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ORIGINAL ARTICLE

Genetic diversity and population structure of watermelon (*Citrullus* sp.) genotypes

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Abstract
Genetic polymorphism amid plant species is a crucial factor for plant improvement and maintaining their biodiversity. Evaluation of genetic diversity amongst plant species is significant to deal with the environmental stress conditions and their effective involvement in the breeding programs. Hence, in present study, an attempt has been made towards the genetic assessment of individual and bulked populations of 25 watermelon genotypes, belonging to Citroides (citron watermelon) and Lanatus (dessert watermelon) group from Konya, Thrace, Turkmenistan, Saudi Arabia and Turkey. The employed Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Polymorphism (ISSR) marker systems provided 69.4 and 95.4% polymorphisms, respectively. Different clustering methods showed clear grouping of the genotypes based on the geographical origin and species. Citron genotypes from Turkmenistan stood apart from all the Turkish Lanatus genotypes. However, Saudi Arab Lanatus genotype grouped with native Turkish varieties indicating the genetic linkage. Among all the Turkmenistan Citron genotypes, Turkmenistan-11 was the most distinct form. Moreover, sufficient genetic variation was found between the commercial and native Lanatus genotypes of Turkey as well as Citron genotypes of Turkmenistan. Hence, it will be beneficial to include these genotypes in the future breeding programs to transfer disease-resistant alleles from Citron to Lanatus genotypes.

Keywords Genetic diversity · Molecular breeding · Population structure · RAPD · ISSR · Watermelon

Introduction

Genetic diversity in plant species provides them the capacity to cope with different environmental stresses. Increment in genetic diversity enhances the prospects of effective plant selection and thus, becomes an imperative factor in plant breeding. However, it is important to estimate its extent and range for its effective utilization. Hence, numerous strategies have been employed to determine genetic polymorphism in several plant species. Estimation of genetic variability using molecular markers is a proven method to understand the genetic constitution, identifying the genes involved in crucial growth mechanisms and conservation of genetic variation in plant species.

Watermelon {*Citrullus lanatus* (Thunb.) Matsum and Nakai} research has evidenced several revolutions and findings in last several years. The fruit species have gained major importance due to its lycopene content per cup that is found higher even than tomato (Chug-Ahuja et al. 1993; Clinton 1998; Holden et al. 1999; Perkins-Veazie et al.

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2003). Including more than 800 species that are widely spread throughout the world, watermelon is a thriftily significant part of Cucurbitaceae group (Jeffrey 1990). It is an innate diploid crop of tropical regions corresponding to the *Citrullus* genus with two sets of eleven chromosomes (Bates and Robinson 1995). The crop has been recorded in Central African, Egypt and the Middle East region for about 10,000 years and later launched in China, Europe and North America in tenth, thirteenth and seventeenth centuries, respectively (Whitaker and Davis 1962).

Nowadays, watermelon species are available in different shapes, sizes, rind thicknesses, skin textures, flesh colors and seed frequencies, but due to continuous cultivation practices and selection of varieties for particular traits, genetic base has narrowed leading to limited improvement in watermelon research and breeding. Nevertheless, morphological characteristics have a crucial role in species conservation and plant breeding, it needs to be associated with genetic information to obtain more definitive conclusion. Although several studies have been conducted throughout the world to estimate the morphological diversity of watermelon species (Huh et al. 2008; Choudhary et al. 2012; Gbotto et al. 2016; Singh et al. 2017; Soghani et al. 2018), experiments on genetic diversity are still limited. Additionally, diseases such as Bacterial fruit blotch (*Acidovorax avenae* subsp. *citrulli*) and Fusarium wilt are responsible to cause a huge loss to watermelon production (Martyn and Netzer 1991; Hopkins and Levi 2008). The two most common species of watermelon are *C. lanatus* var. *citroides* and *C. lanatus* var. *lanatus* that are known as citron melon and dessert melon, respectively (Mashilo et al. 2017). On the one hand, where dessert melon is known for its narrow genetic base, citron melon possesses huge genetic diversity (Levi et al. 2001; Levi and Thomas 2005; Dane and Liu 2007; Ocal et al. 2014).

Moreover, resistance towards drought and several diseases makes the citron melon a suitable resource for watermelon breeding programs (Gusmini et al. 2005; Davis et al. 2007; Yoshimura et al. 2008; Tetteh et al. 2010; Edelstein et al. 2014; Mo et al. 2016; Rhee et al. 2015; Thies et al. 2010). Hence, a number of breeding programs are in progress for the introgression of suitable alleles from resistant form, Citron, to susceptible one, Lanatus (Gusmini et al. 2005; Tetteh et al. 2010; Wechter et al. 2012; McGregor and Waters 2013). Accordingly, in our study, we determined the genetic distance between both the types of accessions so that these can be efficiently involved in the future crossing programs.

Being a vital reservoir of impending beneficial genes, genetic resources can be advantageous for the future studies in plant breeding. Hence, due to scarce genetic and genomic resources and for efficiently employing the available germplasm resources, determination of watermelon diversity is extremely crucial (Che et al. 2003). Turkey has become one of the imperative hubs of watermelon genetic diversity due to the broad expanse of primitive varieties and landraces all over the Mediterranean and Central Anatolian regions (Solmaz and Sarı 2009). Despite watermelon being a significant crop of Turkey, limited number of studies have been performed on its molecular diversity (Solmaz and Sarı 2009; Ulutürk 2009).

For determining the genetic relationship amongst watermelon varieties, molecular markers can be considered as effectual tools. Molecular overviews provide agronomical ideas about genetic resources, directly augment the genetic base, reveal duplicate accessions, recognize purity among genotypes and facilitate crossing and selection of varieties with specific characteristics (Arif et al. 2010). Numerous techniques are now accessible pertaining to molecular marker studies, and researchers can select the mode of specific concern, depending on existing materials and aims. Amongst these available methods, random amplified polymorphic DNA (RAPD) is considered as one of the most commonly used genetic approaches in diversity studies. Though the technique is less reproducible, due to its cost-effectiveness, pace and ease, it has been broadly used for determining the associations among different genotypes, construction of linkage maps, species identification and evaluation of genetic polymorphism. A number of researchers have reviewed the utility of RAPD markers in describing molecular polymorphism of various crop species (Horejsi and Staub 1999; Semagn et al. 2006; Maria et al. 2008; Sikdar et al. 2010; Arif et al. 2010; Jonah et al. 2011; Khan et al. 2014). Similarly, ample amount of revealing primers have been provided by RAPD procedure that are capable of differentiating watermelon genotypes (Levi et al. 2001; Fazeli et al. 2008; Mujaju et al. 2010; Solmaz et al. 2010; Yang et al. 2010). Inter-Simple Sequence Repeat (ISSR) marker system is one more PCR-based organization having extensive relevance for different species, apart from the accessibility of information regarding their genome series (Gui et al. 2007; Kurane et al. 2009; Shi et al. 2010). This marker system has also been verified as more reproducible and consistent, showing profuse polymorphism in comparison to RAPD, in watermelon species (Levi et al. 2004, 2005; Djè et al. 2010; Yang et al. 2010; Huang et al. 2011).

Another interestingly emerged approach, namely bulking the individuals can also facilitate the genotyping of large watermelon populations. As diversity analysis among a huge population is time and cost consuming, this analysis is an easy way for screening the individuals from a population that can be pooled leading to the reduction in the number of screened individuals up to two only per population (Michelmore et al. 1991; Zou et al. 2016).

The aim of the current study was (1) to estimate the polymorphism of watermelon accessions collected from various regions and countries using RAPD and ISSR markers,

and (2) to evaluate two altered methods (single plant usage and ten bulked plant usage) for the confirmation of purity of genotypes and homogeneity of population that would be beneficial for future cultivation and advancement studies.

Materials and methods

The plant resources involved in this study consisted of 25 diverse watermelon genotypes including the samples collected from numerous geographical backgrounds including Thrace (called 'Trakya') and Konya in Turkey and nearby countries, Turkmenistan and Saudi Arabia (called 'Arabistan') (Table 1). Depending on the place of collection and geographical backgrounds, samples were categorized into five populations, namely population 1, 2, 3, 4 and 5 that belong to Konya, Thrace, Turkmenistan, Saudi Arabia and Turkey, respectively. Citroides group of watermelon is well known as a genetic hub of resistance genes that can be utilized for the improvement of Lanatus group. Hence, considering the importance and characteristics of the two groups, genotypes from Lanatus group of watermelon belonging to Turkey and genotypes from Citroides group belonging to Turkmenistan have been included in the study. Additionally, three commercial varieties of Turkey have also been involved. The information regarding the genetic relatedness of the genotypes from the two groups can be efficiently utilized in our future watermelon breeding programs that may include the transfer of resistance genes responsible for different characters from Citroides to Lanatus forms. For DNA extraction, 15 seeds from each variety had been grown in greenhouse under controlled conditions.

Genomic DNA isolation was done using frozen leaf samples employing CTAB extraction method with slight modifications (Doyle 1990). DNA was taken out separately from 10 individual plants belonging to each of the 25 genotypes. After the determination of individual concentrations using NanoDrop ND-1000 UV-Vis Spectrophotometer and identifying the quality by measuring the absorbance ratios at 260/280 and 260/230 nm, samples have been diluted to 25 ng/µl for both RAPD and ISSR analyses. The quality of extracted DNA was assessed on 1% agarose gel by

Table 1 Name of 25 watermelon genotypes along with their place of collection and abbreviations that will be used further in the analysis part

Genotype	Abbreviation	Accession name	Place of collection	Population assigned in structural analysis
Konya Yerli Kırmızı Etli-5	KYKE-5	*Citrullus lanatus* var. *lanatus*	Konya	Pop 1
TrakyaYerli Beyaz Etli-6	TYBE-6	*Citrullus lanatus* var. *lanatus*	Thrace	Pop 2
Trakya Yerli-3	TY-3	*Citrullus lanatus* var. *lanatus*	Thrace	Pop 2
Taşkent	TAS	*Citrullus lanatus* var. *lanatus*	Konya	Pop 1
Trakya-5	T-5	*Citrullus lanatus* var. *lanatus*	Thrace	Pop 2
Trakya-2	T-2	*Citrullus lanatus* var. *lanatus*	Thrace	Pop 2
Trakya-8	T-8	*Citrullus lanatus* var. *lanatus*	Thrace	Pop 2
Konya Yerli-1	KY-1	*Citrullus lanatus* var. *lanatus*	Konya	Pop 1
Konya Yerli Beyaz Etli-2	KYBE-2	*Citrullus lanatus* var. *lanatus*	Konya	Pop 1
Trakya Beyaz Etli-7	TBE-7	*Citrullus lanatus* var. *lanatus*	Thrace	Pop 2
Turkmenistan-11	TURK-11	*Citrullus lanatus* var. *citroides*	Turkmenistan	Pop 3
Konya Yerli Beyaz Etli-4	KYBE-4	*Citrullus lanatus* var. *lanatus*	Konya	Pop 1
Trakya-4	T-4	*Citrullus lanatus* var. *lanatus*	Thrace	Pop 2
Arabistan	ARAB	*Citrullus lanatus* var. *lanatus*	Saudi Arabia	Pop 4
Turkmenistan-1	TURK-1	*Citrullus lanatus* var. *citroides*	Turkmenistan	Pop 3
Turkmenistan-2	TURK-2	*Citrullus lanatus* var. *citroides*	Turkmenistan	Pop 3
Turkmenistan-3	TURK-3	*Citrullus lanatus* var. *citroides*	Turkmenistan	Pop 3
Turkmenistan-5	TURK-5	*Citrullus lanatus* var. *citroides*	Turkmenistan	Pop 3
Turkmenistan-6	TURK-6	*Citrullus lanatus* var. *citroides*	Turkmenistan	Pop 3
Turkmenistan-7	TURK-7	*Citrullus lanatus* var. *citroides*	Turkmenistan	Pop 3
Turkmenistan-8	TURK-8	*Citrullus lanatus* var. *citroides*	Turkmenistan	Pop 3
Turkmenistan-10	TURK-10	*Citrullus lanatus* var. *citroides*	Turkmenistan	Pop 3
Pinaper	PINAPER	Commercial varieties	Turkey	Pop 5
Bursa	BURSA	Commercial varieties	Turkey	Pop 5
Beta	BETA	Commercial varieties	Turkey	Pop 5

Pop 1-Konya, Pop 2-Thrace, Pop 3-Turkmenistan, Pop 4-Saudi Arabia, Pop 5-Turkey

horizontal gel electrophoresis. Further for employing bulked analysis, one individual among every genotype with average DNA concentration was selected as a representative for single plant usage. For bulk usage, equal amount of DNA from all the individual plants of each genotype was mixed together.

PCR analyses

RAPD assay

Several protocols including Williams et al. (1990) have been tried for RAPD assays and finally, protocol of Padmalatha and Prasad (2006) with required modifications has been followed. A total of nine decamer oligonucleotides were used in PCR analyses, as per the number and consistency of amplified fragments (Yan et al. 1997; Goyal et al. 2015; Khan et al. 2015) (Table 2). The total reaction volume for DNA amplification was 15 µl containing 1.5 µl of 10 × PCR buffer containing KCl without $MgCl_2$, 1.8 µl of 25 mM $MgCl_2$, 3.0 µl of 1 mM dNTPs, 0.6 µl of 5 U/µl *Taq* DNA polymerase (Fermentas), 1.5 µl of 5 µM OPA primer and 2 µl of 25 ng/µl DNA. Similar PCR conditions have been employed for all the primers with differences in annealing temperatures (T_a) with initial denaturation at 94 °C for 3 min, succeeded by repetitive cycles of denaturation at 94 °C for 45 s, annealing at T_a for 1 min and primer extension step at 72 °C for 1 min, followed by final extension at 72 °C for 10 min.

ISSR assay

For ISSR study, ten reproducible primers were used for the estimation of genetic diversity of watermelon genotypes (Meloni et al. 2005; Singh et al. 2013; Khan et al. 2015). Amplification reactions were performed in Techne-512 thermocycler and the total reaction volume was 25 µl. Reaction mixture contained 2.5 µl of 10 × PCR buffer containing $(NH_4)_2{\cdot}SO_4$ without $MgCl_2$, 2.5 µl of 25 mM $MgCl_2$, 0.4 µl of 25 mM dNTPs, 0.3 µl of 5 U/µl *Taq* DNA polymerase (Fermentas), 0.5 µl of 10 µM ISSR primer and 4 µl of 25 ng/µl DNA. PCR conditions used for every individual primer have been mentioned in Table 3.

Gel electrophoresis

Following the amplification, PCR products were split by electrophoresis in 1.5% agarose gel with 1 × TBE buffer at 80 V for 5 h. Gel was stained using ethidium bromide and snapped under Transilluminator UV light provided by Vilber Lourmat Gel Documentation System. One kb and 100 bp plus Thermo Scientific DNA ladder were used as standard markers for the quantification of different RAPD- and ISSR-based gel products.

Statistical analyses

As RAPD and ISSR markers are categorized as dominant markers, binary number system 0 and 1 was used for scoring the absence and presence of bands, respectively. Prepared combined matrix for individual and bulk samples was utilized by NTSYS-pc 2.02e software for statistical analysis (Rohlf 1998). Unweighted pair group method using arithmetic averages (UPGMA) and simple matching (SM) coefficient were employed to perform cluster analysis signifying the genetic associations of accessions. Minitab 14 software has been used to construct the scatterplots for the determination of the genotype groups and comparison with pedigree clustering methods. Distinct genetic groups among watermelon genotypes were verified by STRUCTURE software version 2.3.4 (Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009) employing Bayesian model-based clustering method. Total five populations were assumed in the program depending on the place of origin. Ten independent runs were implemented for every population with burn-in period of 50,000 and Markov Chain Monte Carlo (MCMC) replications, 100,000. Structure Harvester v6.0 (Earl and

Table 2 List of RAPD primers used in the study along with the information of polymorphism found in combined (individual and bulk) analyses of 25 watermelon genotypes

# Primer	RAPD primers	Annealing temperature	Primer sequences	Total no. of bands	Polymorphic bands	Polymorphism (%)
1	RAPD B3	34	5′GAT GAC CGC C 3′	28	18	64.3
2	RAPD B4	32	5′CTC ACC GTC C 3′	17	15	88.2
3	RAPD B5	32	5′GAC GGA TCA G 3′	15	8	53.3
4	RAPD B7	32	5′TTG GTA CCC C 3′	17	13	76.5
5	RAPD B8	32	5′ACG GTA CCA G 3′	25	23	92.0
6	RAPD B10	32	5′CTA CTG CGC T 3′	16	9	56.3
7	RAPD B11	32	5′CCT CTG ACT G 3′	12	7	58.3
8	cRAPD1	32	5′GAA ACG GGT G 3′	27	16	59.3
9	cRAPD2	32	5′GTG ACG TAG G 3′	36	25	69.4
Sum				193	134	69.4

Table 3 Specific PCR conditions of all the ISSR primers used in the study

# Primer	ISSR primers	Initial denaturation	First step Denaturation/annealing/primer extension 15 cycles 95 °C—1 min/T_a—1 min/72 °C—2 min	Second step Denaturation/annealing/primer extension 25 cycles 95 °C—1 min/T_a—1 min/72 °C—2 min	Final extension
1	ISSR M1	95 °C-3 min	63.1 °C	61.1 °C	72 °C—10 min
2	ISSR M2		63.1 °C	61.1 °C	
3	ISSR M3		63 °C	60 °C	
4	ISSR M6		67.8 °C	65 °C	
5	ISSR M8		56 °C	50 °C	
6	ISSR M9		56 °C	52 °C	
7	ISSR M11		53.3 °C	51.3 °C	
8	ISSR M12		61.4 °C	59 °C	
9	ISSR M18		56.7 °C	54.7 °C	
10	ISSR F3		56 °C	54 °C	

vonHoldt 2012) program was utilized to authenticate the most appropriate *K* value revealing the unique groups (Evanno et al. 2005). Total percentage polymorphism of the primers was also estimated using total number of bands and total number of polymorphic bands.

Results

RAPD scoring-based analysis

Nine RAPD primers that were used in the study had generated relatively reproducible band patterns. Out of used primers, gel electrophoresis pattern of primer cRAPD2 on both individual and bulked watermelon samples have been shown in Fig. 1. In individual samples, RAPD primers yielded 70 polymorphic bands out of total 97 scorable bands, while in bulk samples, among 96 total scorable bands, 64 were found to be polymorphic. Maximum number of bands (25) was obtained from cRAPD2 while minimum number of bands was obtained from RAPD B11 (7). The polymorphism percentage ranged from 53.3 to 92% where RAPD B8 was found to be highly polymorphic. In the analyses, 14.9 bands per primer have been observed (Table 2). Thus, 72.2 and 66.7% polymorphism were obtained in individual and bulked watermelon samples, respectively. The number of amplified bands per primer varied between 7 and 25.

ISSR scoring-based analysis

Ten ISSR primers that produced highly reproducible results were chosen to generate polymorphic outlines among the 25 watermelon genotypes (Fig. 2). Selected ISSR primers augmented 112 polymorphic out of 117 bands in individual samples and 96 polymorphic out of 101 bands in the bulked set of watermelon genotypes. The range of polymorphic bands obtained was from 8 to 31. Primers ISSR M1, M2, M6, M12 and M18 were found to be 100% polymorphic. The average numbers of bands and polymorphic bands per primer were 21.8 and 20.8, respectively (Table 4). Hence, individual and bulk samples were found to be 95.7 and 95.0% polymorphic utilizing ISSR band patterns.

Principal coordinate analysis (PCoA)

Principal coordinate analysis of various individual and bulked samples using RAPD and ISSR markers separated the 25 accessions into different major groups. PCoA was performed using total 19 primers and scatter plots were portrayed (Fig. 3). Both the individual and bulk two-dimensional scatterplots have shown the clear groupings in agreement with the geographical origin/collection region. In both the plots, varieties are basically clustered into three major groups. In the first group, varieties from Turkmenistan are closely clustered showing less diversity within subpopulations and justifying the common area of growth and collection. Second group contained genotypes from Thrace, Konya and Saudi Arabia while in the third group Turkish commercial genotypes Pinaper, Beta and Bursa were grouped together. However, there were minor but significant differences in individual and bulk analysis plots. In individual and bulk analysis, first two principle coordinates described 49.5% and 50.1% of the total variation, respectively. These differentiating results in individual and bulk plots validate the utility of bulk sample analysis in diversity studies. Grouping of variety Arabistan with Turkish varieties justified its genetic association with them. Commercial Turkish varieties were found in obvious closeness with native varieties in comparison with Turkmenistan samples.

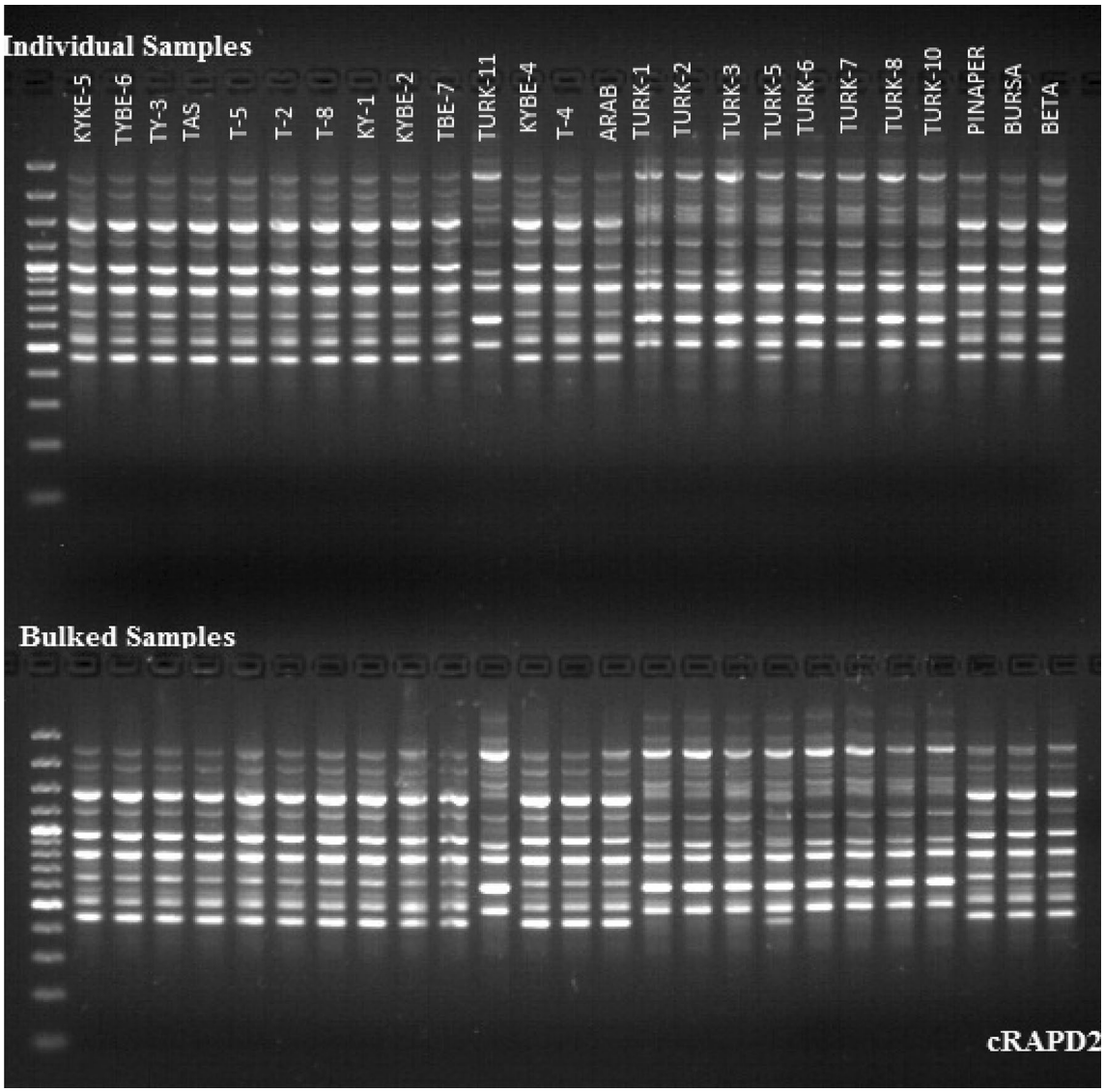

Fig. 1 Band pattern of cRAPD2 primer for 25 individual and bulked watermelon genotypes (size marker: 100 bp plus ladder)

Unweighted pair group method with arithmetic mean (UPGMA) clustering/pedigree analysis

Both individual and bulk dendrograms based on combined RAPD and ISSR data (Figs. 4, 5) showed the grouping of varieties collected from Turkmenistan region in one cluster. In the second cluster, there are two sub-groups (commercial varieties and varieties collected from Konya and Trakya regions). Additionally, as Trakya (Thrace) is closer to Konya in comparison to Turkmenistan region, varieties collected from these areas are demonstrating their proximity in dendrogram as well. Commercial varieties are under the same subgroup and since belonging to Turkey, these varieties share the same group as of Konya and Thrace varieties. In individual dendrogram, only variety Arabistan is separated as an out-subgroup while in bulk tree, Taşkent is separated as out-subgroup. Arabistan variety is closer to Konya and Thrace genotypes.

Bayesian model-based clustering analysis

STRUCTURE 2.3.4 software employing Bayesian clustering revealed the genetic constitution and association of watermelon genotypes. Combined RAPD and ISSR data of individual and bulk populations lead to the formation of discrete subpopulation groups according to the countries of origin. Assumed number of population groups (K) in the program was adjusted from 1 to 5 on the basis of type and place of collection of genotypes, while $K=2$ was confirmed as maximum log likelihood by Evanno test. It justified that all the

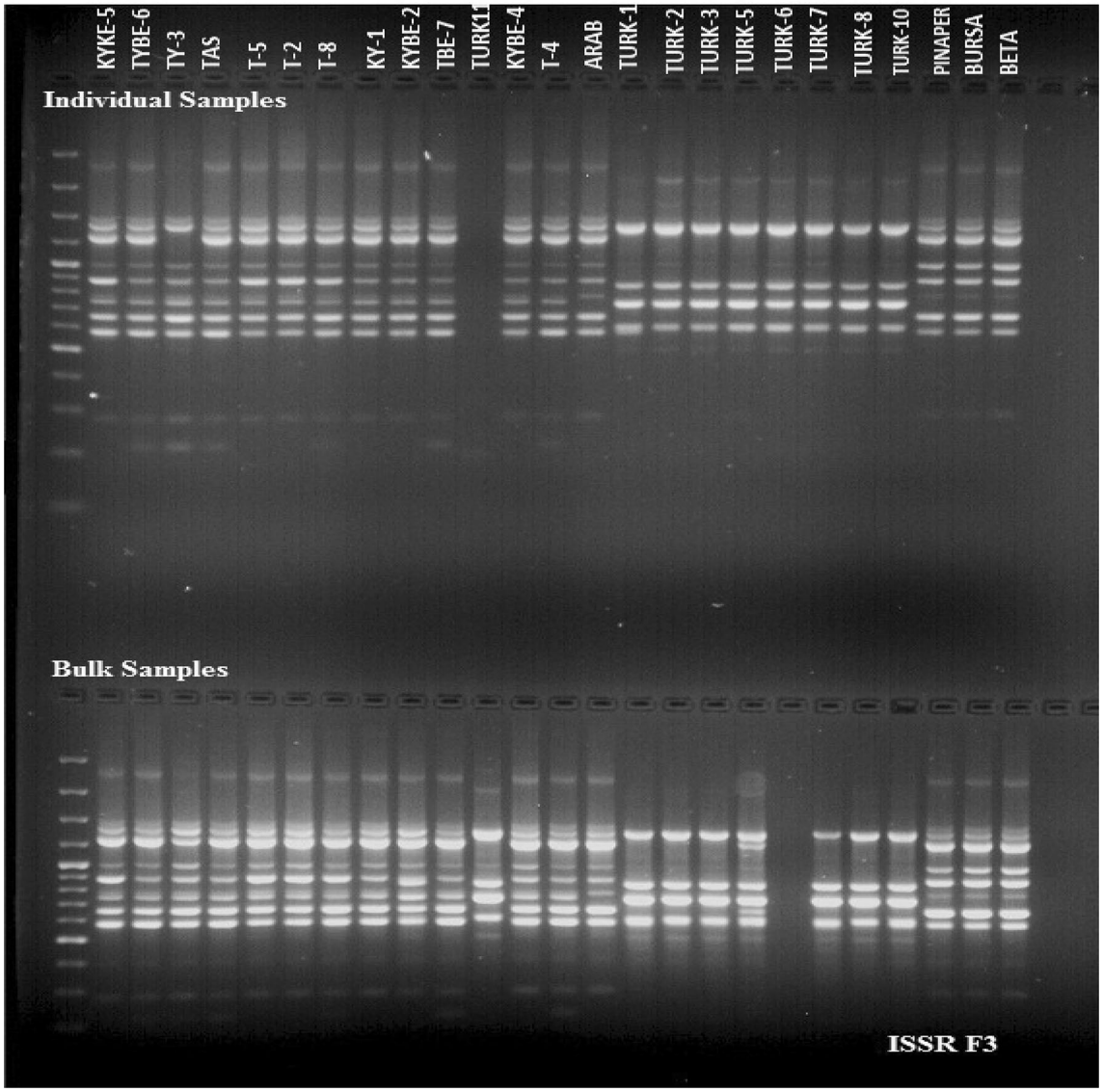

Fig. 2 Band pattern of ISSR F3 primer for 25 individual and bulked watermelon genotypes (size marker: 100 bp plus ladder)

watermelon genotypes mainly belong to two geographical origins, Turkey and Turkmenistan (Fig. 6).

The two genetically variant clusters obtained by STRUCTURE analysis were in favor of the clusters identified by UPGMA dendrogram and PCoA analysis. First group in red color represents watermelon populations of Turkish background while another cluster in green color includes all the Turkmenistan genotypes. Individual populations in the first and second clusters were expected to show 24% and 20% heterozygosity, while Bulk populations were supposed to represent correspondingly 28% and 19% heterozygous character in the first and second groups. In individual populations, Konya, Thrace, Saudi Arabia and Turkish Commercial varieties showed maximum membership: 99%, 99%, 99% and 95% in the first cluster, respectively, while Turkmenistan genotypes showed 96.5% share in the second cluster and 3.5% share in the first cluster, respectively (Table 5). However, bulked Konya and Turkmenistan genotypes showed 96% and 4% involvement in the first and second clusters, respectively (Table 5).

Discussion

As RAPD primers amplify regions from whole genome and ISSR primers amplify the expanses between the simple sequence repeats, combined usage of both primers increases the legitimacy of outcomes (Trindade et al. 2009; Abdel Khalik et al. 2014; Lamare and Rao 2015; Costa et al. 2016). Therefore, combined RAPD and ISSR polymorphism has

Table 4 List of ISSR primers used in the study along with the information of polymorphism found in combined (individual and bulk) analyses of 25 watermelon genotypes

# Primer	ISSR primers	Primer sequences	Total no. of bands	Polymorphic bands	Polymorphism (%)
1	ISSR M1	5′-AGC AGC AGCAGCAGCAGC G-3′	28	28	100
2	ISSR M2	5′-ACC ACC ACC ACC ACC ACC G-3′	21	21	100
3	ISSR M3	5′-AGC AGC AGCAGCAGCAGC C-3′	33	31	93.9
4	ISSR M6	5′-GTC ACC ACCACC ACC ACC ACC AC-3′	28	28	100
5	ISSR M8	5′-ACA CAC ACA CAC ACA CAC G-3′	22	20	90.9
6	ISSR M9	5′-ACA CAC ACA CAC ACA CCG-3′	20	19	95.0
7	ISSR M11	5′-CAC CAC CAC CAC CAC-3′	11	8	72.0
8	ISSR M12	5′-GAC ACG ACA CGA CAC GAC AC-3′	15	15	100
9	ISSR M18	5′-CGT CAC ACA CAC ACA CAC A-3′	18	18	100
10	ISSR F3	5′-AGA GAG AGA GAG AGA GCG-3′	23	21	91.3
Sum			218	208	95.4

been used in the study to estimate the genetic diversity in Citroides and Lanatus group of watermelon populations.

In our study, combined RAPD and ISSR data have revealed 85% and 81.2% polymorphism in individual and bulked samples, respectively. Our results were in agreement with some of the previous analyses while contradictory to some others. In 2001, Levi et al. had emphasized on low genetic diversity level among 46 American cultivars using RAPD primers, while on the basis of ISSR analysis, American watermelon cultivars were found to be 80.2% polymorphic (Levi et al. 2004). Mujaju et al. (2010) had declared 88.4% polymorphism in ten African watermelon accessions employing RAPD markers. They determined similar genetic diversity level in both citron and dessert melons. However, utilizing 22 RAPD primers, Solmaz et al. (2010) had demonstrated 60.6% polymorphism in 303 Turkish watermelon accessions that is similar to our results. Comparable results have been obtained by Djè et al. (2010) revealing 97.7% polymorphism in African indigenous watermelon landraces with 20 ISSR primers. Although RAPD markers are randomly distributed in the genome and ISSR distribution is comparatively more restricted, ISSR shows greater percentage polymorphism as compared to RAPD. RAPD can amplify both coding and non-coding regions of the genome, while ISSR amplifies only coding regions of the genome. However, at a time, RAPD amplifies only one region either coding or non-coding. This decreases the chances of amplifying the polymorphic regions. Hence, it shows less polymorphism as compared to ISSR markers (Costa et al. 2016). Although limited number of diversity studies has been conducted in watermelon species based on the combined utilization of RAPD and ISSR, there have been experiments in other species that revealed higher percentage polymorphism of ISSR markers as compared to RAPD. Levi et al. (2001, 2004) conducted a series of experiments where ISSR and AFLP markers showed relatively higher polymorphism than RAPD in heirloom cultivars. In an experiment performed on melon (*Cucumis melo*.) germplasm, Wanbo et al. (2002) observed 65.5% and 58.6% polymorphism in 37 genotypes using ISSR and RAPD primers, respectively.

Results of principle coordinate analysis and cluster analysis with UPGMA and Bayesian clustering were similar to each other. All the analyses divided genotypes into two main groups, one group containing varieties from Turkey and another from Turkmenistan. Although commercial Turkish varieties are grouped separately, those are in close association with Konya and Thrace genotypes in comparison with the Turkmenistan varieties. Saudi Arabian genotype showed its close linkage with Turkish genotypes in all the analyses. These results were consistent with Solmaz et al.'s (2010) study where *C. lanatus* species in their study made separate clusters from other watermelon species including citron watermelon. Additionally, parallel to our results, they also observed molecular variance of Turkish watermelon genetic ecotypes from other forms. Bayesian clustering revealed admixture between Saudi Arabian genotype and Turkish genotypes demonstrating interbreeding between the cultivars of two countries. High level of heterozygosity in Turkish genotypes in both individual (24%) and bulk (28%) populations can be attributed to the involvement of commercial genotypes in the experiments. This directs towards the utility of participation of commercial varieties in watermelon breeding programs around the world. Separate grouping of citron watermelons with dessert watermelons in all the clustering methods was in accordance with Mujaju et al.'s (2010) study, where both species showed considerable variation.

The results presented in the study can be of major importance for the watermelon research as it is generally assumed that despite the differences in appearance, watermelon genotypes do not show considerable genetic variation. Here, it

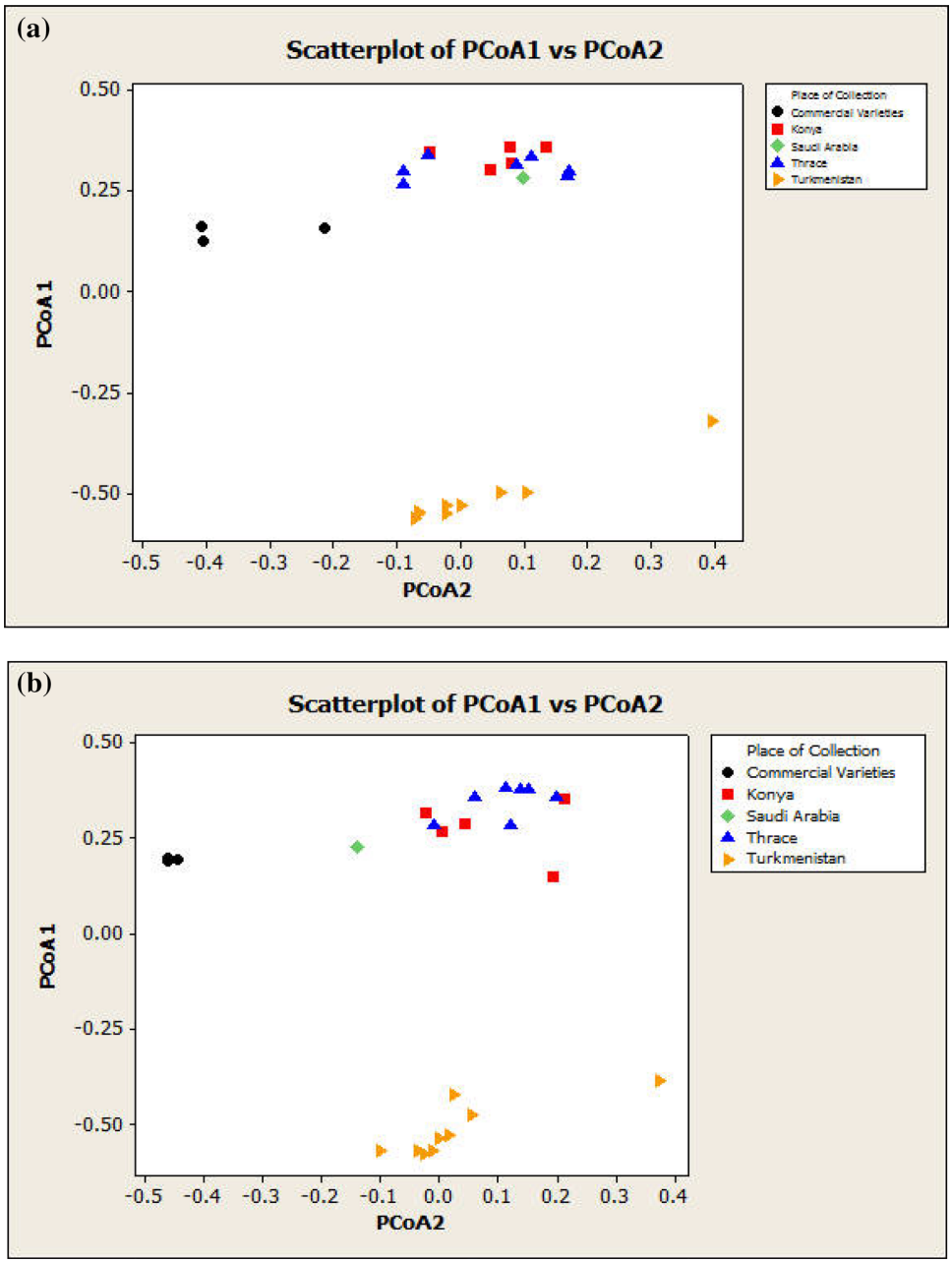

Fig. 3 Scatterplots obtained from combined RAPD and ISSR analyses. **a** Individual sampling and **b** Bulk sampling. Both of them categorized the 25 wheat accessions in similar groups. The obtained clusters/groups were in accordance with the geographical area

can be observed easily that in some cases, varieties from different countries are similar while the commercial varieties of same country vary from other native varieties of Turkey belonging to Konya and Thrace region. Hence, it can be concluded that much higher variation can be observed in within country varieties belonging to similar geographical region. Thus, these genotypes can be utilized for enhancing the diversity and increasing the variation for several characteristics. This was in accordance with Mujaju et al. (2010) and Mashilo et al.'s (2017) experiments where sufficient level of among- and within-group variations was observed in Citroides and Lanatus germplasms. Reliability of biological analysis in crop improvement is largely dependent on the number of samples involved. However, increasing the number of individuals from a sample population enhances the cost and time of the assay. Hence, to sustain the statistical strength of an assay, it is efficient to bulk the individuals of a population for the target traits and evaluate them as a pool (Michelmore et al. 1991; Darvasi and Soller 1992; Xu et al. 2008; Sun et al. 2010; Zou et al. 2016). Accordingly, 10 individuals of 25 watermelon populations have been bulked in this study to determine the efficacy of individual and bulk analysis in diversity analysis. In individual analysis, Taskent (Konya) and Arabistan genotypes were in close association with other Konya genotypes while they got distinct from the Konya group in bulk analysis. Little but higher

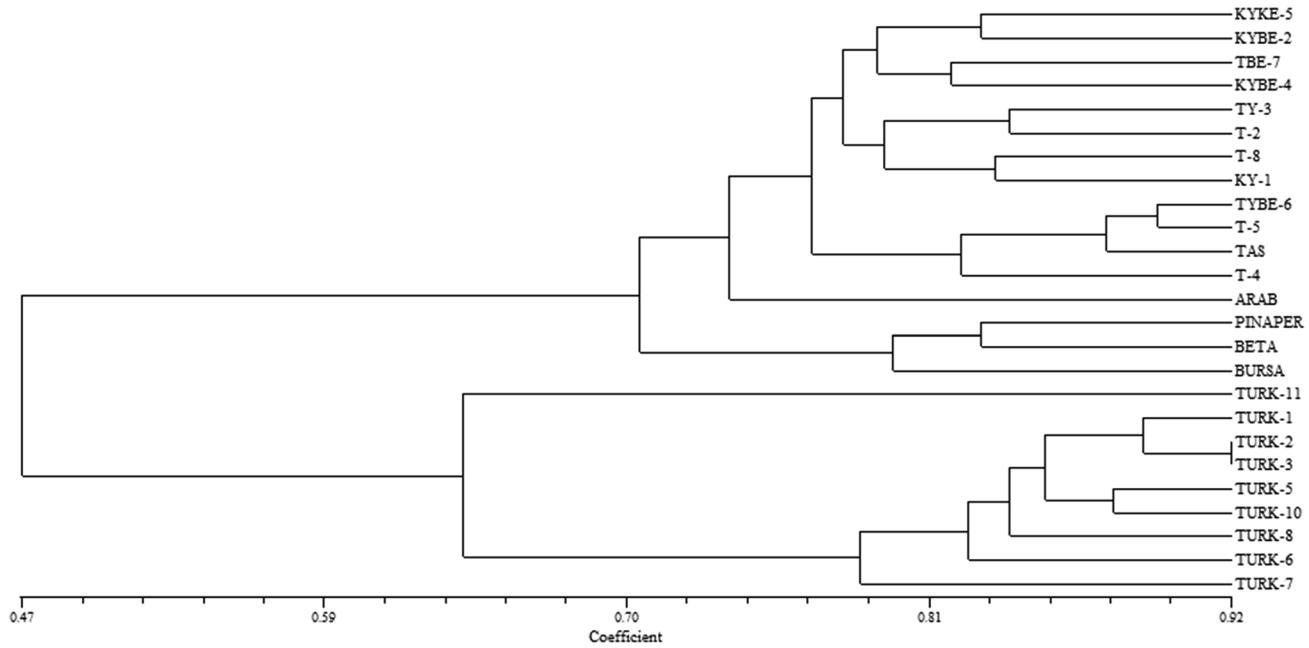

Fig. 4 Combined RAPD- and ISSR data-based dendrogram for 25 watermelon cultivars based on individual samples

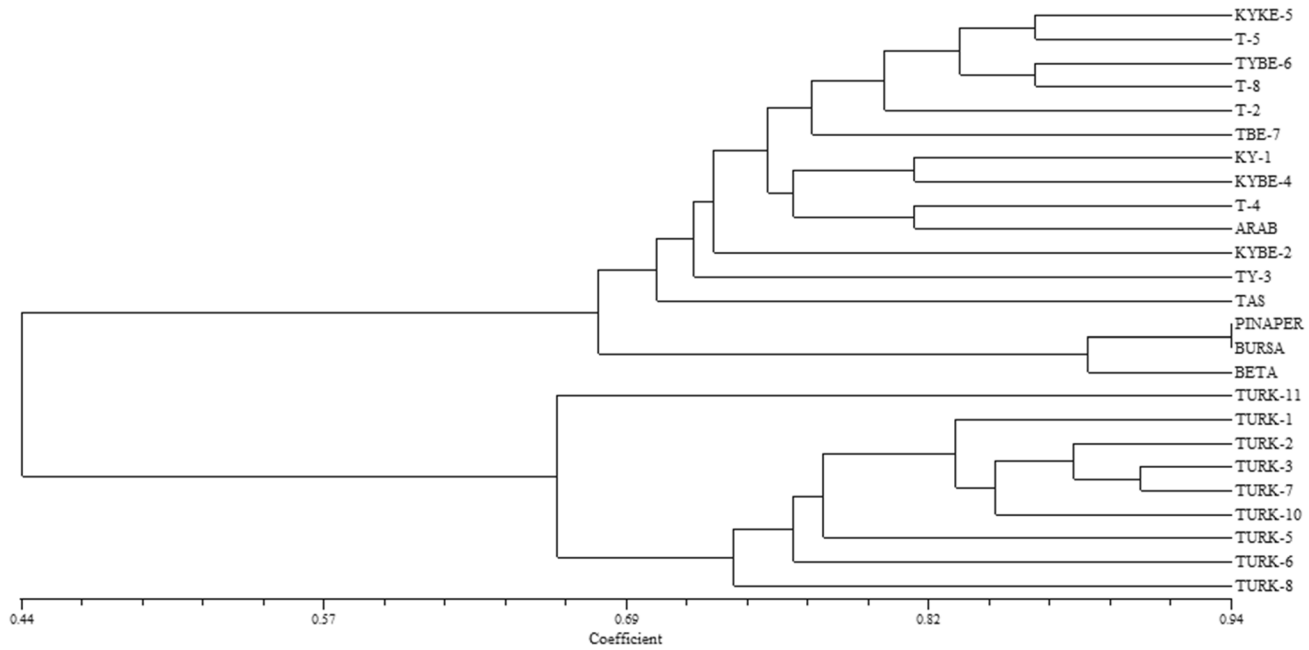

Fig. 5 Combined RAPD- and ISSR data-based dendrogram for 25 watermelon cultivars based on bulked samples

heterozygosity in bulk populations in STRUCTURE analysis as compared to individual populations demonstrates the effectiveness of bulking the individuals in diversity assays (Sun et al. 2010; Zou et al. 2016).

As the genetic background of *C. lanatus* var. *lanatus* is found to be constricted and varieties of this group are more prone towards several diseases, it will be better to cross them with the diverse and resistant genotypes of *C. lanatus* var. *citroides* group. In our study, sufficient differentiation has been obtained between the two forms of accessions, *C. lanatus* var. *citroides and C. lanatus* var. *lanatus* that belong to Turkey and Turkmenistan, respectively. This was in line with the results obtained from the studies of Jarret et al. (1997), Levi et al. (2001, 2005) and Mujaju et al. (2010).

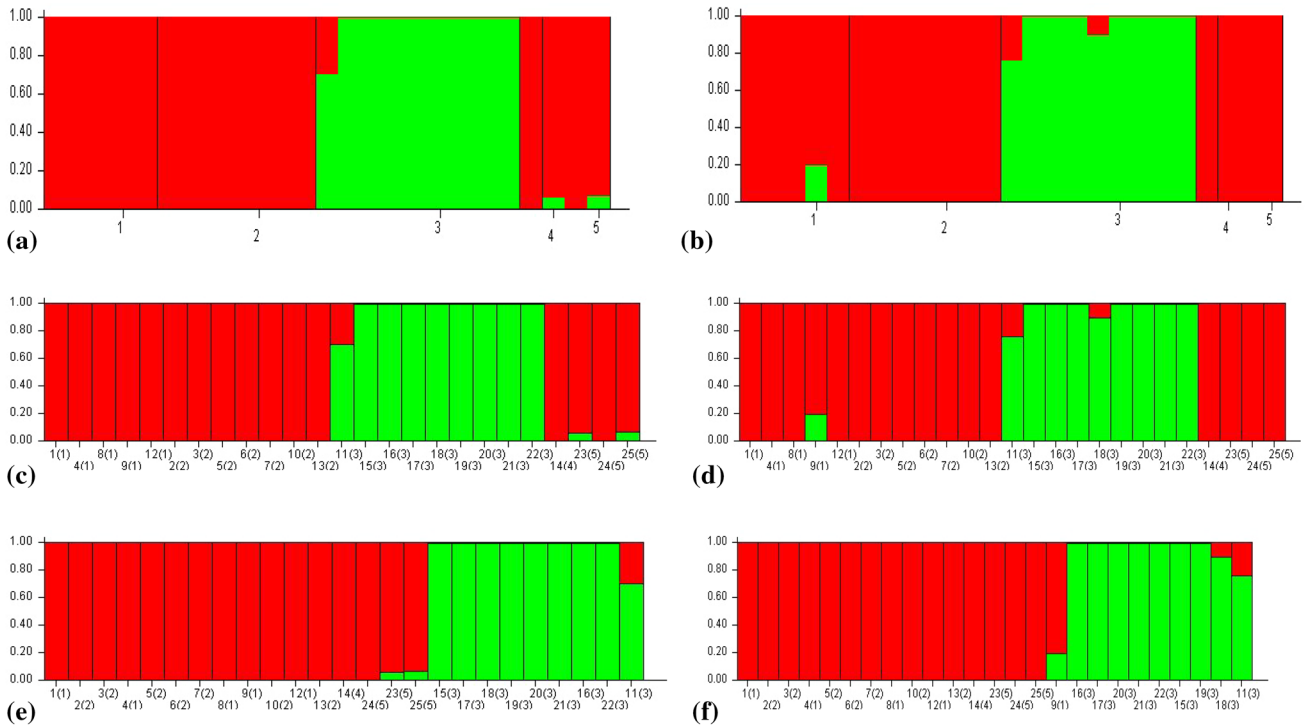

Fig. 6 Sketches **a** and **b** show two main clusters from population STRUCTURE analysis of 25 individual and bulked watermelon genotypes from different geographical origin, respectively. In both the pictures, red zone includes Turkish and Saudi Arab varieties while green zone consists of Turkmenistan varieties. In **c** and **d**, among different clusters, *Y* coordinates represent association coefficients and vertical lines with *X* coordinate resembling individual varieties. Digits in the bracket stand for the assigned population groups, i.e., Konya (1), Thrace (2), Turkmenistan (3), Saudi Arabia (4) and Commercial Turkish Varieties (5). Sketches **e** and **f** indicate several genotypes on the basis of Q which reveals the proportion of every individual genome that belongs to different clusters in both individual and bulk populations, respectively

Table 5 Proportion of membership of each pre-defined population in each of the two clusters of individual and bulked samples obtained from STRUCTURE analysis

Given pop	Inferred clusters		Number of individuals
Individual samples			
	1	2	
1	0.999	0.001	5
2	0.999	0.001	7
3	0.035	0.965	9
4	0.999	0.001	1
5	0.955	0.045	3
Bulked samples			
	1	2	
1	0.961	0.039	5
2	0.999	0.001	7
3	0.040	0.960	9
4	0.999	0.001	1
5	0.998	0.002	3

Although existence of variation in Turkish group can be largely attributed to the presence of commercial genotypes, considerable diversity was found within the other dessert watermelon populations. Moreover, individuals from different geographical locations within Turkey showed inter-mixed populations. This was in contrast to Mujaju et al.'s (2010) study where dessert watermelons collected from different locations did not show inter-mixed populations. This showed the heterogeneous nature of the Turkish genotypes used in the experiment. Similar extent of variability was found in Turkmenistan citron melon genotypes, where populations were basically divided into three scattered clusters. The first cluster was comprised of Turk 1, 2, 3, 7 and 10 populations; the second cluster was made up of Turk 5, 6 and 8 populations; the out-grouping of Turk 11 individuals developed the third cluster. These results were similar to Mashilo et al.'s (2017) study where clustering of experimental citron genotypes into distinct groups demonstrated greater genetic variation for long-term conservation of species and breeding strategies. This vast genetic background of *C. lanatus* var. *citroides* watermelon can be effectively employed to transfer drought and disease resistance characters to Lanatus

watermelon group (Gusmini et al. 2005; Davis et al. 2007; Yoshimura et al. 2008; Tetteh et al. 2010; Edelstein et al. 2014; Thies et al. 2010).

Conclusion

Genetic diversity assessed in watermelon genotypes in the present study can be used for the evolvement of diverse and disease-resistant sweet watermelon genotypes. In conclusion, we can say that in this advanced molecular era, still dominant markers such as RAPD and ISSR can be considered as approachable and justifying method in diversity studies. The variations revealed in this work can be utilized for future molecular and normal breeding programs that may exaggerate the efforts of watermelon betterment in Turkey as well as other parts of the world.

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