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RESEARCH ARTICLE

A comparative assessment of morphological and molecular characterization among three *Ziziphus* species

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Abstract Genetic variability of 84 accessions of three *Ziziphus* species including Z. *spina-christi*, *Z. nummularia* and *Z. mauritiana* were analyzed using a combination of morphological traits and translation initiation codon (ATG) polymorphism. Both morphological and molecular data revealed a high level of inter and intra specific variations among the accessions. Accordingly, 90.49% of amplified fragments were polymorphic among the accessions with the mean values of 0.37 for polymorphic information content (PIC), 3.31 for resolving power (RP), and 1.95 for marker index (MI). The phylogenetic clustering clearly delineated the entire germplasm into three well supported distinct clusters according to the species sources. According to the Nei's genetic identity, *Z. spina-christi* and *Z. nummularia* were the most similar species and had high differentiation with *Z. mauritiana*. Moreover, the highest values for Shannon's information index ($I = 0.505$) and gene diversity ($h = 0.347$) were recorded in *Z. spina-christi* indicating there is higher genetic diversity compared with two other species. Four private alleles were identified in two species which could be beneficial for accessions authentication in argumentative situations. Moreover, results of the Mantel test showed there were moderate correlation between molecular and morphological matrices. In addition, estimation of bivariate correlations revealed there were significant positive and negative correlations between different variables, which offer a practical application of this information during phenotype based selection in ber improvement programs. The results of this investigation highlight the efficiency of translation initiation codon polymorphism for genetic characterization and accurate authentication of *Ziziphus* accessions as well as detecting and tagging morphologically important traits in this genus that would be helpful for implementation of effective conservation strategies and even broaden current genetic diversity.

Keywords Genetic diversity · Population structure · *Ziziphus* · Molecular markers · Phylogenetic

Introduction

Ziziphus, a member of Rhamnaceae family, is a genus with approximately 170 recognizable species which are widely distributed in the tropical and sub-tropical regions of the world with a wide range of climates (Islam and Simmons 2006; Liu and Zhao 2009). Most of the tree species are grown as wild trees in the tropical and sub-tropical forests of Asia and Africa. The fruit of these species are edible with high nutritional value and traditionally being used for medicinal purposes such as antimicrobial, anti-inflamatory, antitumor properties and have been used to treat hysteria, fatigue, wound healing, anorexia and as liver protectant (Gao et al. 2013). Despite the high value of these species as food and medicine resources as well as their remarkable importance in the local markets, *Ziziphus* species receive considerable commercial acceptance neither by local

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farmers nor by plant breeders. The lack of breakthrough can be partly attributed to the underutilization of the current genetic diversity and undeveloped crop improvement strategies, which has led to the cultivation of non-commercial genotypes lacking commercial attributes such as high quality fruits (size, color, organoleptic quality) as well as tree characteristics (high yield, low spine and adaptability to easy plantation and orchard management).

Because of its considerable tolerance to unfavorable conditions such as saline soils, hot and arid climates as well as cold hardiness (Meena et al. 2003) this fruit tree is regarded as an excellent choice for arid and semi-arid regions as well as non-arable lands both for fruit production and for ecological applications. *Ziziphus* species have also strong adaptability to different climates and that is why this genus is getting more popularity and economic importance around the world in recent years. On the other hand, understanding the genetic background of this genus will help to pace with the impacts of climate changes.

Several species of *Ziziphus* originated or even introduced from adjacent countries to the southern provinces of Iran. The Indian jujube (*Z. mauritiana*), Chinese jujube (*Z. jujube*), *Z. spina-christi* (L.), *Z. mauritiana* and *Z. nummularia* are the main tree species in this genus that have been domesticated and widely planted in different geographical regions of the world (Mahajan and Chopda 2009; Huang et al. 2017; Liang et al. 2019) especially in the southern provinces of Iran. There are several natural forests of *Z. spina-christi* (naming as "Konar" in this country) in the southern and south-western of the Iran mainly along with Zagros mountain range. *Z. nummularia* is another widespread *Ziziphus* species in Iran that is naturally distributed along with *Z. spina-christi* in the southern parts of the country. Although being highly similar to the *Z. spina-christi* in some aspects especially the fruit characteristics, *Z. nummularia* is a deciduous high spiny shrub which is more frost hardy and is naturally grown in higher altitudes than *Z. spina-christi* species (Mortazavi Jahromi and Zandi 2012). Because of their outcrossing system as well as high level of gene flow, most of the *Ziziphus* species demonstrate high level of genetic variability (Kulkarni 2016; Asatryan and Tel-Zur 2014). Therefore, selection and utilization of the promising genetic variability and their utilization in the breeding programs need to be practiced for improving the yield quality of this valuable species in future.

Different molecular markers are available for assessing genetic diversity in plants. Assessment of phenotypic diversity as well as molecular marker have been successfully used for evaluating genetic diversity and fingerprinting of various plant species including *Ziziphus* spp. (Li et al. 2010; Razi et al. 2013; Singh et al. 2014, 2017; Ahmad et al. 2016; Norouzi et al. 2017; Liang et al. 2019; Farahani et al. 2019; Shen et al. 2020).

Translation initiation codon (ATG) or Start Codon Targeted (SCoT) polymorphism is one of the molecular marker techniques that has emerged based on the huge sequence data availability of different species (Collard and Mackill 2009). This type of marker has proved its usefulness for various aspects of plant species such as genetic fingerprinting, phylogenetic studies, population genetic investigations, marker assisted selection (MAS) and more precise authentication of controversial species (Mulpuri et al. 2013; Yan et al. 2016; Feng et al. 2018; Jalilian et al. 2018; Safari et al. 2019; Zarei and Erfani-Moghadam 2021). Based on the regions of genome for which SCoT markers are designed, this type of molecular marker provides a good platform both for marker assisted selection and phylogenetic studies among closely related species.

A high phenotypic resemblance among the close relative species, limited genomic sequence data, as well as different views, made the phylogenetic classification of *Ziziphus* as a problematic case (Huang et al. 2017). Intrageneric genetic assessments of *Ziziphus* in their native habitations may provide beneficial information about the genetic population structure and interspecific relationships among different species. In the current study, genetic variability and its association with the morphological attributes were investigated in a collection of 84 accessions from three *Ziziphus* species in Iran in order to obtain a better understanding about the phenotypic diversity, population genetic structure as well as phylogenetic relationship among the native Iranian *Ziziphus*.

Materials and methods

Plant materials

Eighty-four germplasm of three *Ziziphus* species were collected from different regions of Iran with 41 *Z. spina-christi*, 24 *Z. nummularia* and 19 *Z. mauritiana* accessions (Table 1). The samples of *Z. spina-christi* and *Z. nummularia* were collected from the natural forests in their native habitats, while the *Z. mauritiana* specimens were sampled from the individual trees as a wild or cultivated by natives in different regions (Fig. 1). The individuals were selected randomly by considering a minimum distance of 1000 m apart from each other's. The climates of most sampling regions were arid and semi-arid with long dry and warm summer and annual precipitation below 370 mm which mostly occurs during winter. Kazeron and Qaemyeh have higher annual precipitation (370 mm) and moderate temperature, while Larestan and Jouyom have driest climates and lower annual precipitation (198 mm) among the

Table 1 Code and sampling information about *Ziziphus* accessions used in this study

Code	Species	Location	Region	Locality	Altitude (m)	Code	Species	Location	Region	Locality	Altitude (m)
1	*spina-christi*	Anarestan	Qaemyeh	N29°74′ E51°39’	764	43	*nummularia*	Kazeron	Kazeron	N29°70′ E51°49′	780
2	*spina-christi*	Golgoon	Qaemyeh	N29°85′ E51°59′	876	44	*nummularia*	Kazeron	Kazeron	N29°64′ E51°46′	865
3	*spina-christi*	Tolemilak	Qaemyeh	N29°86′ E51°61′	900	45	*nummularia*	Kazeron	Kazeron	N29°60′ E51°46′	921
4	*spina-christi*	Dehno Ghori	Qaemyeh	N29°86′ E51°62′	917	46	*nummularia*	Kazeron	Kazeron	N29°51′ E51°51′	880
5	*spina-christi*	Nowdan	Qaemyeh	N29°80′ E51°69′	995	47	*nummularia*	Kazeron	Kazeron	N29°50′ E51°53′	824
6	*spina-christi*	Deris	Qaemyeh	N29°68′ E51°57′	790	48	*nummularia*	Kazeron	Kazeron	N29°57′ E51°53′	1030
7	*spina-christi*	Mozafar Abad	Qaemyeh	N29°88′ E51°58′	950	49	*nummularia*	Kazeron	Kazeron	N29°56′ E51°53′	980
8	*spina-christi*	Haji Abad	Qaemyeh	N29°66′ E51°57′	795	50	*nummularia*	Kazeron	Kazeron	N29°59′ E51°57′	945
9	*spina-christi*	Meydanak	Qaemyeh	N29°80′ E51°56′	820	51	*nummularia*	Kazeron	Kazeron	N29°56′ E51°62′	919
10	*spina-christi*	Konar Takhteh	Kazeron	N29°54′ E51°40′	520	52	*nummularia*	Kazeron	Kazeron	N29°58′ E51°60′	924
11	*spina-christi*	Grash	Larrestan	N27°64′ E54°13′	910	53	*nummularia*	Simakan	Jahrom	N29°54′ E53°26′	1073
12	*spina-christi*	Grash	Larestan	N27°64′ E54°13′	910	54	*nummularia*	Simakan	Jahrom	N29°59′ E53°17′	874
13	*spina-christi*	Grash	Larestan	N27°64′ E54°13′	910	55	*nummularia*	Simakan	Jahrom	N29°59′ E53°16′	870
14	*spina-christi*	Deh Fish	Larestan	N28°13′ E53°84′	812	56	*nummularia*	Simakan	Jahrom	N29°58′ E53°15′	831
15	*spina-christi*	Deh Fish	Larestan	N28°13′ E53°84′	812	57	*nummularia*	Simakan	Jahrom	N29°58′ E53°15′	827
16	*spina-christi*	Deh Fish	Larestan	N28°13′ E53°84′	812	58	*nummularia*	Simakan	Jahrom	N29°56′ E53°15′	825
17	*spina-christi*	Deh Fish	Larestan	N28°13′ E53°84′	812	59	*nummularia*	Simakan	Jahrom	N29°57′ E53°19′	901
18	*spina-christi*	Deh Fish	Larestan	N28°13′ E53°84′	812	60	*nummularia*	Simakan	Jahrom	N28°57′ E53°20′	920
19	*spina-christi*	Deh Fish	Larestan	N28°13′ E53°84′	812	61	*nummularia*	Simakan	Jahrom	N28°57′ E53°20′	915
20	*spina-christi*	Deh Fish	Larestan	N28°13′ E53°84′	812	62	*nummularia*	Simakan	Jahrom	N28°57′ E53°22′	960
21	*spina-christi*	Jahrom	Jahrom	N28°53′ E53°67′	1183	63	*nummularia*	Simakan	Jahrom	N28°56′ E53°23′	1000
22	*spina-christi*	Jahrom	Jahrom	N28°50′ E53°56′	1043	64	*nummularia*	Simakan	Jahrom	N28°55′ E53°23′	995
23	*spina-christi*	Jahrom	Jahrom	N28°50′ E53°56′	1043	65	*nummularia*	Simakan	Jahrom	N28°55′ E53°25′	1030
24	*spina-christi*	Jahrom	Jahrom	N28°50′ E53°52′	1061	66	*mauritiana*	BandarAbas	BandarAbas	N27°22′ E56°34′	10
25	*spina-christi*	Jahrom	Jahrom	N28°50′ E53°52′	1061	67	*mauritiana*	BandarAbas	BandarAbas	N27°22′ E56°34′	10
26	*spina-christi*	Jahrom	Jahrom	N28°51′ E53°58′	1045	68	*mauritiana*	BandarAbas	BandarAbas	N27°22′ E56°34′	10
27	*spina-christi*	Jahrom	Jahrom	N28°49′ E53°54′	1049	69	*mauritiana*	BandarAbas	BandarAbas	N27°22′ E56°34′	10

Table 1 continued

Code	Species	Location	Region	Locality	Altitude (m)	Code	Species	Location	Region	Locality	Altitude (m)
28	*spina-christi*	Jahrom	Jahrom	N28°49′ E53°54′	1049	70	*mauritiana*	BandarAbas	BandarAbas	N27°22′ E56°34′	10
29	*spina-christi*	Jahrom	Jahrom	N28°49′ E53°54′	1049	71	*mauritiana*	BandarAbas	BandarAbas	N27°22′ E56°34′	10
30	*spina-christi*	Shahrak	Jahrom	N28°51′ E53°58′	1045	72	*mauritiana*	BandarAbas	BandarAbas	N27°22′ E56°34′	10
31	*spina-christi*	Dastgheib	Jahrom	N28°50′ E53°61′	1100	73	*mauritiana*	BandarAbas	BandarAbas	N27°22′ E56°34′	10
32	*spina-christi*	Dastgheib	Jahrom	N28°50′ E53°61′	1100	74	*mauritiana*	BandarAbas	BandarAbas	N27°22′ E56°34′	10
33	*spina-christi*	Dastgheib	Jahrom	N28°53′ E53°67′	1100	75	*mauritiana*	BandarAbas	BandarAbas	N27°22′ E56°34′	10
34	*spina-christi*	Dastgheib	Jahrom	N28°53′ E53°67′	1100	76	*mauritiana*	BandarAbas	BandarAbas	N27°22′ E56°34′	10
35	*spina-christi*	Freshtejan	Juyom	N28°25′ E53°86′	955	77	*mauritiana*	Jahrom	Jahrom	N28°50′ E53°58′	1050
36	*spina-christi*	Freshtejan	Juyom	N28°25′ E53°86′	950	78	*mauritiana*	Jahrom	Jahrom	N28°50′ E53°58′	1050
37	*spina-christi*	Freshtejan	Juyom	N28°24′ E53°86′	947	79	*mauritiana*	Jahrom	Jahrom	N28°50′ E53°58′	1050
38	*spina-christi*	Freshtejan	Juyom	N28°25′ E53°86′	974	80	*mauritiana*	Jahrom	Jahrom	N28°50′ E53°58′	1050
39	*spina-christi*	Freshtejan	Juyom	N28°25′ E53°86′	954	81	*mauritiana*	Jahrom	Jahrom	N28°50′ E53°58′	1050
40	*spina-christi*	Freshtejan	Juyom	N28°24′ E53°86′	945	82	*mauritiana*	Jahrom	Jahrom	N28°50′ E53°58′	1050
41	*spina-christi*	Freshtejan	Juyom	N28°24′ E53°87′	940	83	*mauritiana*	Jahrom	Jahrom	N28°50′ E53°58′	1050
42	*nummularia*	Kazeron	Kazeron	N29°72′ E51°51′	730	84	*mauritiana*	Jahrom	Jahrom	N28°50′ E53°58′	1050

regions. However, Bandar Abas is the only coastal region with 200 mm annual precipitation, which represents tropical climate and the highest relative humidity among the sampling sites.

Morphological attributes

Seventeen quantitative and qualitative attributes were measured among various germplasm (Table 2). To record the phenotypic diversity, a minimum number of ten fruits were selected randomly from different sides of each plant at the ripening stage. Leaf and branch characteristics were measured on the tree using a vernier caliper with an accuracy of 0.10 mm. The fruit and seed weight were evaluated using a digital balance with a sensitivity of 0.01 g (model JKH-500, Jadever Co.). To measure the fruit dry weight, experimental samples were oven-dried at 60–70 °C until reaching a constant weight.

DNA extraction and SCoT markers

DNA was extracted from leaf samples using a modified CTAB based protocol (Doyle and Doyle 1987). From thirty SCoT primers that were initially tested on the samples, 21 primers produced clear scorable polymorphic amplicons that were used for polymerase chain reaction (PCR) amplification (Table 3; Collard and Mackill 2009; Guo et al. 2016; Singh et al. 2017). The amplification reactions were conducted using 40 ng of template DNA, 10 picomoles of SCoT primer, 10 µl PCR master mix (SinaClon BioScience Co. Iran) in a final volume of 20 µl by a thermocycler (iCycler, Bio Rad, Hercules, CA, USA) with the following program: an initial denaturing step at 98 °C for 5 min, followed by 35 cycles consisting of three consecutive steps of 94 °C for 50 s, specific anneal temperature of each primer for 1 min, and 72 °C for 2 min, and a final extension of 72 °C for 7 min. The amplified

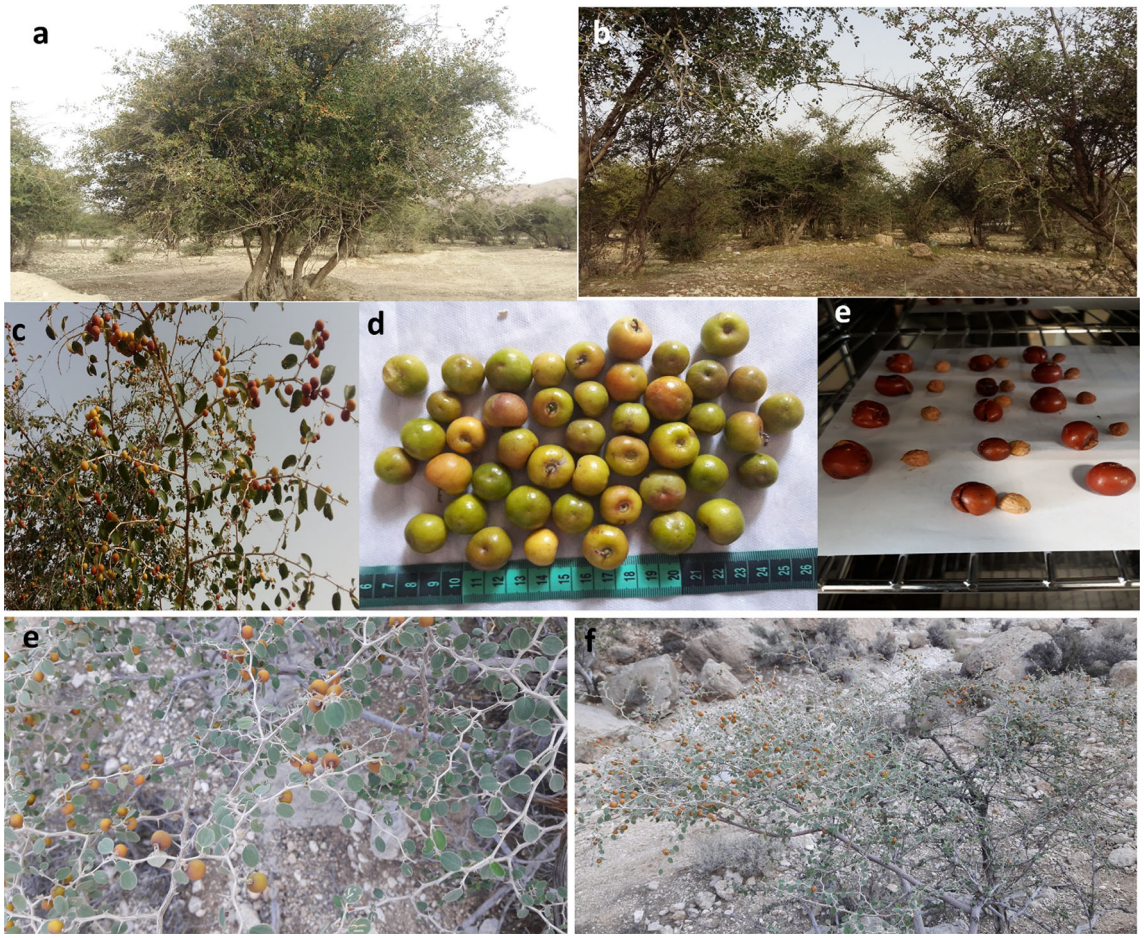

Fig. 1 Photographs of some *Ziziphus* species; **a**, **b** and **c** natural forests of the *Z. spina-christi* species; **d** fresh fruits; and e: oven dried fruits and seeds of *Z. spina-christi*; **e** and **f** fruit and tree of *Z. nummularia* at its natural location

fragments were stained with ethidium bromide, and were electrophoretically separated in a 1.5% agarose gel and finally visualized under UV light in a gel document instrument. To estimate the approximate length of the amplicons, a 1 kb DNA gene ladder (SinaClon BioScience Co. Iran) was loaded in parallel to the PCR products on either side of each gel.

Scoring and data analysis

The mean values of the morphological attributes were used as the input data in SPSS software (ver. 17) (Norusis 1998) for statistical analyses such as descriptive statistics. Cluster analysis was performed based on the distance obtained from morphological traits following the Euclidean coefficients using NTsys software (ver. 2.2) (Rohlf 2000). Factor analysis was conducted using SPSS software to estimate the effective factors for differenting the accessions and the first two main factors were used to construct a biplot for a better understanding of the germplasm affinities. Bivariate correlation was conducted using the R package (Wei and Simko, 2017) to visualize the linear relationships between pairs of morphological traits.

SCoT produced amplicons were scored based on the presence (1) and absence (0) of a given fragment in each germplasm. These data were used for the binary matrix construction which then were employed for further statistical analysis. The effectiveness of the examined SCoT primers for genetic discrimination among *Ziziphus* samples was estimated by measuring different primer efficiency parameters including polymorphic information content (PIC), heterozygosity index (HI), discriminating power (DP), and resolving power (RP) using an online program iMEC (https://irscope.shinyapps.io/iMEC/) (Amiryousefi et al. 2018). Effective multiplex ratio (EMR) was calculated according to Powell et al. (1996) and marker index (MI) was estimated from EMR multiply by PIC.

The neighbor-joining (NJ) phylogenetic tree among the species was created based on Nei's genetic distance matrix using Mega software (ver. 6) (Tamura et al. 2012).

Table 2 Descriptive statistics of different morphological attributes among the accession belonging to three *Ziziphus* species

Characters	Abbreviation	Unit	*Z. spina-christi*					*Z. nummularia*					*Z. mauritiana*					Average CV%
			Mean	Min	Max	SD	CV%	Mean	Min	Max	SD	CV%	Mean	Min	Max	SD	CV%	
Fruit fresh weight	FFW	g	1.55	0.47	3.51	0.70	45.01	1.39	0.85	2.05	0.33	23.96	18.16	11.03	25.35	4.67	25.72	31.56
Fruit length	FL	mm	12.86	8.45	19.08	2.61	20.28	11.18	8.50	15.90	2.09	18.72	34.41	29.80	46.60	4.55	13.22	17.41
Fruit diameter	FD	mm	13.92	9.25	19.60	2.72	19.57	11.66	9.00	16.00	2.08	17.85	28.41	22.50	32.60	3.19	11.22	16.21
Fruit length/diameter	FL/D	ratio	0.93	0.70	1.20	0.10	10.44	0.96	0.83	1.11	0.06	5.98	1.22	0.94	1.61	0.18	14.39	10.27
Fruit shape	FS	–	2.37	1.00	3.00	0.94	39.83	2.33	1.00	3.00	0.96	41.28	3.74	1.00	5.00	1.52	40.72	40.61
Seed length	SL	mm	10.65	6.93	17.08	2.17	20.33	8.76	7.00	11.00	1.41	16.06	19.64	15.50	25.00	3.15	16.02	17.47
Seed diameter	SD	mm	10.78	6.15	17.38	2.76	25.66	9.14	7.60	11.20	1.30	14.25	6.36	4.40	8.73	1.35	21.30	20.40
Seed length/diameter	SL/D	ratio	1.01	0.70	1.37	0.14	14.07	0.96	0.90	1.00	0.03	3.18	3.19	2.20	5.68	0.73	22.88	13.38
Fruit dry weight	FDW	g	0.73	0.28	1.44	0.28	37.90	0.91	0.47	1.39	0.25	27.87	5.94	2.08	9.74	2.37	39.84	35.20
Dry matter percent	DMP	%	49.70	31.37	79.10	10.22	20.56	65.32	54.00	72.50	5.92	9.06	31.88	16.24	43.59	7.53	23.61	17.74
Fruit moisture	FM	%	50.65	20.90	68.64	10.17	20.08	34.68	27.50	46.00	5.92	17.07	68.12	56.41	83.76	7.53	11.05	16.07
Leaf length	LL	cm	2.91	1.32	5.20	0.96	32.99	1.89	1.25	2.75	0.45	24.00	6.16	5.70	6.70	0.33	5.43	20.81
Leaf width	LW	cm	2.21	1.14	3.90	0.75	34.08	1.63	0.82	2.49	0.51	31.38	4.29	3.80	4.90	0.27	6.28	23.91
Leaf length/width	LL/W	ratio	1.33	1.03	1.65	0.15	11.43	1.19	1.04	1.65	0.16	13.66	1.44	1.24	1.58	0.09	6.11	10.40
Petiole length	PL	mm	6.55	2.00	20.00	4.01	61.24	4.24	2.00	7.80	1.85	43.70	17.66	8.32	23.35	4.25	24.05	43.00
Internode length	IL	cm	22.45	1.70	51.00	12.07	53.76	20.82	15.29	27.25	3.43	16.47	17.20	11.54	26.73	4.16	24.17	31.47
Shoot color	SC	–	5.00	5.00	5.00	0.00	0.00	1.17	1.00	5.00	0.82	69.99	18.16	11.03	25.35	4.67	25.72	31.90

Table 3 The SCoT primers diversity indices for genetic differentiation among 84 *Ziziphus* accessions

Primer ID	Primer sequence 5′ → 3′	TB	PB	PPB	SB (bp)	RP	MRP	HI	EMR	PIC	MI	DP	Ra
SCoT8	CAACAATGGCTACCACGT	4	4	100.00	320–600	2.29	0.57	0.50	4.00	0.35	1.40	0.73	0
SCoT20	ACCATGGCTACCACCGCG	6	6	100.00	300–650	2.83	0.47	0.47	6.00	0.36	2.17	0.62	0
SCoT22	AACCATGGCTACCACCAC	8	7	87.50	140–1200	3.81	0.54	0.50	6.13	0.35	2.14	0.75	0
SCoT19	ACCATGGCTACCACCGGC	7	7	100.00	320–870	3.52	0.50	0.44	7.00	0.38	2.63	0.55	0
SCoT13	ACGACATGGCGACCATCG	6	5	83.33	250–700	1.31	0.26	0.43	4.17	0.38	1.59	0.53	1
SCoT16	ACCATGGCTACCACCGAC	6	6	100.00	360–1100	1.60	0.27	0.23	6.00	0.45	2.68	0.25	2
SCoT28	CCATGGCTACCACCGCCA	5	5	100.00	270–740	2.38	0.48	0.40	5.00	0.40	1.98	0.47	0
SCoT29	CCATGGCTACCACCGGCC	5	5	100.00	530–820	2.93	0.59	0.41	5.00	0.39	1.94	0.50	0
SCoT23	CAACAATGGCTACCACGA	6	4	66.67	410–900	2.90	0.73	0.47	2.67	0.36	0.97	0.61	0
SCoT18	ACCATGGCTACCACCGCC	7	6	85.71	280–950	3.62	0.60	0.42	5.14	0.38	1.98	0.51	1
SCoT2	CAACAATGGCTACCACCC	8	6	75.00	250–1000	4.40	0.73	0.50	4.50	0.35	1.57	0.73	0
SCoT11	AAGCAATGGCTACCACCA	7	7	100.00	300–1100	5.14	0.73	0.49	7.00	0.35	2.47	0.68	0
SCoT3	CAACAATGGCTACCACCG	8	7	87.50	330–1500	3.31	0.47	0.45	6.13	0.37	2.28	0.57	0
SCoT36	GCAACAATGGCTACCACC	6	4	66.67	280–650	2.93	0.73	0.49	2.67	0.35	0.94	0.70	0
SCoT15	ACGACATGGCGACCGCGA	7	7	100.00	350–800	6.02	0.86	0.50	7.00	0.35	2.45	0.71	0
SCoT6	CAACAATGGCTACCACGC	9	8	88.89	280–750	4.86	0.61	0.49	7.11	0.35	2.51	0.68	0
SCoT24	CACCATGGCTACCACCAT	4	4	100.00	550–780	2.88	0.72	0.46	4.00	0.37	1.47	0.87	0
SCoT21	ACGACATGGCGACCCACA	5	5	100.00	400–650	4.33	0.87	0.50	5.00	0.35	1.74	0.76	0
SCoT31	CCATGGCTACCACCGCCT	7	5	71.43	370–850	3.88	0.78	0.48	3.57	0.36	1.29	0.63	1
SCoT9	CAACAATGGCTACCAGCA	8	7	87.50	420–900	2.98	0.43	0.33	6.13	0.42	2.56	0.38	1
SCoT33	CCATGGCTACCACCGCAG	6	6	100.00	340–720	1.55	0.26	0.50	6.00	0.35	2.10	0.71	0
Mean	–	6.43	5.76	90.49	–	3.31	0.58	0.45	5.25	0.37	1.95	0.62	0.29
SD	–	1.36	1.22	11.91	–	1.20	0.18	0.07	1.34	0.03	0.52	0.14	0.56

TB, Total bands; PB, Polymorphic bands; PPB, Percentage of polymorphic bands; SB, Size of bands; RP, Resolving power of primer; MRP, Mean of resolving power; HI, Heterozygocity index; EMR, Effective multiplex ratio; PIC, Polymorphic information content; MI, Marker index; DP, Discriminating power; Ra, Rare allele

Accordingly, the phylogenetic tree among the accessions was constructed based on the Jaccard's similarity coefficient after 2000 permutation using TreeView software (ver. 32) (Page 1996).

The NTsys software (ver. 2.2) (Rohlf 2000) was employed to estimate the genetic similarity among the accessions and subsequent constructing of an unweighted pair group method with arithmetic mean (UPGMA) dendrogram. In addition this software was used to carry out the Mantel test between similarity matrices from morphological and molecular data with 5000 permutations (Mantel, 1967).

Analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA) via covariance matrix was performed by GeneAlex software (ver. 6.6) (Peakall and Smouse 2012).

Bayesian-based clustering was conducted by STRUCTURE program (ver. 2.3.4) following an admixture model with a burn-in period of 100,000 interactions and 100,000 interactions of Markov Chain Monte Carlo (MCMC) testing ten independent runs with k from 1 to 10 (Evanno et al. 2005). The Structure Harvester program (ver. v.0.9.94) (Earl and VonHoldt 2012) was employed to determine the most likely number of subpopulations in the germplasm set. A membership coefficient of 80% was considered as the threshold for sample assignment in each cluster and the accessions with the values below threshold ($q \leq 0.8$) were considered as genetically admixture.

Results

Morphological diversity

Wide phenotypic variations were observed not only among the *Ziziphus* species but also among the samples belonging to the same species (Table 2). The mean values of most morphological traits were different among the species (Table 2). Z. *mauritiana* had the highest values for fruit weight (18.16 g), fruit dimensions (fruit length = 34.41 mm; fruit diameter = 28.41 mm) and fruit moisture (56.41%) while Z. *nummularia* had the smallest leaves

(leaf length = 1.89 cm; leaf width = 1.63 cm) and fruits (fruit length = 11.66 mm; fruit diameter = 11.18 mm) but the highest dry matter (65.32%) among the species. However, the longest internode (22.45 cm) was recorded in *Z. spina-christi* accessions.

Cluster analysis based on the morphological attributes divided the germplasm into three main groups mainly based on the species sources (Fig. 2). According to constructed dendrogram, the members of Z. *mauritiana* formed a distinct group and were completely separated from other samples. Most of the *Z. spina-christi* accessions were grouped together and formed the second main cluster. However, the third main cluster consisted of the Z. *nummularia* samples alongside the remaining accessions of *Z. spina-christi*. Results of PCA analysis revealed that 48.64% and 17.19% of variations were captured by the first two factors. The bi-plot were depicted based on the first two factors and separated the entire germplasm into two groups including Z. *mauritiana* group and *Z. spina-christi* and Z. *nummularia* group (Fig. 3a).

Results of bivariate correlations revealed there were significant positive and negative correlations between pairs of variables (Fig. 4). In particular, fruit fresh weight showed significant positive correlation with fruit dry weight, fruit length and fruit diameter. Moreover, fruit fresh weight was significantly correlated with leaf dimensions and petiole length. Significant negative correlation was also recorded between dry matter percentage with fruit moisture, fruit diameter and fruit dimensions (Fig. 4).

SCoT markers analysis

Out of 30 SCoT primers that were initially tested, 21 successfully amplified polymorphic fragments on with the germplasm. A total of 135 high quality scorable and reproducible bands were generated by these primers on 84 *Ziziphus* accessions (Table 3). The average number of produced fragments were 6.43, varying from 4 (SCoT-8 and SCoT-26) to 9 (SCoT-6) among the primers. The sizes of scorable amplicons were in the range of 280 bp to 1.5 kb. The number of polymorphic fragments ranged from four to eight with an average of 5.76 ± 1.22 (Table 3).

The resolving power (RP) varied from 1.31 (SCoT-13) to 6.02 (SCoT-15) with a mean value of 3.31 ± 1.20. The average values of heterozygocity index (HI) and polymorphic information content (PIC) were estimated to be

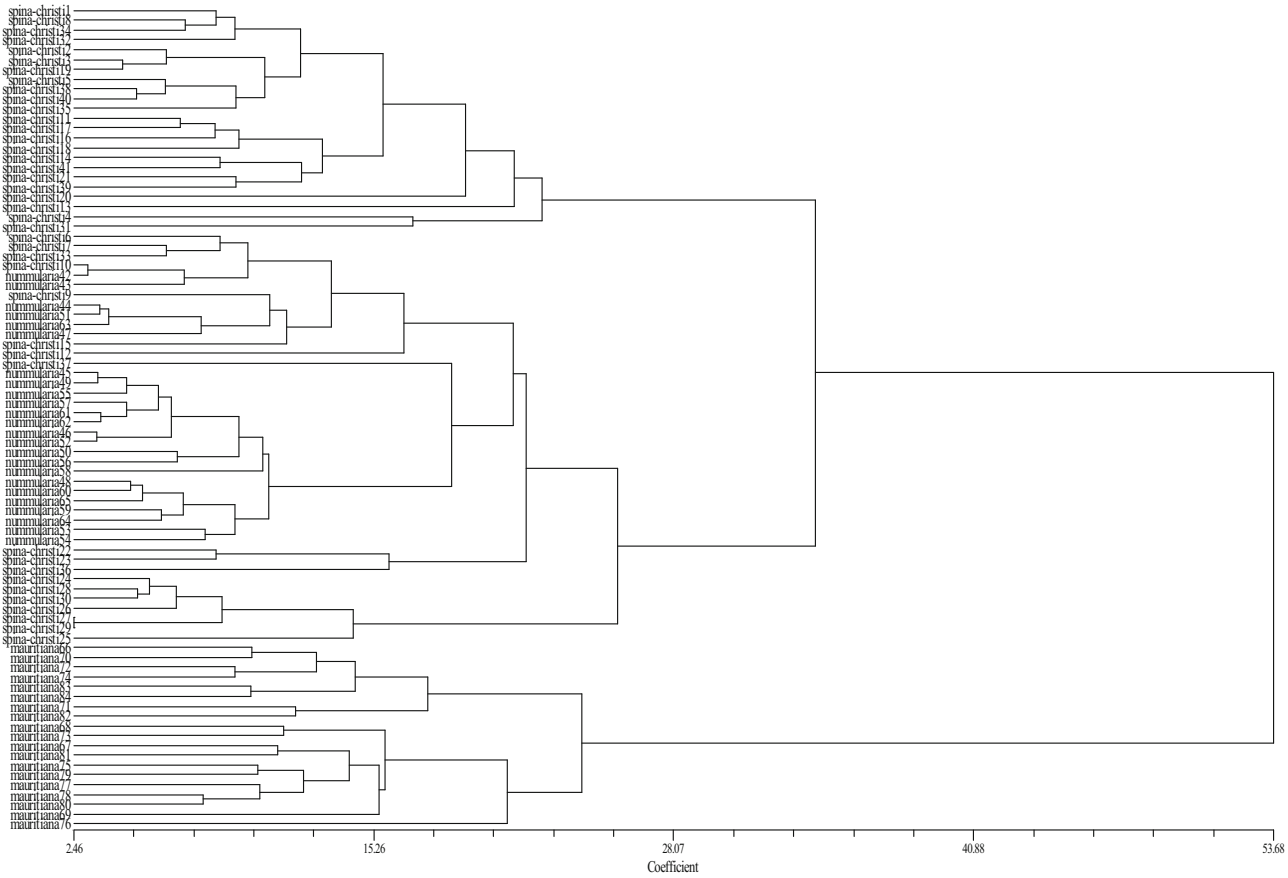

Fig. 2 UPGMA cluster analysis delineating 84 *Ziziphus* accessions based on their morphological characteristics. Each accession is represented by the species name and a number according to Table 1

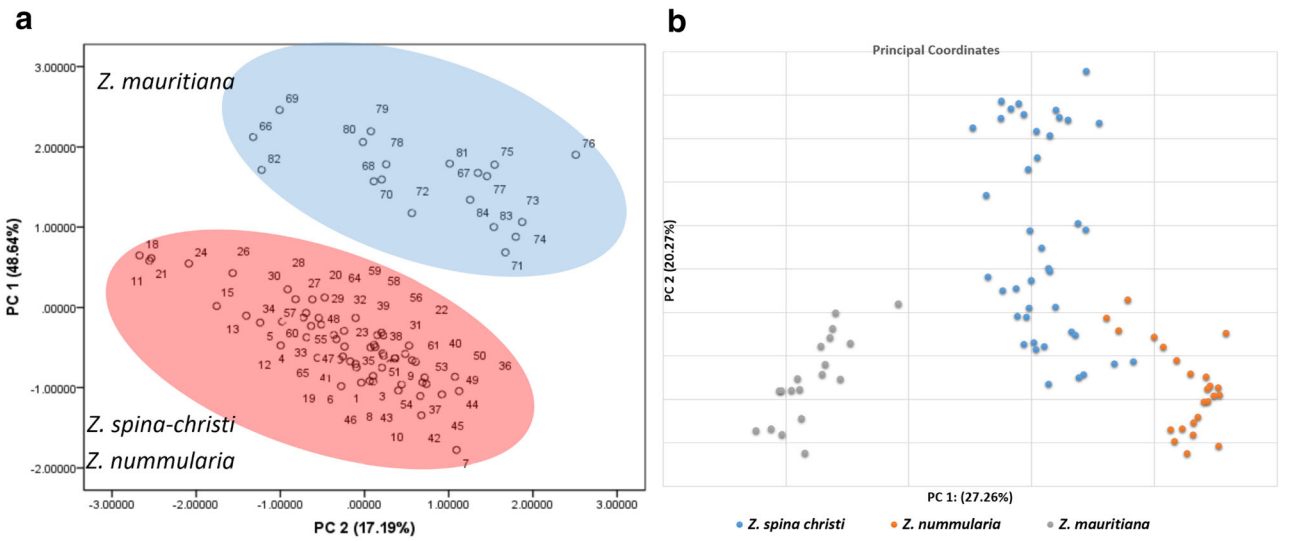

Fig. 3 A two dimensional plot representing the pattern of *Ziziphus* accessions according to the main principal components in morphological traits (**a**) and SCoT markers (**b**). The samples were labeled by a number based on the Table 1

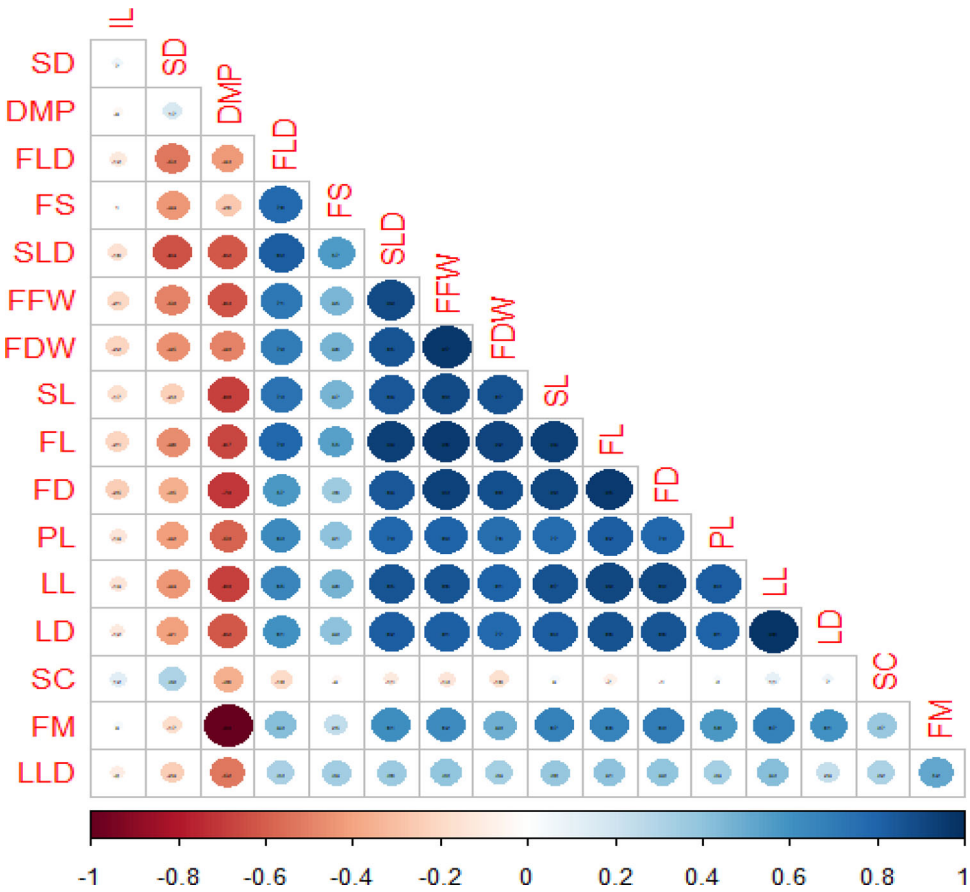

Fig. 4 Plot of bivariate correlation between each morphological traits depicted by R package. In this plot the degrees of correlation are presented by colored circles, in which their color represents the direction of correlation (positive correlation in blue and negative correlation in red). In addition the size and intensity of color represent the level of correlation from − 1 to 1. The traits are represented by the abbreviations, which their full terms are presented in Table 2

0.45 (range 0.23 to 0.50) and 0.37 (range 0.35 to 0.45), respectively. The marker index (MI), was in the range of 0.94 (SCoT-36) to 2.68 (SCoT-16) with a mean value of 1.95 ± 0.52. Discriminating power (DP) of SCoT primers varied from 0.38 (SCoT-9) to 0.87 (SCoT-24) across all primers with an average of 0.62 ± 0.14. Out of the 121 generated polymorphic fragments, six alleles had a frequency below 5% and were identified as the rare allele (Ra).

Population genetic indices were computed for *Ziziphus* germplam and are presented in Table 4. The observed number (*Na*) of allele and effective number of alleles (*Ne*) across the accessions were 1.98 and 1.69, respectively. The Nei's gene diversity (*h*) was 0.38 and the Shannon's information index (*I*), an indicative of genetic diversity, had an average of 0.56 ± 0.17. Following with the results obtained from AMOVA analysis, the majority of genetic diversity was appropriated to the within species (0.28). The estimate of genetic differentiation (G_{ST}) and gene flow were 0.26 and 1.41, respectively.

The estimated genetic diversity indices varied among the species (Table 5). *Z. spina-christi* showed the highest values for the number of observed (1.88 ± 0.33) and effective (1.62 ± 0.25) alleles. The highest (0.347) and the lowest (0.234) Nei's gene diversity was recorded from Z. *spina-christi* and *Z. mauritiana* species, respectively. Accordingly, the Shannon index of Z. *spina-christi* was the highest (0.505), while that of *Z. nummularia* was similar to that of *Z. mauritiana* (0.383 and 0.342, respectively).

Four alleles were identified as the private alleles which were detected only in one species (Table 5). Specifically, *Z. mauritiana* showed two private alleles in loci SCoT2-4 and SCoT26-2 and Z. *nummularia* also showed two private alleles in loci SCoT15-6 and SCoT9-3. However, no specific allele was detected in *Z. spina-christi* by these primers. In addition, several unique alleles were only detected in just two species and were not detected in the remaining one. Such fragments were designated as absent fragments (AF) and were 2, 1 and 4 for *Z. spina-christi*, Z. *nummularia* and *Z. mauritiana*, respectively.

The total variance of the SCoT pattern was apportioned among and within *Ziziphus* species using analysis of molecular variance (AMOVA). Results showed that 73% of the total variations were contributed by differentiation within species, which were significantly higher than the differences that were ascribed among the species (27%) (Table 6).

According to PCoA, a total of 67.55% of the variation were explained by the first three main Eigen factors (27.26%, 20.27%, and 20.02%, respectively). A two-dimensional scatter plot that was created based on the two main eigenvectors, completely differentiated the accessions into three main groups corresponding to their species origin (Fig. 3b). The scatter plot revealed that *Z. mauritiana* was the most distant species and formed a separate group, while samples of *Z. spina-christi* and Z. *nummularia* had high genetic similarity representing a number of probable intermediate accessions.

Population structure and cluster analysis

The mean value of Jaccard's similarity coefficient across the accessions was 0.57. The lowest genetic similarity (0.30) was recorded between two accessions from *Z. spina-christi* (sample No. 3) and *Z. mauritiana* (samples No. 70), while the highest genetic similarity was recorded between two accessions from *Z. nummularia* (samples No. 53 and 54).

A dendrogram was created based on the Jaccard's similarity coefficient matrix from SCoT data using UPGMA method (Fig. 5). The entire *Ziziphus* germplasm was divided into four main clusters at 0.55 genetic similarity. According to the dendrogram, all the samples from Z. *mauritiana* were grouped together and formed a distinct clade in the cluster. Accessions of *Z. spina-christi* were divided into three clusters mainly based on their sampling sites. All of the specimens belonging to Z. *nummularia* species were clustered together as a separate sub-cluster alongside one of the sub-cluster of *Z. spina-christi* species.

The phylogenetic cluster that was created by the TreeView program also clustered the accessions based on the species source (Fig. 6). Accordingly, three main groups were generated completely consisted with the species origin of the specimens. The biggest cluster included 41 accessions all belong to *Z. spina-christi* with three recognizable sub-clusters each covering the accessions from the same geographical region. The second and the third main groups included 19 and 24 individuals belonging to *Z. mauritiana* and Z. *nummularia* species, respectively.

Bayesian clustering analysis using STRUCTURE program was also performed to understand the genetic structure of the germplasm. Delta K method suggested K = 3 as the best number of sub groups (supplementary Fig. 1). Similarly three main sub populations were identified in concordance with three *Ziziphus* species (Fig. 7).

Table 4 Population genetic analysis of *Ziziphus* germplasm

Diversity indices	N_a	N_e	h	I	H_T	H_S	G_{ST}	N_m
Mean value	1.98	1.69	0.38	0.56	0.38	0.28	0.26	1.41
Standard deviation	0.13	0.30	0.14	0.17	0.02	0.02	–	–

N_a, Observed number of alleles; N_e, Effective number of alleles; h, Nei's (1973) gene diversity; I, Shannon's information index of genetic diversity (Lewontin, 1972); H_T, Total genetic diversity; H_S, Average genetic diversity within populations; G_{ST}, Genetic differentiation; N_m, Estimate of gene flow from G_{ST}

Table 5 Estimates of genetic diversity indices for each *Ziziphus* species based on the SCoT patterns

Species	N	*na*	*ne*	*h*	*I*	NPA	AF
Z. spina-christi	41	1.88 ± 0.33	1.62 ± 25	0.347 ± 0.172	0.505 ± 0.234	0	2 (SCoT11-6, SCoT 21–3)
Z. nummularia	24	1.65 ± 48	1.48 ± 0.41	0.26 ± 0.21	0.383 ± 0.302	2 (SCoT 15–6; SCoT9-3)	1 (SCoT 31–4)
Z. mauritiana	19	1.60 ± 0.49	1.41 ± 0.39	0.234 ± 0.213	0.342 ± 0.302	2 (SCoT 2–4); SCoT 26–2)	4 (SCoT3-4, SCoT29-3, SCoT20-5, SCoT13-2)
Mean ± SE	28 ± 11.53	1.71 ± 0.15	1.50 ± 0.11	0.280 ± 0.059	0.41 ± 0.085	1.33 ± 1.15	2.33 ± 1.53

N, Number of individuals; *na*, Observed number of alleles; *ne*, Effective number of alleles *h*, Nei's gene diversity Nei's (1973); *I*, Shannon's information index of genetic diversity (Lewontin, 1972); NPA, Number of private alleles; AF, Absent fragments

Table 6 Analysis of molecular variance (AMOVA) based on SCoT molecular markers for three species of *Ziziphus*

Source of variation	Degrees of freedom	Sum of squares	Mean square	Percentage variation (%)	*P*- value
Among species	2	394.984	197.492	27	< 0.001
Within species	81	1458.587	18.007	73	< 0.001
Total	83	1853.571		100	

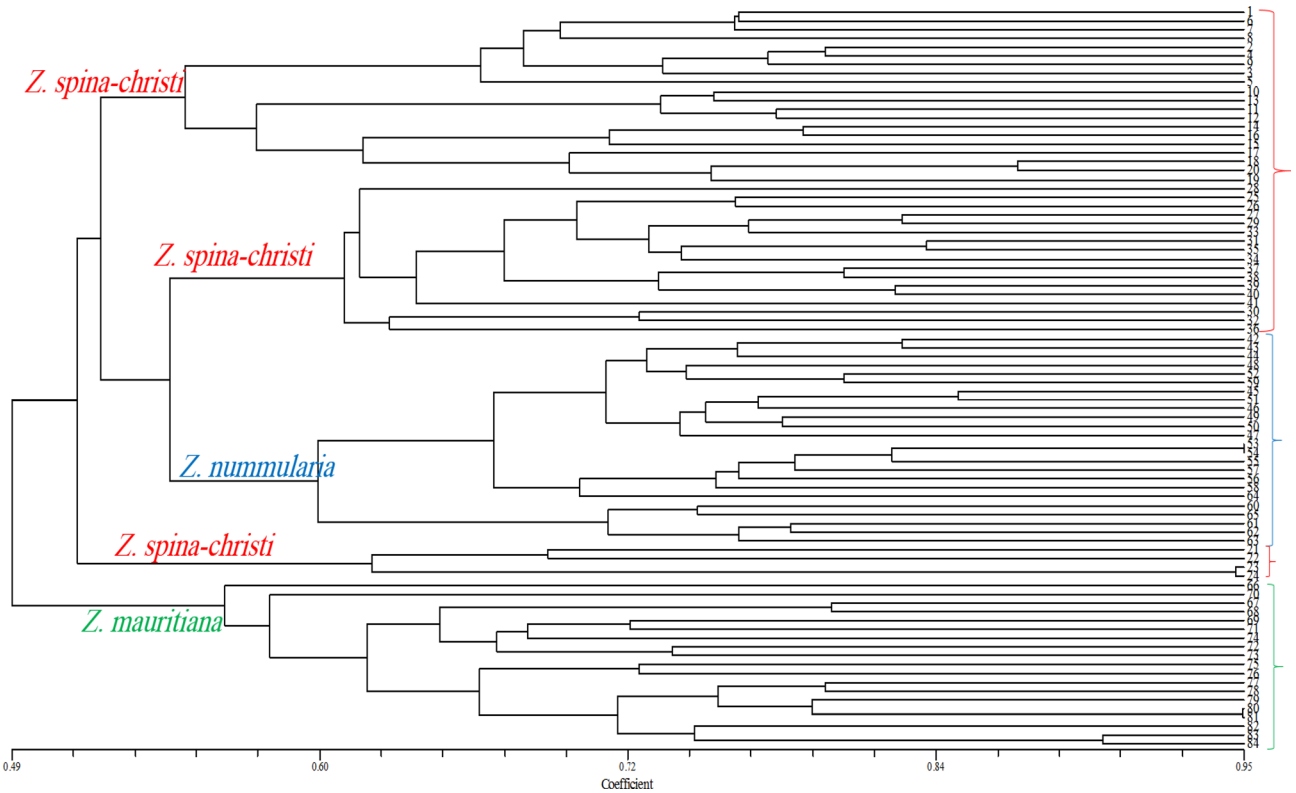

Fig. 5 UPGMA cluster derived from SCoT data, delineating the genetic relationship among 84 accessions from three *Ziziphus* species. The accessions are displayed by a number according to Table 1

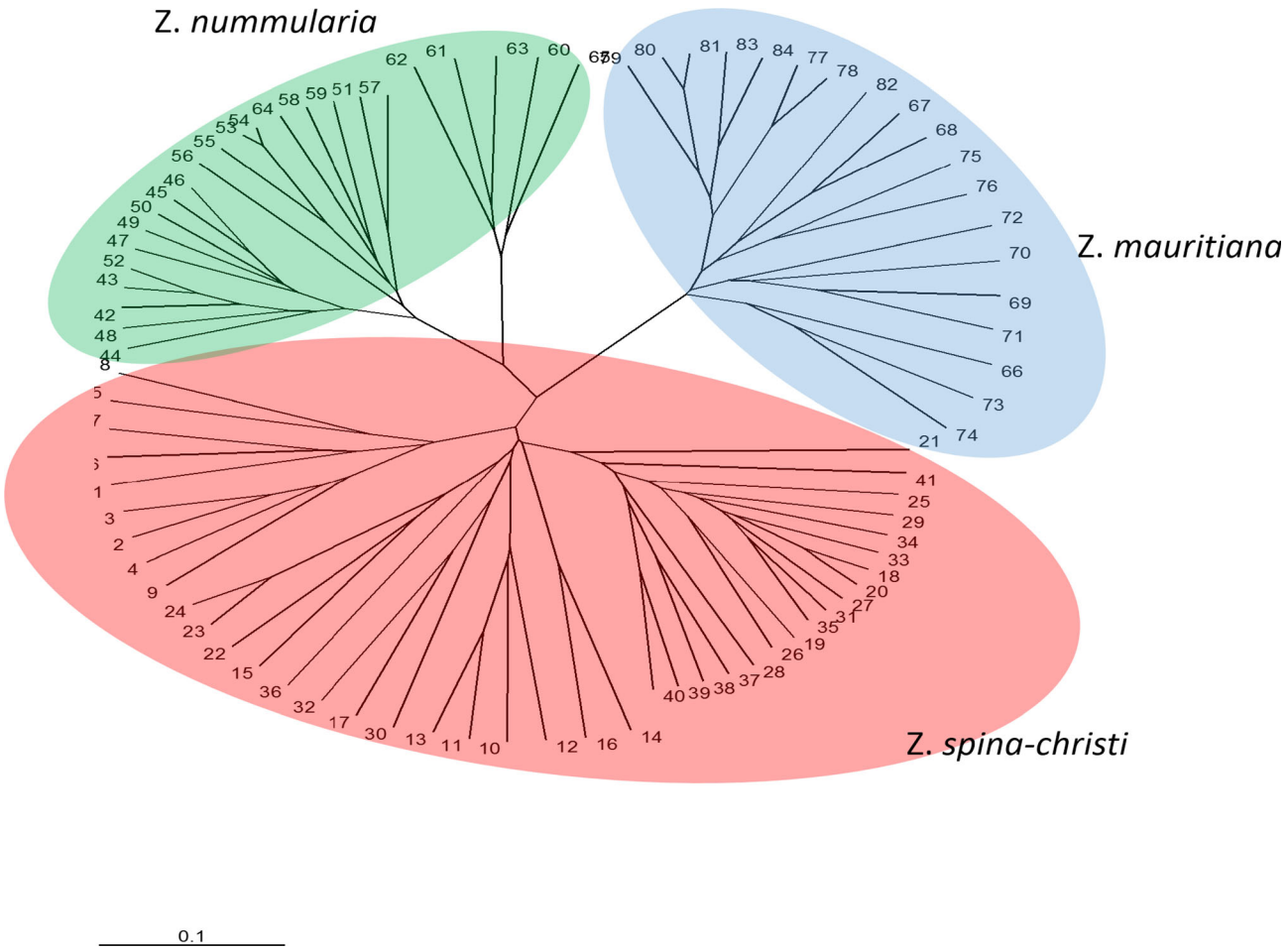

Fig. 6 Genetic relationships among *Ziziphus* samples from three species based on 21 SCoT primers. The samples were labeled by a number based on the Table 1

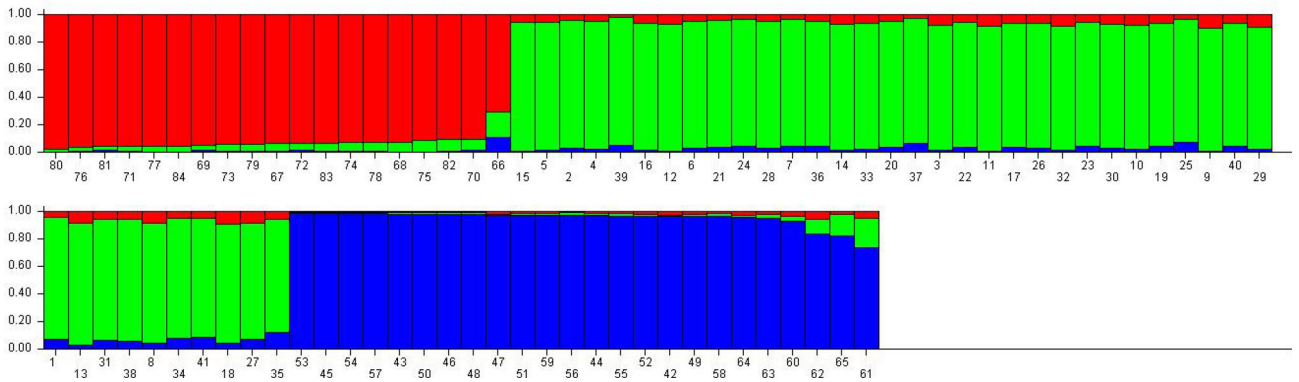

Fig. 7 Bayesian assignment of *Ziziphus* germplasm based on SCoT molecular markers using STRUCTURE program for K = 3. The individuals are presented by a number according to Table 1

Species affinity

Nei's genetic distance (GD) was calculated to estimate the species pairwise affinity (Table 7) and the distance matrix was used for respective dendrogram construction using Mega 6 software (Fig. 8). The unbiased genetic distance showed that *Z. spina-christi* and *Z. nummularia* were the most similar species and had the lowest genetic distance (GD = 0.163), while *Z. nummularia* and *Z. mauritiana* were the most distinct species (GD = 0.301).

Table 7 The Nei's unbiased measures of genetic distance (below diagonal) and genetic identity (above diagonal) among three *Ziziphus* species

Ziziphus species	*Z. spina-christi*	*Z. nummularia*	*Z. mauritiana*
Z. spina-christi	1	0.849	0.812
Z. nummularia	0.163	1	0.740
Z. mauritiana	0.208	0.301	1

Results of Mantel test indicated there were moderate correlation ($r = 0.45$; $p = 0.001$) between morphological data and SCoT derived similarity matrices.

Discussion

Morphological attributes

Estimation of the existing genetic diversity is one of the preliminary fundamental steps and an important component of each plant resource management, which pave the way for better utilization of the elite plant materials and their subsequent applications in crop improvement. Three *Ziziphus* species that were investigated in this study have a wide geographic distribution and are considered as hardy and low-expectation tree species bearing edible fruit with significant nutritional and medicinal properties. These valuable traits were considered by plant scientists towards this genus for land validation and fruit production especially in the regions that most of the other fruit crops cannot be cultivated commercially. Underutilization of the current diversity within and between the *Ziziphus* species is one of the main reasons for such backwardness. Morphological evaluation of the current plant genetic resources is regarded as the essential component for management, utilization and improvement of each plant species (Zarei 2017). A wide phenotypic variation was detected both within and among the *Ziziphus* species in the present work. *Ziziphus* is a naturally widespread genus with inter and intra specific hybridization capacity (Asatryan and Tel-Zur 2014); thereby high morphological variations and ecotypes development are not far from expectation. Our finding confirmed the reports indicating high morphological variations among different *Ziziphus* species in previous records (Pareek 2001; Razi et al. 2013; Ahmad et al. 2016; Norouzi et al. 2017; Gupta 2018). Among the studied characteristics, petiole length, fruit weight and fruit size were highly variable among the accessions of three *Ziziphus* species. Most of the morphological evaluation concerning different *Ziziphus* species indicated that fruit weight and size are

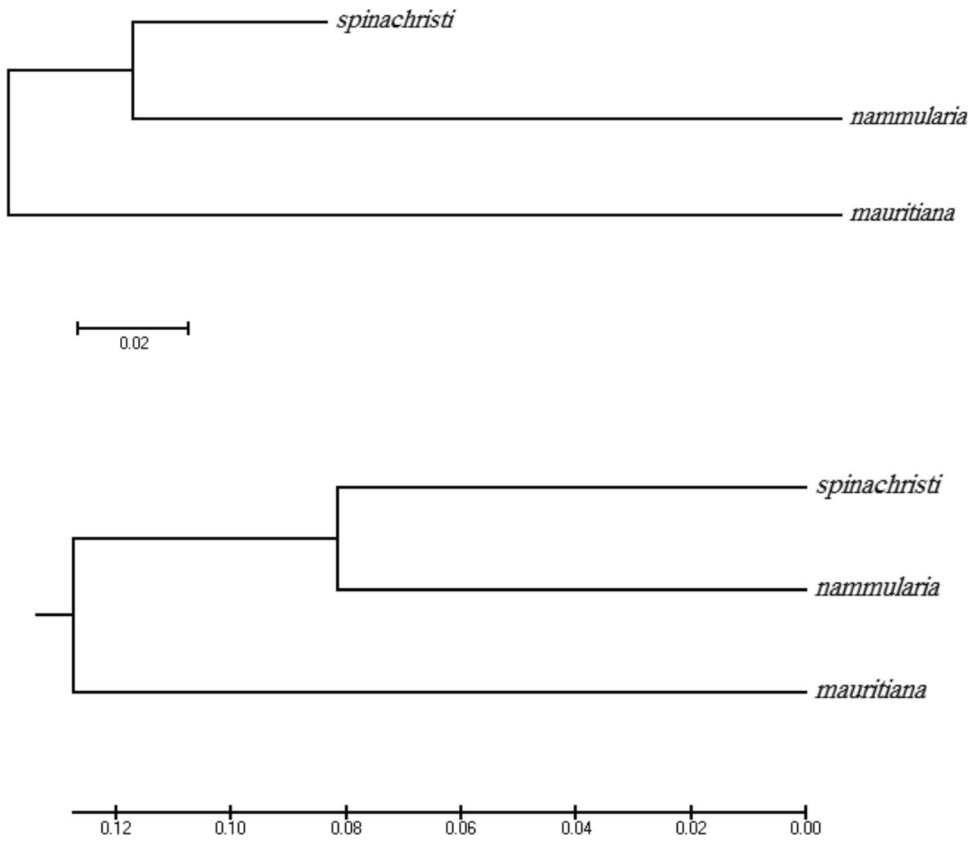

Fig. 8 NJ (above) and UPGMA (below) dendrograms based on the Ni's genetic identity delineating the phylogenetic relationships among three *Ziziphus* species obtained by Mega 6 software

highly variable among the samples (Dahlia et al. 2009; Razi et al. 2013; Ahmad et al. 2016; Norouzi et al. 2017). We observed a wide range of fruit weight (0.48–25.35 g), which is relatively higher than previous similar reports (6–37 g for *Z. mauritiana*) (Ahmad et al. 2016) and (0.96–3.14 g for three *Ziziphus* species) (Norouzi et al. 2017). It is noteworthy that the experimental specimens in the current work have been sampled from three different species across a wide geographical distance. Therefore, it could be interesting to investigate the effect of genotype-environment in the future experiments. According to Gao et al. (2011) both genotype and ecological condition are among the main factors affecting plant characteristics and may result in high variation in fruit weight. Improving the fruit weight and size are considered as the most important objectives in *Ziziphus* breeding programs (Norouzi et al. 2017). Therefore, current germplasm has great potential to serve as a rich source for selection, domestication and hybridization activities aiming to improve the fruit characteristics in *Ziziphus* species.

In addition, significant positive and negative correlations were detected between different pairs of morphological attributes. Besides the fruit dimensions, leaf dimensions also had significant positive correlations with fruit fresh weight. Our results corroborate the previous reports indicating high correlation between these two characteristics (Norouzi et al. 2017; Zarei et al. 2019). Plant leaves are the main specialized tissue for capturing sunlight energy and converting it to photosynthetic products. Therefore, the availability of a high level of carbohydrates in the plant and increase the ratio of C to N will provide desirable conditions for the growth of reproductive organs and subsequently will led to the improvement in the size and weight of fruit. Information about the correlation between morphological traits would be of great importance in the breeding programs, as these informations enable the breeders to predict one trait from other one and subsequently will reduce the period required for selection which is highly advantageous especially in the long lived woody species plants. In addition, high correlation between phonotypic traits could facilitate or hinder the gene introgression programs as selection for the trait of interest is always accompanied by another one.

SCoT markers

Twenty-one start codon-based primers were successfully employed for inter and intraspecific characterization of *Ziziphus*. Results indicated that 90.49% of produced amplicons were polymorphic which was higher than reports from *Mangifera indica* (73.82%) (Luo et al. 2012), and *Nigella sativa* (82.7%) (Golkar and Nourbakhsh 2019) and in the range of *Physalis* (90.2%) (Feng et al. 2018) and *Bromus* (92.24%) (Safari et al. 2019) using the same marker system. Moreover, SCoT markers showed a higher polymorphic pattern in different *Ziziphus* species when compared with other molecular marker systems such as ISSR (61.49%) and RAPD (40.11%) in *Z. spina-christi* (Moustafa et al. 2016), ISSR (63.62%) in *Z. mauritiana* (Singh et al. 2017), RAPD (74.15%) and ISSR (89.94%) in *Z. mauritiana* and *Z. nummularia* (Singh et al. 2009), and ISSR (61.31%) in *Z. lotus* (Dahlia et al. 2019). The efficiency of a molecular marker for genetic differentiation among different plant accessions depends on the degree of generated polymorphism (Mehdi et al. 2014; Zarei and Sahraroo 2018). Therefore, according to our results, SCoT markers showed to be highly suited for genetic discrimination among *Ziziphus* species. SCoT11, SCoT15, and SCoT6 were among the highly informative primers that showed high values for marker efficiency indices (RP, MI and DP) and hence are suggested for further genetic differentiation among the *Ziziphus* species.

Genetic diversity indices indicated that there are high levels of genetic differentiation among the *Ziziphus* accessions. The Nei's gene diversity ($h = 0.38$) and Shannon's information index ($I = 0.56$) across the accessions showed relatively higher values when compared with other long lived out crossing plant species (Hamrick and Godt 1996; Kumar et al. 2014; Jalilian et al. 2018). The highest values for these indices were recorded in *Z. spina-christi* samples ($h = 0.347$ and $I = 0.505$); indicating the genetic diversity of this species was the richest, the results that were reflected in phenotypic assessment as well. On the other hand, for most of the evaluated attributes, the highest coefficients of variations were recorded among the *Z. spina-christi* accessions and the cluster analysis and scatter plot of the accessions based on the main principal components also represented higher diversification for *Z. spina-christi* compared with its closely related species. The high value of recorded genetic differentiation ($G_{ST} = 0.26$) represents a substantial differentiation among the species. The G_{ST} values greater than 0.25 and gene flow below 1, indicate a significant genetic differentiation among the sub-populations (Slatin 1987). Population isolation, extensive recurrent gene flow and the small size of population are among the main documented reasons for high G_{ST} values (Ellstrand and Elam 1993; Mehdi et al. 2014). However, a relatively high level of gene flow ($Nm = 1.41$) was estimated among the species that may be contributed to the high cross ability among the samples. The existence of an efficient pollen dispersion mechanism in the outcross species can be led to the value of $N_m > 1$ (Mehdi et al. 2014). The higher values of gene flow can reduce the detrimental effect of extreme genetic differentiation among the populations and subsequent genetic drift (Slatkin and Barton 1989). Genetic diversity of a given species is affected by

many traits including, lifespan, seed dispersal method, and the geographic distribution (Kumar et al. 2014; Ebrahimi et al. 2017a). The high value of gene flow was also reflected in Bayesian clustering and the entire germplasm shared a trace of common alleles (Fig. 7). Three *Ziziphus* species that were investigated in this study have a wide distribution, crossable flowers, and easily dispersible seeds. Besides being the most populous species in this investigation, the *Z. spina-christi* samples have been collected from naturally wild forests of different wide geographic regions. The *Z. mauritiana* showed the lowest genetic diversity indices among the three species.

The level of genetic differentiation is affected by selective (Nm, genetic drift) and non-selective (natural selection) forces in the sub population (Mehdi et al. 2014). The small size of a population may affect the selective forces and subsequently result in the underestimation of genetic differentiation of a population (Ebrahimi et al. 2016). In contrary to the *Z. spina-christi* and *Z. nummularia* which were sampled from wild forests in their natural ranges, samples of *Z. mauritiana* were collected from local farmers and personal collections. Therefore, the lower genetic diversity in this species compared with two other close relatives, at least to some extent may be attributed to the genetic erosion which may be resulted from founder effects. However, we can not ignore the low number of samples in this species.

Genetic relationships among the germplasm

The distribution pattern of *Ziziphus* accessions across the main principal coordinates indicates a broad diversification among the germplasm. The samples from *Z. spina-christi* showed to be the most diverse one. Relatively high levels of genetic similarity among some of the accessions from *Z. spina-christi* and *Z. nummularia* can also be seen in the Biplot (Fig. 3), UPGMA cluster analysis (Fig. 5), and similarity matrix (Table S1).

The AMOVA results revealed that the majority of genetic variations (73%) existed within species. Previous records from other out-crossing plant species including *Z. mauritiana* and *Z. jujube* also indicated a higher genetic variations within rather than between populations (Ebrahimi et al. 2017b; Jalilian et al. 2018; Singh et al. 2017; Shen et al. 2020). Allocation of genetic differentiation among and within populations can provide valuable information for designing sampling scenarios in crop breeding (Ebrahimi et al. 2016). However, the amount of genetic variation that was partitioned among populations was relatively higher in our study (27%) when compared to the other tree species such as *J. regia* (15%) (Ebrahimi et al. 2016), *Pyrus* spp. (17%) (Jalilian et al. 2018), *Z. spina-christi* (10%) (Alansi et al. 2016), *Z. mauritiana* (5%) (Singh et al. 2017), and *Z. jujube* (12%) (Shen et al. 2020). Similarly, Farahani et al. (2019) reported that 28% of genetic variability occurred among populations of *Z. jujube*. Population isolation, genetic drift, local selection and founder effects are among the main factors that are suggested to give rise to high level of differentiation among the populations (Jolivet and Bernasconi 2007). However, the high value of genetic variations that were partitioned among groups in the current study, can be attributed in part to the source of populations that were analyzed. On the other hand, we analyzed three different species in our study as three populations and it is not unexpected to find higher genetic differentiation among the groups than groups within a given species.

We used different clustering methods to classify the germplasm. Based on the results, *Z. spina-christi* and *Z. nummularia* were the most genetically similar species and these two species had higher genetic differentiation with *Z. mauritiana*. The intrageneric classification of *Ziziphus* is a controversial issue and has been considered as a "thorny dilemma" (Islam and Simmons 2006; Huang et al. 2017). In fact, the current classifications were constructed based on the analysis of morphological attributes or a few nuclear and plastid loci (Islam and Simmons 2006). Incorporation of more coding regions in the phylogenetic analysis of this genus, will improve our knowledge about the intrageneric relationships in *Ziziphus*. Based on the data from morphological characters and it's region, the Indian jujube (*Z. mauritiana*) was in the same clade with *Z. glabrata* (another species native to India) and *Z. spina-christi* and was completely separated from the Chines jujube, *Z. jujuba* (Islam and Simmons 2006). In addition, classification based on the whole chloroplast genomic revealed that *Z. mauritiana and Z. spina-christi* were clustered together, -while *Z. jujuba* were grouped with *Z. acidojujuba* (Huang et al. 2017). However, none of the previous studies included *Z. nummularia* species in their research. However, Devanshi et al. (2007) employed the RAPD markers to investigate the Z. *mauritiana* genotypes of India and used a wild specimen from each of the *Z. spina-christi* and *Z. nummularia* species along with their samples and observed the latter species were completely separated from *Z. mauritiana* genotypes.

Six alleles had a frequency below 0.05 in the germplasm. The number of rare alleles should be considered as the main factor for the implementation of genetic conservation scenarios. Typically, a substantially greater number of accessions would be required to capture rare allelic variation efficiently. In addition, the use of spatially stratified sampling would not lead to the efficient capturing of the rare alleles in the populations and general guidelines of conservation should be adjusted to accommodate the rare variants (Whitlock et al. 2016). Although rare alleles may

have beneficial, deleterious, or neutral effects on the population fitness, the majority of such alleles which are accumulated through mutations have detrimental effects (Keightley and Lynch 2003); hence should not be captured during conservation actions. However, there are also beneficial rare alleles that can improve plant performance either through enhancing plant resistance to biotic/abiotic stresses or improving adaptability to the changing ecological and environmental conditions both of which subsequently can increase the population fitness (Piertney and Oliver 2005; Loewe and Hill 2010). Therefore, designing conservation scenarios are more sensitive in the populations with a high level of rare alleles.

Four species specific SCoT amplicons were detected in *Z. nummularia* and *Z. mauritiana*. Moreover, some of the SCoT amplicons were detected in two species with no amplification in the remaining one. These types of markers have high practical application for species authentication especially in the controversial cases including *Ziziphus* genus classification. Similarly, species specific SCoT markers have been detected in other plant species (Xu et al. 2019; Jalilian et al. 2018). Detection of specific amplicons would be of great importance for designing longer specific co-dominant primers and are highly useful to terminate disagreement about the controversial classifications in the closely related species. Sequence-characterized amplified regions (SCARs) primers were successfully designed based on the SCoT specific fragments and have been used to trustworthy differentiate among the *Physalis* species (Feng et al. 2018). SCARs that are produced from SCoT markers have an additional value than those obtained by other molecular marker systems. As these types of markers are designed in a way that can amplify the coding regions of genome by anchoring the start codon of the genes, they have high potential to be linked with morphological traits; hence have a great chance to be used in MAS programs. The fact that was reflected in the Mantel test and significant correlations were detected between morphological and molecular data. There are contradictory reports regarding the correlation between matrices derived from different molecular marker systems and morphological attributes in different plant lineages. Some of the previous works reported a low Mantel test coefficient between morphological and molecular data (Hagidimitriou et al. 2005; Zamani et al. 2010; Ouji et al. 2016; Jin et al. 2017; Laaribi et al. 2017), while several records are indicating a significant correlation between these two marker types (Wen et al. 2004; Pakseresht et al. 2013; Mahmodi et al. 2021). Both morphological and molecular data greatly contribute to the level of correlation between these two marker classes. In the case of morphological traits, where researchers used limited phenotypic characteristics in their experiments (e.g. only fruit related attributes), Mantel coefficient was relatively low (Zamani et al. 2010), however, by using a wide range of traits including both vegetative and reproductive organs, high levels of correlation were recorded (Wen et al. 2004). The nature of molecular marker technique also highly affect the degree of correlation between molecular and morphological markers. Results of available records derive us to the conclusion that using molecular marker systems, which are mostly targeting non-coding regions of the genome (e. g. SSR, ISSR and RAPD), will results in a lower Mantel coefficient than those capturing coding sequences. The SCoT primers are designed based on the conserved surrounding sequences of start codon (ATG), hence the markers derived from this technique may have a high association with the functional genes and their corresponding traits. Identification of marker-trait association would be a great importance for application of DNA markers in the plant improvement programs, as well as development of sequence-characterized amplified regions (SCARs) primers.

Conclusion

The exploitation of the existing genetic resources is of utmost need for direct utilization of superior accessions and to meet the modern breeding programs in *Ziziphus* species. Data collected from SCoT markers and phenotypic traits revealed there is a broad genetic base in three Iranian *Ziziphus* species. Start codon based fingerprinting showed to be highly helpful for genetic fingerprinting of genus *Ziziphus*. According to the diversity indices, *Z. spina-christi* showed to be the most diverse species. Different clustering methods were conducted and the accessions were clustered based on their species source. The data represented there is considerable interspecific genetic exchanges in *Ziziphus* species. Accordingly, STRUCTURE analysis revealed a number of the accessions are admixture, which may be the result of interspecific hybridization. Analysis of the phylogenetic relationship among the species revealed that *Z. spina-christi* and *Z. nummularia* have the highest similarity and can be considered as the sister species, while *Z. mauritiana* showed to be the most distinct species. Moreover, a number of the species private alleles were identified in this work that would help for authentication of the species and to resolve the uncertainty cases. At the same time, a high association was recorded between morphological and molecular markers, which suggests these amplicons can serve as a helpful axillary tool in the improvement programs. Results of our investigation revealed that SCoT markers have the potential to be successfully used for clarification across blurred taxonomic boundaries and complicated cases.

Authors' contributions All authors contributed to the study conception and design. Material preparation and data collection were performed by Abdolkarim Zarei, Asma Rezaei and Mohammad Esmailpour. Data analysis were performed by Aziz Ebrahimi and Abdolkarim Zarei.The first draft of the manuscript was written by Abdolkarim Zarei, revised by Aziz Ebrahimi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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