# Assessment of New Kiwifruit Genotypes (*Actinidia chinensis* (A.Chev.) A.Chev.) From Open Pollination by Molecular Markers and Phenotypic Traits

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**Summary**

Molecular markers are useful tool to study genetic diversity and the relationship between genotypes. The evaluation of genetic diversity in species is a start step for breeding program. In this study, molecular markers were used to determine diversity and genetic relationships of 120 kiwifruit genotypes. During two consecutive growing seasons, thirty-two discretional morphological traits were studied by the International Union for the Protection of New Varieties of Plants (UPOV) in new kiwifruit genotypes. Average value of polymorphism per primer was 95.1%. Polymorphism information content values for all the primers used ranged from 0.32 to 0.41. High levels of polymorphism were found for all marker systems. Dendrogram generated using unweighted pair group method with arithmetic cluster analysis based on Jaccard's similarity coefficient and dendrogram generated using Neighbor-joining cluster analysis accessions were separated 120 genotypes into 11 and 5 groups, respectively. For all markers, 171 marker–trait associations were found with the GLM, the significance criteria defined for the traits only at the $P \leq 0.01$ level. This study results provide useful genetic information about new kiwifruit genotypes of Iran and indicate that the use of new kiwifruit genotypes in breeding program could be useful for generating new cultivars with novel characteristics.

**Key words**

cluster, diversity, GLM, MTAs, polymorphism

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## Introduction

Kiwifruit is one of the most important horticultural crops in Iran, which annually exports high volume of kiwifruit to more than 30 countries (Maleki et al., 2017). Kiwifruit has been demonstrated to provide high nutritional and health benefits for humans, due to providing bio-active components (Vitamin C) and mineral elements (such as Fe, Zn, Cu) (Ma et al., 2019). Iran is one of top kiwifruit producers in the world, with a yield of 344189t in 2018 from a cultivated area of 12773ha (FAO, 2019).

To date, the main kiwifruit cultivar grown in Iran is *Actinidia deliciosa* cv. Hayward with green flesh fruits. However, little area is grown with red and golden flesh. There is a tendency for consumers to prefer higher variation in kiwifruit's color and taste (Ferguson, 2015). In addition, colored kiwi fruit has a higher price than the traditional green kiwifruit has in global market. Therefore, Iran needs to target breeding program for introducing of the new unique cultivars to maintain its position in the world market.

High demand for novel kiwifruit, especially for red and golden flesh kiwifruit both in domestic markets and export has caused breeders to start different breeding projects to find new kiwifruit cultivars. In addition, for sustainable production and export and access to new market, Iran needs to diversify its cultivars. This is because other kiwifruit produces of a number of different cultivars of *A.chinensis* and *A. deliciosa* have flesh that is lime green, bright yellow, red, purple, yellow, or orange when ripe. Some of cultivars have a variety of advantages such as early maturity, as well as resistance to diseases which is obtained through natural selective breeding or breeding program (Li et al., 2018).

In general, genetic diversity is a prerequisite for starting any breeding program (Verma et al., 2017). Therefore sexual propagation (seeding) is an opportunity for germplasm variation. Kiwifruit cultivars on the world market are mostly generated from wild germplasm, produced from open pollination (seedlings) and controlled crosses (Wang et al., 2012).

Investigation of plant phenotypic diversity has become a major field of research in plant breeding (Li et al., 2014). Researchers still require the phenotypic data; a single phenotyping cycle is used to identify markers for subsequent selection through generations within the maker assisted recurrent selection. Although molecular breeding techniques have placed their greater focus on selections based on genotypic information (Li et al., 2014), the evaluation of genetic diversity of any species is a start step for breeding program (Verma et al., 2017).

PCR-based markers determine the variation in genomic DNA sequences and ideally link them to phenotypic trait in breeding programs (Pourian et al., 2019). Several different molecular markers are used to investigate the relationship between diversity and genetic, since there are no molecular markers available to complete all the genomic regions (Pourian et al., 2019).

In recent years, a number of molecular techniques such as Inter-Simple Sequence Repeat (ISSR), Inter Retrotransposon Amplified Polymorphism (IRAP), REMAP, and RAPDs have been widely used to detect genetic diversity and polymorphisms at the DNA level (Pourian et al., 2019; Sharafi et al., 2016; Sharafi et al., 2017; Hassanpour et al., 2013).

The utility of markers such as ISSR for assessing genetic diversity arises. There is no need for prior DNA sequence information and this technique provides a powerful, rapid, simple, reproducible, and inexpensive means for easy assay (Sharafi et al., 2017).

IRAP and Retrotransposon Microsatellite Amplified Polymorphism (REMAP) are one of the several molecular marker methods based on retrotransposons (RTNs). IRAP and REMAP have been applied as molecular markers in genetic diversity studies, due to the dispersion, ubiquity, and prevalence of RTNs in plant genomes and also two RTN-based markers and they require no DNA digestion (Holasou et al., 2019).

RAPD is PCR-based marker, one of the first developed methods that has been extensively used in assessing genetic relationship among various accessions of different plant species, due to the arbitrary quick sequence of the primers and requires only small amounts of DNA sample (Verma et al., 2017).

An alternative manner for QTL analysis is association analysis, particularly for trees and perennial horticultural crops to eliminate limitations of their pedigree-based mapping. Association analysis is an efficient scientific approximate for recognizing the relationships between molecular markers and morphological traits in a population (Abouzari et al., 2020 Association analysis is also done to recognize markers associated with cold tolerance- related traits in Citrus (Abouzari et al., 2020), fatty acid metabolism in flax seeds (Xie et al., 2019) and cold tolerance in cotton (Sun et al., 2019). Furthermore, the association analysis approach has the potential to eliminate the main bug of classical linkage analysis, which is developing prolonged specific mapping populations (Abouzari et al., 2020). In recent years, association analysis has been extensively used to detect and validate QTLs or genes for important traits in many plants. For this purpose, association analysis has several analytical advances including the stratification of population structure and statistical models such as generalized linear model (GLM) and mixed linear model (MLM) approaches (Abouzari et al., 2020;).

The aim of current study was to identify genomic locations associated with morphological traits using RAPD, ISSR, REMAP and IRAP markers in new kiwifruit Genotypes from open pollination. In the present study, equence-based DNA markers were used to evaluate the genetic diversity of some kiwifruit Genotypes with the aim of using them in breeding programs as well as for conservation management of kiwifruit germplasm in Iran.

## Materials and Methods

### Plant Material and Phenology

The plant materials were supplied of the cultivated seeds from two elite genotypes as female parents and two elite genotypes as male parents of kiwifruit (*Actinidia chinensis*). To reduce juvenility and accelerate flowering, scions from young seedlings were grafted on mature Hayward kiwifruit vines cultivated in research kiwifruit collection in university of Guilan, Iran. The genetic diversity accession consisted of 98 progenies and twenty genotypes as parents have been characterize used. Thirty-two morphological traits were recorded during two consecutive

seasons. All characteristics were considered, based on rating and coding according to kiwifruit descriptor. For determining the difference between genotypes, UPOV test guidelines were used (www.upov.int), to detect whether was any difference between seedlings (UPOV 2012).

### DNA Extraction

Total genomic DNA was extracted from young leaves of genotype following the standard CTAB method with minor modifications (Sharafi et al., 2016). The quantity of DNA was checked by spectrophotometer using Nano Drop-1000 spectrophotometer (NanoDrop Technol-ogies, USA). Thereafter samples were diluted to 25 ng $\mu L^{-1}$ and stored at −20 °C for next PCR reaction.

### PCR Reaction Conditions and Program

Seven molecular markers (Table 1) were used to find the genetic relationships between 120 kiwifruit genotypes; namely, Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeat (ISSR), Inter-Retrotransposon Amplified Polymorphism (IRAP), and Retrotransposon Microsatellite Amplified Polymorphism (REMAP). The PCR reaction was mixture of 20-40 ng of each sample DNA, 0.3 mM of primers, 8.5 ml of PCR kit master mix (CinnaGen Co., Iran), and DNA-free water. PCR reactions were performed in a BioRad thermocycler (BioRad Laboratories Inc., Hercules, CA, USA). To confirm the size of the amplicon, the PCR products were run through a 1.5% agarose gel and visualized using safe stain (CinnaGen Co., Iran) and viewed under UV light (BioDoc Analyzer 2.1 (Germany). Then the amplicon of predicted size was compared with the Ladder 100 bp plus R-T-U 100-3000, (Sharafi et al., 2017).

### Data Analysis

Bands of all markers were scored as one (presence) and zero (absence) and the resulting matrix was used to study the population structure. Polymorphism information content (PIC) of each marker was computed according to the formula:
$PIC = 1 - \sum pi2$, where 'pi' is the frequency of the ith allele.

$EMR = np \times \beta$, where 'np' is the total of polymorphic bands, and 'β' is the ratio of polymorphic bands to total bands and $MI = PIC \times EMI$. All of the following calculations were implemented in POPGENE software: Effective number of alleles (Ne), Nei's gene diversity and Shannon's information index (Hassanpour et al., 2013; Noroozisharaf et al., 2015). Similarity matrix based on simple matching coefficient was constructed from the markers data. Then, the similarity matrix was constructed with different coefficients such as Jacquard, simple matching coefficient and Nei. In order to check the fitness of the cluster and the similarity matrix, the cophenetic correlation coefficient was calculated. Population genetic structure was analyzed according to the method of the cluster analysis such as the neighbour-joining method and maximum distance clusters (farthest neighborhood) using the NTSYS software. The dendrograms were constructed through SAHN clustering program using the unweighted pair group method with arithmetic means (UPGMA) (Pourian et al., 2019).

Principal component analysis (PCA) and cluster analysis were undertaken for the morphological data, to show the relationship between genotypes in SPSS software.

Therefore, to cluster and for better inference, the genetic structure of the analyzed new kiwifruit genotypes was performed through STRUCTURE software. For this intention, the number K was considered from 1 to 6 and simulation was performed using 10,000 turns in steps followed by 100,000 Markov Chaine Monte Carlo (MCMC) repetitions. The Evannos DK method was used to determine conclusive K value and Lnp (D) (Ln probability data) was used to detect the presence of genetically distinct populations (Surapaneni et al., 2016).

Tassel. 3 software was used for the association analysis between markers and phenotyping traits. A general linear model was used to investigate the association of markers and traits, the GLM analysis being based on Q matrix. In a general linear model, when the P value obtained for the relationship between the marker and the trait is 0.05, there is a significant correlation between them (Liao et al., 2019;). A P value of 0.01 in the relationship between a marker and trait represents a very significant correlation between them (Liao et al., 2019).

**Table 1.** ISSR, IRAP, RAPD and REMAP markers used for evaluation of the diversity and genetic structure of in 120 kiwifruit genotype accessions

| Marker | No. | Primer name | Annealing temperature (°C) | Sequence (5_–3_) |
|---|---|---|---|---|
| RAPD | 1 | OPN | 36 | ACTGAACGCC |
| | 2 | OPC5 | 45 | GATGACCGCC |
| ISSR | 1 | UBC855 | 52 | (AC)8YT |
| | 2 | UBC823 | 49 | (TC)8C |
| IRAP | 1 | WLTR455 | 50 | TTGAATTTCTGCTACGTTCCCC |
| REMAP | 1 | WLTR455+UBC855 | 50 | TTGAATTTCTGCTACGTTCCCC+(AC)8YT |
| | 2 | WLTR455+UBC824 | 59 | TTGAATTTCTGCTACGTTCCCC+(TC)8C |

Note: N – number

## Results and Discussion

The very first and the most essential step to make the conservation, characterization and benefit of biodiversity in a seedling collection is assessment of genetic variation. During two growing seasons, thirty-two morphological traits were studied on the basis of the descriptors established by the International Union for the Protection of New Varieties of Plants (UPOV, 2018) at the experimental sites. According to the UPOV guidelines, all evaluated traits were morphologically distinct.

For indicating differences measured among groups, factor analysis was used based on principle components that are a decrease distance model. The data analysis based on principal component analysis showed that 9 components could explain 71.7% of the total variation contributed among studied traits. Flower traits contributed the most of total variation and were predominant in the first component. In fact, 22.4% of the variation in the first component was floral traits. The second component showed 9.3% of the total variation, and included inflorescence characteristics as an inflorescence. Respectively, the other components were named bud traits (7.4%), stem traits (5.9%), petiole (5.9%), leaf hair (5.6%), leaf blade (5.4%), leaf color (5.2%) and stem hair (4.3%). Due to the importance of traits related to flowers and inflorescence, floral traits and inflorescence (the first two components) are used in two-dimensional PCA plot. Based on two-dimensional PCA plot and with regard to the first two components, all the offspring genotypes and parents, except 12 progenies were placed in one group with each other. Flowering traits were not recorded for these offsprings, because these progenies got the longest juvenile period (Fig. 1). PCA was used to study the diversity of many plants and species (Tahernezhad et al., 2010; Khadivi-khub et al., 2013;), but so far it has not been used to study the morphological traits of kiwifruit.

Morphological cluster analysis showed that two distinct groups most offspring genotypes and parents were placed in the first group. N55, N1, S6, R40 and R44 were located at the shortest distance from the red female parent (FR1 to FR6). Also, N40 and E2 were located at the shortest distance from the gold female parent (FG1 to FG6). N51, E14 and N58 had the shortest distance from the red male parents (MR1 to MR6) and the gold male parents (MG1 to MG6), respectively. The second group N30, S19, E10, N53, N59, R21, R52, S7, E9, N26, N33, R29, R47, N43, S26, S20 and E8 were grouped in the same cluster according to their morphological characters. The classification based on morphological traits was conforming to two-dimensional PCA plot and genotypes with the longest juvenile period located in closer distance to each other (Fig. 2).

### Molecular Markers Analysis

A population with high biodiversity is the first tool in breeding programs. The high molecular variability was detected among plant materials. We used 7 primers (Table 1) with appropriate genomic distribution and coverage, in order to evaluate the diversity and genetic structure of 120 different kiwifruit genotypes. Average value of polymorphism per primer was 95.1% (Table 2). From 166 (95.4%) polymorphic bands, a mean of 23.7 polymorphic band belonging to each of the primers. From 7 markers, Maximum value for polymorphism (100%) belonged to marker OPC5 and also the maximum number of bands (29 fragments) was obtained from marker OPC5. The marker UBC855 gave the minimum number of bands and the minimum value for polymorphism (22 fragments and 86.3% respectively). According to previous studies, the primer sequence and also the extent of molecular variation in each genotype played the main role in the number of generated bands per primer (Nikoumanesh et al., 2011).

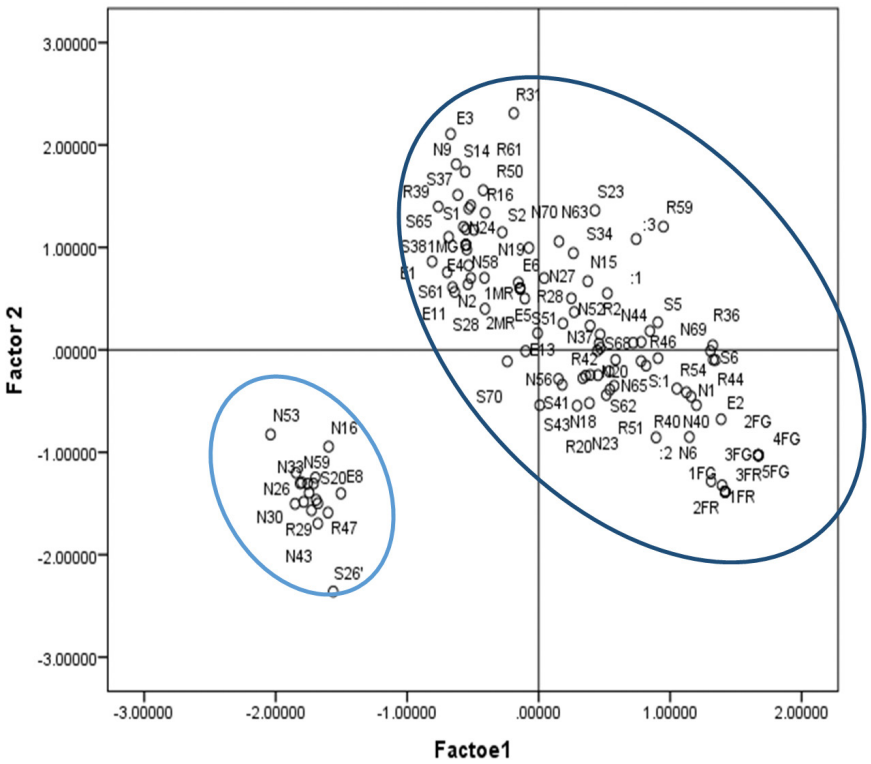


**Figure 1.** Two-dimensional plot of 120 accessions of new kiwifruit genotypes by principal component analysis (PCA)

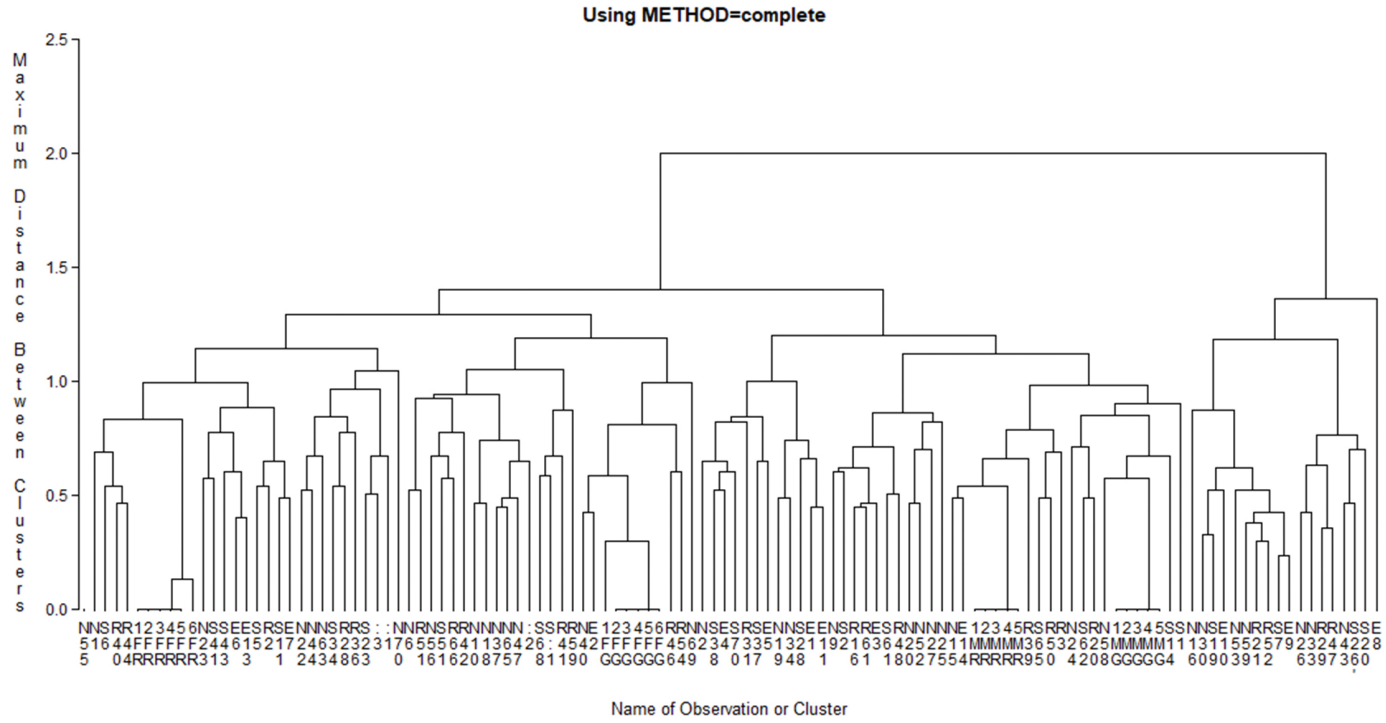


**Figure 2.** Dendrogram of genetic distance for 120 new kiwifruit genotypes individuals based on morphological data

PIC values for all the primers used ranged from 0.32 to 0.41, indicating that the primers could make medium polymorphism which is beneficial for genetic variation of studied genotypes (Table 2). The PIC value (PIC > 0.5, 0.5 > PIC > 0.25 and PIC < 0.25) determines the polymorphism rate (High, medium or low polymorphism, respectively) in the study population (Hassanpuor et, al., 2013). The highest PIC value (0.41) was observed in the REMAP primer of WLTR455+ubc855 and the lowest PIC value (0.32) was observed in UBC855, OPN and REMAP (WLTR455+ubc524) primers.

The observed average of the effective number of alleles per locus (Ne) was 1.46 that ranging from1.36 to 1.57. The Nei's gene diversity (H) ranged from 0.24 to 0.34 with an average of 0.29 per marker. All used markers revealed Shannon's information index (I) between 0.40 and 0.52. The highest value off pp% (100), EMR (29) and IM (9.9) was observed in OPC5 primer and the highest value of Ne(1.57), h(0.34) and I(0.52) belonged to REMAP(WLTR455+UBC524) marker. According to these results, it could be claimed that (WLTR455 UBC524), (WLTR455+UBC855) and OPC5 primers had a greater potential among primers used in this study (Table 2). However, all primers used to evaluate the genetic relationships among the studied kiwifruits genotypes were very efficient. It should be noted that, as a measure of genetic variation, the values of EMR, MI, Ne, He and I are more appropriate, since they are independent of the sample size (Hassanpour et al., 2013).

According to dendrogram generated using UPGMA cluster analysis based on Jaccard's similarity coefficient, accessions were separated into 29 clusters and the values of the similarity coefficient were from 0.72 to 1.00 (Fig. 3).

**Table 2.** Levels of polymorphism and comparison of information obtained with our markers in 120 new kiwifruit genotypes

| | TB | PB | PP% | EMR | IM | ne* | h* | I* | PIC |
|---|---|---|---|---|---|---|---|---|---|
| OPN | 22 | 21 | 95.5 | 20.0 | 6.4 | 1.44 | 0.27 | 0.42 | 0.32 |
| OPC5 | 29 | 29 | 100.0 | 29.0 | 9.9 | 1.42 | 0.26 | 0.41 | 0.34 |
| UBC855 | 22 | 19 | 86.4 | 16.4 | 5.3 | 1.48 | 0.30 | 0.47 | 0.32 |
| UBC823 | 24 | 23 | 95.8 | 22.0 | 8.5 | 1.40 | 0.27 | 0.44 | 0.39 |
| WLTR455 | 24 | 23 | 95.8 | 22.0 | 8.9 | 1.53 | 0.32 | 0.49 | 0.40 |
| WLTR455+UBC855 | 26 | 25 | 96.2 | 24.0 | 9.9 | 1.36 | 0.24 | 0.40 | 0.41 |
| WLTR455+UBC524 | 27 | 26 | 96.3 | 25.0 | 8.1 | 1.57 | 0.34 | 0.52 | 0.32 |

Note: TB – total number of bands; PB – total number of polymorphic bands; PPB – percentage polymorphic bands; EMR – effective multiplex ratio; MI – marker index; Ne – effective number of alleles; H – Nei's gene diversity; I – Shannon's information index; PIC – poly-morphism information content

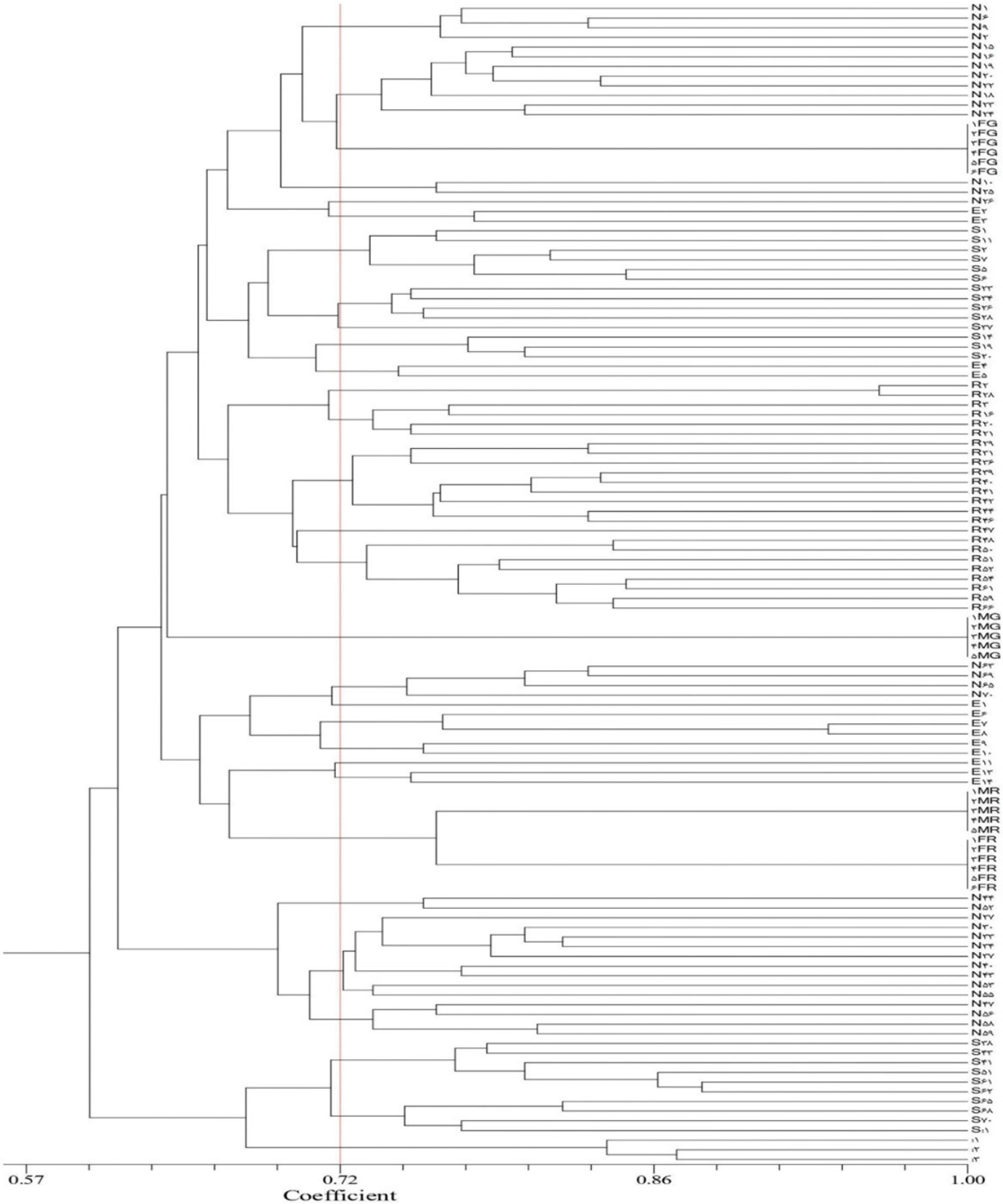


**Figure 3.** UPGMA dendrograms based on our markers

The number of accessions in these groups varied, with the largest and smallest groups having 13 and one member, respectively. The high cophenetic correlation coefficient (r = 0.84) shows that this dendrogram is a good depiction of our markers for the data matrix (Fig 3). The dendrogram accuracy in genetic relationships among accessions is determined by its cophenetic correlation coefficient (Surapaneni et al., 2016). The extensive genetic diversity is evident in the kiwifruit population. High biodiversity is used in modern breeding programs to produce genotypes with superior agronomic traits as it is more resistant to different environmental conditions (Surapaneni et al., 2016).

Results showed that these markers measured sufficient polymorphism for DNA typing and could be used in kiwifruit genetic diversity studies, as noted for other plant species or for helping breeders to select diversity genotypes. The dividing based on morphological diversity showed that it was not conforming to genetic dividing. In fact, it can be said the dividing based on morphological diversity and Principal Component Analysis (PCA) has been complemented by molecular techniques (Khadivi-Khub et al., 2013). But the molecular techniques due to cover a large proportion of the genome, including coding and noncoding regions, show different results from those of morphological marker. (Tahernezhad et al., 2010). In the present study, results demonstrate high levels of polymorphism among studied genotypes. The information obtained in here can be of great help in improving the production of kiwifruit in Iran, because this is the first study of genetic relationships of new kiwifruit genotypes using molecular marker technique in Iran.

The results will also be useful in selecting diverse genotypes and inter-specific breeding programs and QTL mapping studies in kiwifruit. To establish a genetic diversity study, it is necessary to combine the required and also less laborious morphological evaluation with molecular markers evaluation. This procedure can give more valid conclusions in the evaluation of genetic diversity (Nikoumanesh et al., 2011). Population structure is used to describe the relationships of individuals within and between populations. In genetic studies, population structure provides a perspective on the evolutionary relationships of individuals in a population. There should not be a structure in the studied population or the population structure must be considered to avoid false positive associations in association analysis studies ideally (Sun et al., 2014).

According to dendrogram generated using Neighbor-joining cluster analysis, accessions were separated into 5 clusters, including 24, 15, 40, 27 and 14 accessions, respectively (Fig. 4). Based on the molecular dendrogram, grouping of the parents in a group with the shortest distance was evident. To study the genetic structure of the population and determine the appropriate number of subpopulations, a structure analysis was performed before association analysis. The highest value of ΔK for the 120 Genotypes accessions was K =5. In order to achieve K optimization, the share of individuals in each cluster (matrix Q) was considered in estimating the population structure and the matrix calculation.

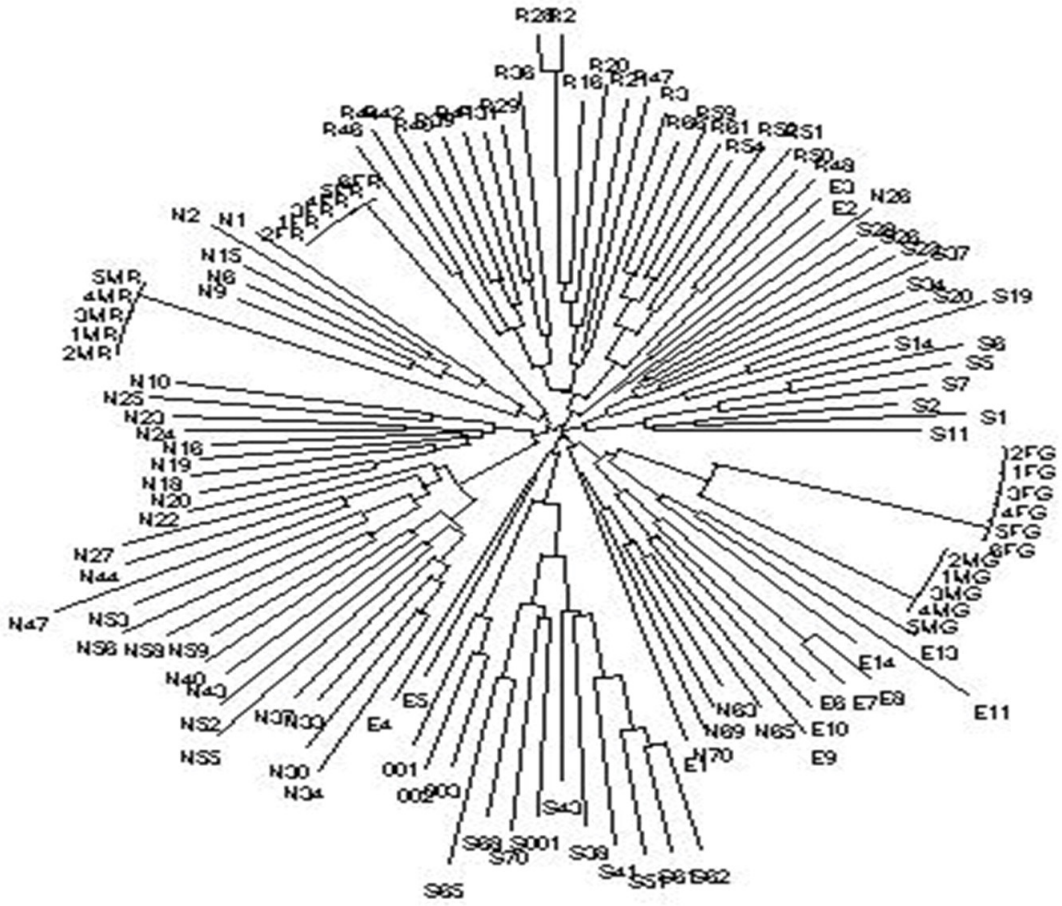


**Figure 4.** Neighbor-joining tree analysis based on our markers

**Table 3.** Marker–trait association in120 kiwifruit genotypes using generalized linear model (GLM) approach

| Marker | Marker_p | MarkerR2 | Trait | Marker | Marker_p | MarkerR2 | Trait |
|---|---|---|---|---|---|---|---|
| P1o1 | 0.001 | 0.096 | Leaf blade: basal lobes | P3o21 | 0.008 | 0.052 | Petiole: anthocyanin coloration of upper side |
| P1o10 | 0.008 | 0.061 | Leaf blade: basal lobes | P3o21 | 0.003 | 0.066 | Inflorescence: type |
| P1o16 | 0.002 | 0.084 | Leaf blade: basal lobes | P3o21 | 0.004 | 0.068 | Flower bud: position of first spike |
| P2o10 | 0.005 | 0.066 | Leaf blade: basal lobes | P3o21 | 0.004 | 0.058 | Flower: number of sepals |
| P2o13 | 0.010 | 0.058 | Leaf blade: basal lobes | P3o21 | 0.000 | 0.120 | Flower: main color of sepals |
| P3o8 | 0.004 | 0.070 | Leaf blade: basal lobes | P3o21 | 0.001 | 0.074 | Flower: number of styles |
| P4o12 | 0.002 | 0.084 | Leaf blade: basal lobes | P3o21 | 0.002 | 0.073 | Flower: number of anther |
| P4o16 | 0.009 | 0.059 | Leaf blade: basal lobes | P3o21 | 0.000 | 0.093 | Flower: attitude of anther |
| P4o17 | 0.004 | 0.071 | Leaf blade: basal lobes | P3o21 | 0.006 | 0.055 | Petal: main color on adaxial side |
| P5o15 | 0.001 | 0.091 | Leaf blade: basal lobes | P3o21 | 0.001 | 0.086 | Flower: diameter |
| P6o8 | 0.000 | 0.127 | Leaf blade: basal lobes | P3o21 | 0.001 | 0.080 | Flower: shape in profile |
| P6o20 | 0.000 | 0.140 | Leaf blade: basal lobes | P5o12 | 0.008 | 0.058 | Stem: texture of bark |
| P6o27 | 0.000 | 0.204 | Leaf blade: basal lobes | P5o15 | 0.004 | 0.066 | Stem: texture of bark |
| P7o4 | 0.002 | 0.083 | Leaf blade: basal lobes | P5o21 | 0.007 | 0.060 | Stem: texture of bark |
| P7o7 | 0.010 | 0.058 | Leaf blade: basal lobes | P6o12 | 0.010 | 0.054 | Stem: texture of bark |
| P7o10 | 0.006 | 0.065 | Leaf blade: basal lobes | P6o20 | 0.007 | 0.058 | Stem: texture of bark |
| P7o13 | 0.006 | 0.066 | Leaf blade: basal lobes | P6o25 | 0.005 | 0.064 | Stem: texture of bark |
| P7o19 | 0.001 | 0.087 | Leaf blade: basal lobes | P7o2 | 0.010 | 0.054 | Stem: texture of bark |
| P1o8 | 0.003 | 0.071 | Stem: texture of bark | P7o6 | 0.006 | 0.062 | Stem: texture of bark |
| P1o16 | 0.002 | 0.077 | Stem: texture of bark | P7o18 | 0.005 | 0.064 | Stem: texture of bark |
| P2o5 | 0.002 | 0.078 | Stem: texture of bark | P7o26 | 0.009 | 0.055 | Stem: texture of bark |
| P2o8 | 0.000 | 0.135 | Stem: texture of bark | P3o5 | 0.001 | 0.092 | Stem: texture of bark |
| P2o14 | 0.003 | 0.073 | Stem: texture of bark | P5o4 | 0.005 | 0.063 | Stem: texture of bark |
| P2o21 | 0.009 | 0.056 | Stem: texture of bark | | | | |

Note: e.g. P1o9 - First primer and band 9 observed on the gel

The association of molecular markers with phenotyping traits in the presence of population structure was tested using the software program TASSEL 3.0.1. In order to identify associations between the markers and the evaluated traits in the studied genotypes, the general linear model (GLM) based on the Q-matrix obtained from the STRUCTURE software was used. Numerous marker–trait associations (MTAs) were detected based on this method. For all markers, numbers of 171 MTAs were found but the significance basis in the GLM method for all markers was performed only on the $P \leq 0.01$ level. The GLM results showed that alleles of various loci were significantly associated with multiple traits (Table 3). Most MTAs were observed in the 21st band of UBC855 primer (P3o21), which is associated with 11 different traits. Among the 32 evaluated traits leaf blade, basal lobes and stem: the texture of bark had the highest association with different bands of markers (18 bands of 5 markers). In the general linear model, a number of common markers were identified for different traits, which may be due to pleiotropic effects or the association of genomic regions involved in trait control (Abouzari et al. 2020).

This probable interaction resulted in the observed high level of variations in this study. A structural difference was observed between the morphological and molecular analysis. A notable divergence in groupings obtained from RAPD dendrogram with those obtained by morphological dendrogram was reported by Nikomanesh et al., 2011. Molecular markers investigate the whole genome including both coding and non-coding regions but morphological traits are related to coding regions and environmental effects (Verma et al., 2017). The low correlation could be due to differences in the nature of these two methods given the fact that most of the genome size consists of non-coding regions (Nikomanesh et al. 2011; Vermaet al., 2017). In previous studies, low level of correlation was found between morphological characterestics and molecular markers (Verma et al., 2017).

## Conclusion

With increasing consumers' demand for novel, kiwifruit breeders try to access new cultivar development. A population with high diversity is the first tool for breeding programs. Although further studies and more validation are required, the markers with the strongest effects in our study provide ability for candidates to further study suitability capacity in future research on marker assisted selection (MAS). Species differences at molecular level in some of genotypes could be explained by random mating and gene exchange through natural interspecific hybridization, owing to open pollination in kiwifruits of our study and its related species. These results show that there is valuable biodiversity among the members of this group to start a breeding program. This source of diversity can be used in the next steps as parents in breeding programs.

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