental factors on the traits.

Table 2. Variation of morphological and chemical traits of nine selected *Camellia sinensis* clones

Row	Traits	Number	Range	Mean±SD	%Phenotypic C.V
1	Number of plucking shoots	9	134.66	143.85±58.12	40.40
2	Fresh weight of plucking shoots	9	70.61	79.14±25.30	31.97
3	Dry weight of plucking shoots	9	12.15	15.35±4.14	26.96
4	Length of plucking shoots	9	1.93	7.62±0.60	7.96
5	5^{th} leaf length	9	3.75	7.50±1.16	15.51
6	5^{th} leaf width	9	1.30	3±0.46	15.41
7	Leaf area	9	18.37	23.14±6.20	26.82
8	Internode distance	9	2.96	3.86±0.96	24.96
9	Green leaf yield	9	561.62	379.29±214.18	56.47
10	Water extract	9	5.19	38.43±1.69	4.40
11	Polyphenol	9	2.03	13.50±0.64	4.74
12	Total ash	9	1.30	6.39±0.42	6.58
13	Dry matter	9	4.87	19.77±1.77	8.96

Table 3. Analysis of variance of the traits in tea clones

S.O.V	df	Mean of squares						
		N.S ($n.m^{-2}$)	F.W ($gr.m^{-2}$)	D.W ($gr.m^{-2}$)	L.S (cm)	L.L(cm)	L.W (cm)	L.A (cm^2)
Block	2	173.03^{ns}	301.90^{ns}	10.52^{ns}	0.06^{ns}	0.95^{ns}	0.09^{ns}	23.66^{ns}
Clones	8	10135.25^{**}	1921.07^{**}	51.46^{*}	1.11^{ns}	4.06^{**}	0.64^{*}	115.59^{**}
Error	16	597.70	433.13	13.53	0.71	0.64	0.17	27.31
CV(%)		16.99	26.29	23.95	11.10	10.66	14.01	22.58
S.O.V	df	Mean of squares						
		I.D (cm)	L.Y ($gr.m^{-2}$)	W.E (%)	P (%)	T.A (%)	D.M (%)	
Block	2	0.16^{ns}	13.43^{ns}	4.21^{ns}	0.26^{ns}	0.08^{ns}	0.17^{ns}	
Clones	8	2.78^{**}	137633.30^{**}	8.56^{**}	1.23^{**}	0.53^{**}	9.42^{**}	
Error	16	0.55	85.80	1.05	0.15	0.03	2.06	
CV(%)		19.15	12.44	2.66	2.90	2.88	7.25	

ns,*, and **: non-significant, significant at 5 and 1% probability levels, respectively.
N.S: number of plucking shoot, F.W: fresh weight of plucking shoot, D.W: dry weight of plucking shoot, L.S: length of plucking shoot, L.L: 5^{th} leaf length, L.W: 5^{th} leaf width, L.A: leaf area, I.D: internodes' distance, L.Y: green leaf yield, W.E: water extract, P: polyphenol, T.A: total of ash and D.M: dry matter.

Table 4. Comparison of means of nine selected clones based on morphological and chemical characters

Clones	N.S ($n.m^{-2}$)	F.W ($gr.m^{-2}$)	D.W	L.S	L.L	L.W
272	86.67^{c}	49.41^{cd}	9.49^{c}	8.37^{a}	8.39^{a}	3.12^{a-c}
277	164^{b}	88.05^{a-c}	15.62^{a-c}	7.72^{ab}	7.83^{ab}	3.01^{a-c}
100	102.67^{c}	72.25^{b-d}	14.20^{bc}	8.16^{a}	7.28^{ab}	3.37^{ab}
285	214.67^{a}	111.55^{a}	21.64^{a}	8.27^{a}	5.02^{c}	2.18^{d}
74	122.67^{bc}	67.37^{b-d}	14.66^{a-c}	7.64^{ab}	6.47^{b}	2.62^{b-d}
399	221.33^{a}	114.04^{a}	20.25^{ab}	7.19^{ab}	8.77^{a}	3.36^{ab}
276	86.67^{c}	43.43^{d}	9.78^{c}	7.30^{ab}	7.41^{ab}	2.52^{cd}
278	89.33^{c}	68.99^{b-d}	14.73^{a-c}	6.44^{b}	8.44^{a}	3.48^{a}
269	206.67^{a}	97.23^{ab}	17.82^{ab}	7.55^{ab}	7.92^{ab}	3.39^{ab}

Table 4 (continued). Comparison of means of nine selected clones based on morphological and chemical characters

Clones	L.A ($c.m^2$)	I.D (c.m)	L.Y (gr/m^2)	W.E (%)	P (%)	T.A (%)	D.M (%)
272	26.21^{ab}	2.98^{cd}	157^{h}	41.60^{a}	13.74^{a}	6.02^{cd}	18.79^{cd}
277	24.05^{ab}	2.30^{d}	218.80^{g}	37.73^{bc}	14.19^{a}	6.75^{b}	17.86^{d}
100	24.81^{ab}	3.71^{bc}	647.73^{b}	40.50^{a}	13.95^{a}	6.29^{c}	19.66^{b-d}
285	11.34^{c}	4.23^{a-c}	705.33^{a}	37.80^{bc}	13.89^{a}	6.30^{c}	19.59^{b-d}
74	17^{bc}	4.39^{a-c}	297.42^{e}	38.58^{b}	13.71^{a}	6.20^{cd}	21.82^{ab}
399	29.62^{a}	4.89^{ab}	583.33^{c}	38.53^{b}	13.65^{a}	6.78^{b}	17.94^{d}
276	18.69^{bc}	3^{cd}	143.71^{h}	36.58^{c}	12.78^{b}	5.88^{d}	22.74^{a}
278	29.71^{a}	4.04^{a-c}	379.25^{d}	36.42^{c}	12.15^{b}	7.18^{a}	21.21^{a-c}
269	26.80^{ab}	5.2^{a}	281.40^{f}	38.11^{bc}	13.46^{a}	6.14^{cd}	18.35^{d}

Means with a common letter do not differ from other means ($p \leq 0.05$).
N.S: number of plucking shoot, F.W: fresh weight of plucking shoot, D.W: dry weight of plucking shoot, L.S: length of plucking shoot, L.L: 5^{th} leaf length, L.W: 5^{th} leaf width, L.A: leaf area, I.D: internodes' distance, L.Y: green leaf yield, W.E: water extract, P: polyphenol, T.A: total of ash and D.M: dry matter.

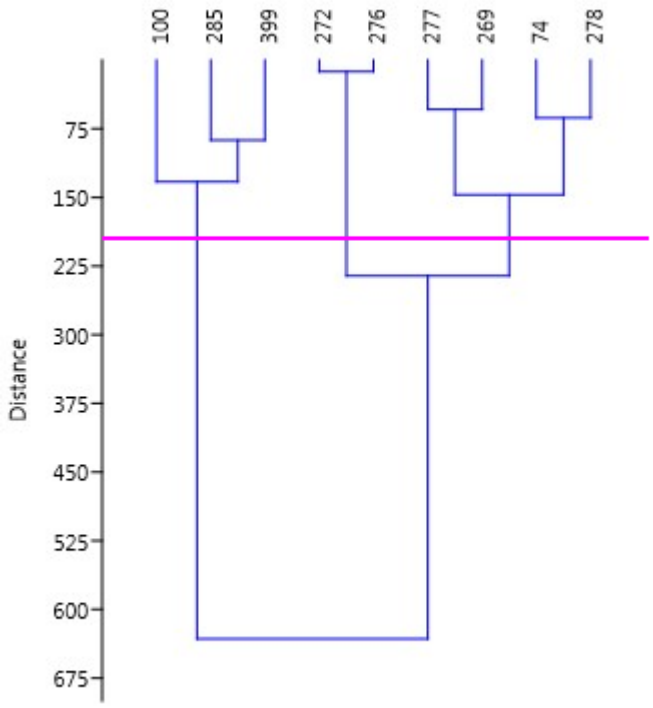

Fig. 1. Cluster analysis based on morphological and chemical traits with ward's method in tea clones.

To show the value of each cluster from 13 measured attributes, the percentage of deviation from the mean of clusters was calculated from the total mean (Table 5). Since clones in each cluster had a greater genetic relationship with clones in the other clusters, the first cluster was composed of three clones and the value of number of plucking shoot, fresh and dry weight of plucking shoot, length of plucking shoot, internodes' distance, green leaf yield and polyphenols showed a higher value than the total average value. The second group consisted of two clones and the average value of the number of plucking shoot, fresh and dry weight of plucking shoot, leaf width, leaf area, internodes' distance, green leaf yield, polyphenol and total ash was less than the total average value. Traits likes' leaf width, leaf area and total ash in clones of the third group showed a higher value than the total average. With regard to the cases mentioned above, crossing among clones sorted in first and second clusters will result offsprings with high yield and quality.

It is emphasized that the tendency toward using similar parents, lack of recognition and using new cultivars in breeding programs leads to a reduction in genetic diversity (Yan et al., 2016). However, farther cultivars with more polymorphism show more genetically distinction, and in terms of hybridization, cultivars with more differences will have the potential for more heterosis or transferring rare traits to the database. To the best of our knowledge, this is the first report that shows promising results of applying morphology and biochemistry for investigating genetic diversity in Iranian selected tea clones.

Yu and Xu (1999) used morphological characters to evaluated diversity in tea germplasm resources of China. In another research, morphology, biochemistry and allozyme studies has been used to present genetic diversity and segregation of *C. sinensis* (cultivated tea) and its wild relatives in Yunnan province of China (Chen et al., 2005). According to Kim et al. (2012), the relationship between catechin-rich and poor lines of tea bushes was mainly analyzed using plants' morphological characteristics and DNA. Genetic diversity of 51 accessions of tea landraces was studied based on agronomic and quality characteristics by Jinang et al. (2013). In a study of 15 tea accessions in Vietnam based on 21 morphological traits, the accessions were clustered by UPGMA cluster and Euclidean distance in two main groups, in the first group 12 accessions of Asami and in the second group three accessions of Chinese type (Phong et al., 2016).

Genetic relationship in selected tea clones

DNA was extracted from leaves of the nine selected tea clones and then analyzed by SCoT analysis using 13 random primers (Table 6). The various sizes of DNA bands were produced in the 13 primers (Table 7).

Amplification of genomic DNA procreated a total of 165 bands with an average of 12.69 bands per primer and generated 122 polymorphic bands patterns with an average of 9.38 bands per primer. The maximum number of polymorphic bands was scored with primer SCoT15 (12 bands, 85.71%). However, a minimum polymorphic band was created by primer SCoT9 and SCoT37 (7, 63.64%). Wachira et al. (2001) announced that 72% of variation inhabited within populations of *C. sinensis* and its wild *Camellia* relatives based on RAPD and AFLP markers. Kaundun and Park (2002) stated that 16% of the total diversity of the RAPD-PCR marker was observed among populations of Korean tea (*C. sinensis*). SCoT is polymorph marker that was used in this study to investigate the genetic diversity of tea for the first time. The results showed that the SCoT marker is capable of detecting polymorphs well. The SCoT marker was used to study the genetic diversity of 8 Iranian modified wheat cultivars. Molecular evaluation results showed that SCoT marker had a high ability to evaluate diversity and differentiability of wheat cultivars (Hamidi et al., 2014).

Table 5. Mean and percentage average deviation of any group from total average for different traits of tea clones derived from cluster analysis

Traits	TA	AFG	PADF	ASG	PADS	ATG	PADT
N.S	143.85	179.56	35.70	86.67	-57.19	145.67	1.81
F.W	79.15	99.28	20.13	46.42	-32.73	80.41	1.26
D.W	15.36	18.70	3.34	9.64	-5.72	15.71	0.35
L.S	7.63	7.88	0.25	7.84	0.21	7.34	-0.29
L.L	7.50	7.02	-0.48	7.90	0.40	7.67	0.16
L.W	3.01	2.97	-0.04	2.82	-0.19	3.13	0.12
L.A	23.14	21.93	-1.21	22.46	-0.69	24.40	1.25
I.D	3.87	4.28	0.41	2.99	-0.88	4	0.13
L.Y	379.29	645.47	266.17	150.36	-228.94	294.13	-85.16
W.E	38.43	38.95	0.52	39.10	0.67	37.71	-0.72
P	13.51	13.84	0.33	13.26	-0.24	13.38	-0.12
T.A	6.40	6.46	0.06	5.95	-0.44	6.57	0.17
D.M	19.78	19.07	-0.71	20.77	0.99	19.81	0.04

TA: Total average, AFG: Average of first group, PADF: Percentage average deviation of first group from total average, ASG: Average of second group, PADS: Percentage average deviation of second group from total average, ATG: Average of third group, PADT: Percentage average deviation of third group from total average, N.S: number of plucking shoot, F.W: fresh weight of plucking shoot, D.W: dry weight of plucking shoot, L.S: length of plucking shoot, L.L: 5^{th} leaf length, L.W: 5^{th} leaf width, L.A: leaf area, I.D: internode distance, L.Y: green leaf yield, W.E: water extract, P: polyphenol, T.A: total of ash and D.M: dry matter.

Table 6. Characteristics of SCoT primers studied

Row	Primers	Nucleotide sequence (5′ to 3′)	Annealing temperature	%GC content
1	SCoT4	CAACAATGGCTACCACCT	48	50
2	SCoT5	CAACAATGGCTACCACGA	48	50
3	SCoT9	CAACAATGGCTACCAGCA	48	50
4	SCoT10	CAACAATGGCTACCAGCC	50	56
5	SCoT11	AAGCAATGGCTACCACCA	48	50
6	SCoT13	ACGACATGGCGACCATCG	52	61
7	SCoT14	ACGACATGGCGACCACGC	54	67
8	SCoT15	ACGACATGGCGACCGCGA	54	67
9	SCoT16	ACCATGGCTACCACCGAC	52	61
10	SCoT18	ACCATGGCTACCACCGCC	54	67
11	SCoT21	ACGACATGGCGACCCACA	52	61
12	SCoT28	CCATGGCTACCACCGCCA	55	67
13	SCoT37	CAATGGCTACCACTAGCC	50	56

Table 7. The percent of polymorphism, polymorphism information content (PIC), generated of molecular data in studied tea clones

Row	Primers	Polymorphic bands	Total bands	%Polymorphism	PIC	MI	Shannon	Nei
1	SCoT4	10	14	71.43	0.37	2.76	0.51	0.33
2	SCoT5	9	13	69.23	0.44	2.92	0.48	0.31
3	SCoT9	7	11	63.64	0.47	2.46	0.60	0.42
4	SCoT10	8	10	80.00	0.48	2.85	0.60	0.41
5	SCoT11	7	9	77.78	0.35	1.81	0.61	0.43
6	SCoT13	11	15	73.33	0.36	2.95	0.50	0.32
7	SCoT14	10	13	76.92	0.41	3.04	0.54	0.35
8	SCoT15	12	14	85.71	0.32	2.88	0.47	0.30
9	SCoT16	11	15	73.33	0.30	2.48	0.62	0.43
10	SCoT18	10	14	71.43	0.35	2.56	0.58	0.40
11	SCoT21	9	12	75.00	0.35	2.30	0.65	0.46
12	SCoT28	11	14	78.57	0.35	2.81	0.58	0.39
13	SCoT37	7	11	63.64	0.40	2.10	0.63	0.44

Molecular genetic diversity between selected tea clones was designated through SCoT-PCR analysis. Genetic similarity across selected tea clones was further analyzed thanks to the results of SCoT analysis. Genetic similarity amongst nine selected clones showed a range between 0.16 and 0.86. The lowest similarity amongst selected tea clones (0.16) was observed in clones 272, 276 and 278. Intercrossing of clones with the lowest similarity (maximum spacing) will give the best result in order to achieve hybrids or attain maximum separation in the next generation. The UPGMA cluster analysis with Jaccard coefficient based on SCoT marker showed the highest amount of cophenetic correlation coefficient (0.91) and placed the tea clones in three distinct groups (Fig. 2). The first group included of clones 272 and 276. The second one consisted of clones 100 and 399, and the third group contained clones 269, 277, 278, 285 and 74 (Fig. 2). Possibility to achieve optimal results can be achieved by crossing between distant clones selected from spaced clusters. It is expected that these results could be used in breeding programs of highly valuable tea clones.

Correlation between the similarity coefficient matrix of molecular markers and matrix of morphological and chemical data with the mantel test was significant (0.44). This indicates that there is a correlation between the pattern of variation represented by the markers and the morphological and chemical data. The grouping of two methods (based on morphological, chemical and molecular marker) was partially identical, and one clone was in different group in two methods. This result may imply that the two systems have different estimates of genetic relationships between clones. The main reason for the discrepancy between grouping clones based on morphological and chemical traits can be that the most quantitative traits are controlled by a large number of gene and are strongly influenced by the environment. In addition, SCoT markers are randomly distributed throughout the genome (Rahimi et al., 2019). Other researchers also examined genetic variation of various accessions and varieties of tea with different markers, showing the diversity between them and placing the accessions in different groups (Mondal, 2002; Balasaravanan et al., 2003; Matsumoto et al., 2004; Hu et al., 2014; Beris et al., 2016).

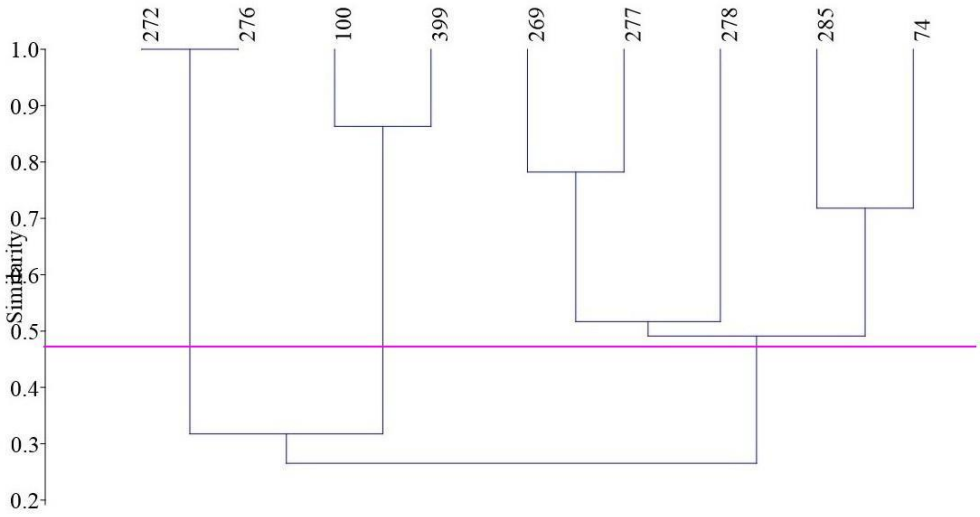

Fig. 2. Cluster analysis based on SCoT markers with UPGMA method and Jaccard's genetic similarity in tea clones.

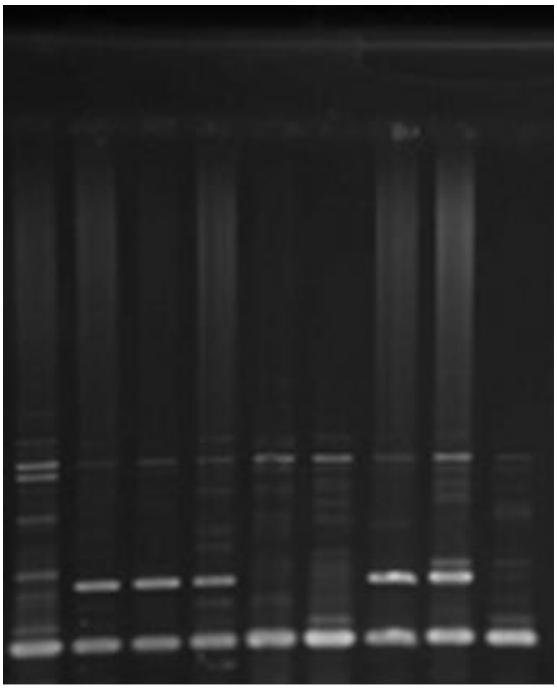

Fig. 3. Amplified fragment for primer SCoT 13.

CONCLUSIONS

The results showed that there was a high genetic variation among the tea clones in terms of morphological and chemical traits as well as the SCoT markers. The grouping of two methods (based on morphological, chemical and molecular marker) was partially identical. Having information about genetic diversity of tea germplasm for designing suitable breeding programs is very helpful to obtain suitable plants for specific purposes.

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Conflict of interest
The authors declare that they have no conflict of interest.

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