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Full Length Article

Molecular Markers Systems Revealed High Genetic Similarity among Fifty Date Palm (*Phoenix dactylifera*) Genotypes

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Abstract

The availability of different PCR based markers systems to reveal the differences among various genotypes has prompted to compare the utility of these markers in diversity analysis. In the present study, we reported the diversity among collected date palm germplasm and compared the efficiency of SSRs and ISSRs in revealing the genetic diversity. Dendrograms based on ISSRs and SSRs grouped fifty date palm genotypes into seven and three clusters, respectively. Genotypes having similar genetic make-up were grouped together. Two genotypes Begum Jangi and Burhami had distinct genetic background and remained independent in the ISSRs based dendrogram. Population structure analysis revealed higher allelic admixture among fifty date palm genotypes collected from two different regions *i.e.,* Jhang and Bahawalpur. This allelic admixture among the genotypes of two regions is possibly due to exchange of germplasm. Among ISSRs, UBC-808 has the maximum *PIC* (0394) and *Dj* (0.722) values. While among SSRs, PDAAG-1010 has *PIC* and *Dj* values of 0.510 and 0.677, respectively. Comparison of two markers systems depicted that SSRs have high value (0.51) of expected heterozygosity of polymorphic loci (*Hep*). However, higher effective multiplex ratio (*E*) and markers index (*MI*) advocated the usability of ISSRs for diversity analysis. In conclusion, in the presence of high level of genetic similarity among collected germplasm, the use of markers indices can be helpful for the selection of particular markers system to reveal the genotypic differences.

Keywords: DNA fingerprinting; Germplasm dissemination; ISSRs; SSRs; Population structure analysis

Introduction

Genetic diversity in plants is important for breeding of elite genotypes and conservation of novel germplasm (Iqbal *et al.* 2018). Genetic diversity possibly occurs due to selection process, genetic drift, interaction of climatic conditions and geographical features (Malik *et al.* 2018). In date palm, genetic diversity is greatly influenced by selection process, clonal propagation and germplasm exchange. It is thought that genotypes are developed from continuous selection process by farmers on the basis of fruit traits (Haider *et al.* 2015). In date palm, identification of germplasm/ specific genotypes is a hectic job for farmers as well as researchers due to use of different names for the same genotype by the people of different geographical regions (Purayil *et al.* 2018). Specific language of a region is also a major cause of misnaming in date palm nomenclature. Secondly, seed and offshoot propagation are factors leading to the mixing of date palm germplasm within the country (Chaluvadi *et al.* 2014). Hence, plant researchers developed different molecular tools for accurate characterization of date palm germplasm.

Most consistent tools used for evaluation of genetic diversity are morphological, physical, biochemical and molecular markers (Ahmad and Anjum 2018). However, morphological, physical and biochemical markers are not much reliable for fingerprinting because these are highly influenced by environmental conditions and growth stages (Maina *et al.* 2019). Introduction of molecular markers brings a great revolution in phylogenetic relationships and evaluation of genetic variation (Hazzouri *et al.* 2015). Among molecular markers, SSRs and ISSRs are frequently used for evaluation of genetic diversity of date palm genotypes (Yusuf *et al.* 2015; Mirbahar *et al.* 2016). ISSRs have high genome abundance, dominant nature, high polymorphism, high reproducibility and less developmental cost. So, these are appropriate markers for DNA fingerprinting of date palm genotypes (Karim *et al.* 2010). SSRs have moderate genome abundance, co-dominant nature, crop specific, moderate developmental cost and very high reproducibility (Naeem *et al.* 2018). Cluster and structure analyses based on SSRs and ISSRs are effective tools used for evaluation of genetic relationship and genetic

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structure of huge set of genotypes (Ashraf *et al.* 2016).

Markers discriminating indices *i.e.,* polymorphic information content (*PIC*), confusion probability (*Cj*) and discriminating power (*Dj*) are reliable parameters and have been used for determination of markers potential in fingerprinting of pistachio genotypes (Belaskri *et al.* 2018). The highest *PIC* and *Dj* of molecular markers indicate that these have excellent potential to determine genetic diversity among the studied genotypes. However, the highest *Cj* of molecular markers exhibit that these markers have poor reliability for evaluation of genetic variation among the studied genotypes (Ahmad *et al.* 2019). Direct relationship exists between *PIC* and *Dj*, while these have inverse relation with *Cj* (Ahmad *et al.* 2019). Hence, selection of molecular markers could be fruitful for different genetic analyses based on these markers indices *i.e., PIC*, *Cj* and *Dj*.

In Pakistan, different research organizations/stations *i.e*., Date palm Research Sub-Station, Jhang, Horticultural Research Station, Bahawalpur, Date Palm Research Station, Khairpur and District Government Orchard, Layyah are working on selection and breeding of date palm genotypes (Markhand *et al.* 2010; Naqvi *et al.* 2015). Mostly, they are focusing on morphological markers for identification of date palm genotypes. In Pakistan, there are 325 date palm genotypes that need to be secured scientifically focusing on molecular aspects (Jamil *et al.* 2010; Haider *et al.* 2015). In the world, there is extensive use of molecular markers for different genetic analyses *i.e.,* DNA fingerprinting, phylogenetic studies, genotyping-by-sequencing, genome sequencing and re-sequencing and genome wide association (Gros-Balthazard *et al.* 2018). Hussein *et al.* (2004) used RAPDs and ISSRs (dominant markers system) for DNA fingerprinting of seven date palm genotypes collected from Egypt. Younis *et al*. (2008) used RAPDs and ISSRs for identification of male plants grown in Egypt region. Phylogenetic relationship was determined among date palm genotypes using RAPDs and ISSRs (Abdulla and Gamal 2010; Kumar *et al.* 2010). RAPDs and chloroplast ribosomal protein gene were used for determination of genetic similarity among Pakistani date palm genotypes (Akhtar *et al.* 2014; Mirbahar *et al.* 2014). In Pakistan, application of different molecular markers systems like dominant and co-dominant for different genetic analyses of date palm genotypes is very negligible. However, few researches were conducted on genetic similarity among date palm genotypes. Accurate information of genotypes is a basic need for better utilization of germplasm in the country. Knowledge of genetic variation, population structure and its linkage within or among the populations is important to better understand the available genetic inconsistency for further exploration in potential breeding programs. In this scenario, current study encourages the comparison of dominant (ISSRs) and co-dominant (SSRs) molecular markers for evaluation of genetic similarity among indigenous date palm genotypes.

Methods and Methods

Plant materials and DNA isolation

Fifty date palm genotypes were collected from two different research stations of Punjab, Pakistan (Table 1). Mature leaves were collected from selected date palm trees and stored at -80°C for DNA extraction. DNA was isolated according to CTAB method as described by Doyle (1987). Spectro nanophotometer (Implen Nano-photometer, Germany) was used to calculate concentration and purity of extracted DNA.

Amplification of ISSRs and SSRs

PCR reaction of 20 μL volume was performed using 30 ng/μL of genomic DNA as template, 10× PCR buffer and 1 unit of Taq DNA polymerase (Fermentas, USA). PCR reactions were carried out in a thermal cycler (MyCycler, BioRad, USA). Detailed description of ISSRs sequences and annealing temperatures are listed in Table 2. The SSRs sequences and annealing temperatures are given in Table 3 & 4. Amplified PCR products were visualized using 1% agarose gel after electrophoresis at 80 voltage for 3 h and photographed with gel documentation system (Photonyx, USA). The binary data were collected as presence of bands (1) and absence of bands (0) for each locus.

Genetic diversity analyses

Two separate dendrograms of SSRs and ISSRs were constructed under un-weighted pair group method of arithmetic means (UPGMA) with statistical software NTSYS-pc Version 2.10 (Rohlf 2002).

Population structure analyses

A statistical software "STRUCTURE program ver. 2.3.4." was used for evaluation of genetic structure and neighbor joining tree of fifty date palm genotypes. The appropriate K value was calculated through "Structure Harvester" as described (Earl 2012). The number of sub-populations (ΔK) was calculated through ad-hoc statistic method (Evanno *et al.* 2005). K value graph was developed through "Microsoft Excel program, 2016".

Markers discriminating catalog

Polymorphic information content (*PIC*), confusion probability (*Cj)*, discriminating power (*Dj)* of each primer pair were calculated as described earlier (Ahmad *et al.* 2019).

Comparison of ISSRs and SSRs markers systems

Comparison between two markers systems ISSRs and

Table 1: Date palm genotypes collected from different research stations of Punjab, Pakistan

Genotype name	Collection site	Latitude	Longitude	Elevation
Akhrot	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Dhakki	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Aseel	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Hilawi-1	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Hilawi-2	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Kantar	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Makran	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Chohara	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Zahidi	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Burhami	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Neelum	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Zarin	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Haleeni	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Jaman	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Kohraba	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Koznabad	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Karbalaen	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Jan Sahr	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Gokhna	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Danda	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Begum Jangi	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Deglet Noor	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Peela Dhora	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Shamran-1	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Shamran-2	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Rachna	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Seib	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Zardo	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Shado	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Peeli Sundar	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Khudrawi-1	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Khudrawi-2	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Wahn Wali	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Angoor	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Champa Kali	Date palm Research Sub-Station, Jhang	31, 15.557	72, 19.960	492
Baidhar	Horticultural Research Station, Bahawalpur	29, 22.796	71, 38.787	335
Dedhi	Horticultural Research Station, Bahawalpur	29, 22.796	71, 38.787	335
Sundari	Horticultural Research Station, Bahawalpur	29, 22.796	71, 38.787	335
Kupra	Horticultural Research Station, Bahawalpur	29, 22.796	71, 38.787	335
Shakri	Horticultural Research Station, Bahawalpur	29, 22.796	71, 38.787	335
Eedel Shah	Horticultural Research Station, Bahawalpur	29, 22.796	71, 38.787	335
Pathri	Horticultural Research Station, Bahawalpur	29, 22.796	71, 38.787	335
Kur	Horticultural Research Station, Bahawalpur	29, 22.796	71, 38.787	335
Tarmali	Horticultural Research Station, Bahawalpur	29, 22.796	71, 38.787	335
Fasli	Horticultural Research Station, Bahawalpur	29, 22.796	71, 38.787	335
Sufaida	Horticultural Research Station, Bahawalpur	29, 22.796	71, 38.787	335
Hamin Wali	Horticultural Research Station, Bahawalpur	29, 22.796	71, 38.787	335
Gajar	Horticultural Research Station, Bahawalpur	29, 22.796	71, 38.787	335
Halmain	Horticultural Research Station, Bahawalpur	29, 22.796	71, 38.787	335
Makhi	Horticultural Research Station, Bahawalpur	29, 22.796	71, 38.787	335

Naqvi *et al*. (2015)

SSRs was conducted by calculating different indices (Maras *et al.* 2008).

Results

Cluster analysis and similarity matrix

Dendrograms were generated on the basis of these two markers systems for fingerprinting of date pam genotypes. This ISSRs based dendrogram was truncated at similarity coefficient 0.75 and grouped fifty date palm genotypes into seven main clusters (cluster A–G). Cluster G was sub-divided into two sub-clusters *i.e.*, G_1 & G_2 (Fig. 1). Two genotypes Begum Jangi and Burhami of Jhang region remained independent and did not group with any other genotypes. Cluster G comprised of twenty-six genotypes, being the largest as compared to other clusters (Fig. 1). Cluster G is admixtures of genotypes collected from Bahawalpur and Jhang regions. Genotype Halmain shared (93%) genetic similarity with genotype Makhi which is the highest than among other genotypes. These two genotypes were collected from same region Bahawalpur. Sub cluster G_1 exhibited the highest genetic similarity between Zardo and Shado (91%) collected from Jhang region. The greater

Table 2: Markers sequences and annealing temperatures of ISSRs

Marker name	Marker sequence (5′ -3′)	Annealing temperature (°C)
UBC-808	AGAGAGAGAGAGAGA GC	52
UBC-809	AGAGAGAGAGAG AGA GG	52
UBC-810	GAGAGAGAGAGAGAG AT	52
UBC-811	GAGAGAGAGAGAGAG AC	52
UBC-812	GAGAGAGAGAGAGAGAA	52
UBC-813	CTCTCTCTCTCTCTCTT	52
UBC-814	CTCTCTCTCTCTCTCTA	52
UBC-815	CTCTCTCTCTCTCTCTG	52
UBC-816	CACACACACACACACAT	52
UBC-817	CACACACACACACACAA	52
UBC-818	CACACACACACACACAG	52
UBC-819	GTGTGTGTGTGTGTGTA	54
UBC-820	GTGTGTGTGTGTGTGTC	54
UBC-822	TCTCTCTCTCTCTCTCA	52
UBC-823	TCTCTCTCTCTCTCTCC	50
UBC-825	ACACACACACACACACT	52
UBC-826	ACACACACACACACACC	52
UBC-827	ACACACACACACACACG	48
UBC-828	TGTGTGTGTGTGTGTGA	52
UBC-829	TGTGTGTGTGTGTGTGC	52
UBC-834	AGAGAGAGAGAGAGAGYT	54
UBC-836	AGA GAG AGA GAG AGA GYA	52
UBC-840	ACAATGGCTACCACCAGC	52
UBC-841	GAGAGAGAGAGAGAGACTC	52
UBC-842	ACAATGGCTACCACTACC	48
UBC-845	CTCTCTCTCTCTCTCTRG	50
UBC-846	CACACACACACACACART	50
UBC-847	CACACACACACACACARC	52
UBC-848	CAACAATGGCTACCACCG	52
UBC-850	GTGTGTGTGTGTGTGTYC	52

UBC = University of British Colombia

genetic similarity existed in Kupra and Shakri (91%) in sub cluster G_2 collected from Bahawalpur region. Cluster F comprised of five genotypes *i.e.*, Dhakki, Makran, Aseel, Hilawi-1 and Kantar. The highest genetic similarity was found between Hilawi-1 and Kantar (88%) as compared to other genotypes of cluster F. Cluster E contained only two genotypes Chohara and Zahidi having same origin of collection as Jhang region. Four genotypes *i.e.*, Neelum, Zarin, Haleeni and Koznabad were grouped into cluster D. Jaman, Jan Sahr, Gokhna and Danda were clustered into cluster C. Cluster B comprised of five genotypes Deglet Noor, Peela Dhora, Shamran-1, Shamran-2 and Rachna. Kohraba and Karbalaen were grouped into cluster A. Cluster A, B, C, D, E and F genotypes were collected from Jhang region. However, cluster G showed the mixing of genotypes collected from two different regions *i.e.* Jhang and Bahawalpur.

Cluster analysis based on SSRs grouped fifty date palm genotypes into three major clusters (cluster A–C) truncated at similarity coefficient 0.95 (95%) (Fig. 2). Five genotypes from Jhang region showed the highest genetic similarity with one genotype Dedhi from Bahawalpur region. Therefore, these genotypes grouped together in cluster A. Genotype Koznabad from Jhang region shared 96% genetic similarity with genotype Dedhi from Bahawalpur region. Cluster B comprised of 17 mixed genotypes *i.e.*, Makran, Kupra, Shakri, Eedel Shah, Sufaida, Burhami, Neelum, Jaman, Kohraba, Karbalaen, Shamran-1, Shamran-2, Rachna, Seib, Zardo, Sundari and Halmain of Jhang and Bahawalpur regions. Four genotypes *i.e.*, Kupra, Shakri, Eedel Shah and Sufaida were collected from Bahawalpur region among 17 genotypes of cluster B. Cluster C contained 21 mixed genotypes *i.e.*, Akhrot, Dhakki, Aseel, Hilawi-1, Kantar, Chohara, Zahidi, Zarin, Danda, Deglet Noor, Peela Dhora, Peeli Sundar, Hilawi-2, Pathri, Kur, Tarmali, Fasli, Hamin Wali, Gajar, Makhi and Haleeni of Bahawalpur and Jhang regions. Pathri, Kur, Tarmali, Fasli, Hamin Wali, Gajar and Makhi genotypes from Bahawalpur region exhibited genetic similarity with Jhang region genotypes as in cluster C (Fig. 2).

Population structure analysis

ISSRs and SSRs results were used to perform population structure analysis for fifty date palm genotypes under an admixed Bayesian model. Bar plot, best K value and neighbor joining tree were developed using results of ISSRs and ISSRs to determine the sub-population of fifty genotypes collected from two different regions (Fig. 3A–C and Fig. 4A–C). Population structure analysis using SSRs results exhibited that the Logarithm of the Data likelihood [Ln (PD)] on average continued to increase with increasing the numbers of assumed sub-populations (K) from 2 to 10. The adhoc quantity based on the second order rate of change in the log probability (ΔK) exhibited a clear peak at $K = 3$. So, Ln (PD) suggested that a K value of three was the most

Table 3: SSRs sequences for evaluation of genetic diversity in date palm germplasm

Marker name	Marker sequence (5′ -3′)	Reference
PDAAG 1001-Forward	TGCCGAGTGGTTTAATTGTG	Arabnezhad *et al.* (2012)
PDAAG 1001-Reverse	TGAAGCAGAGAATCCAACAGAG	Arabnezhad *et al.* (2012)
PDAAG 1002-Forward	GGACATAGTTTTGGCTGGCTAC	Arabnezhad *et al.* (2012)
PDAAG 1002-Reverse	ACCAGTTTACCACTTGCTCCA	Arabnezhad *et al.* (2012)
PDAAG 1003-Forward	GACTGGGAATATAAAGCGATGTC	Arabnezhad *et al.* (2012)
PDAAG 1003-Reverse	CCATCTCCCCTAACTCTCCTC	Arabnezhad *et al.* (2012)
PDAAG 1005-Forward	GTATGTTCCATGCCGTTCTAC	Arabnezhad *et al.* (2012)
PDAAG 1005-Reverse	AGCCACATCACTTGGTTCA	Arabnezhad *et al.* (2012)
PDAAG 1008-Forward	GATGCTGAACTCGGACAAAG	Arabnezhad *et al.* (2012)
PDAAG 1008-Reverse	TGGGTAGAGATGGTTGGTTG	Arabnezhad *et al.* (2012)
PDAAG 1010-Forward	TGAAGCAGTGAGTTCCATTG	Arabnezhad *et al.* (2012)
PDAAG 1010-Reverse	GATGTGCTTTGTGCCATTC	Arabnezhad *et al.* (2012)
PDAAG 1011-Forward	TCGATCGCTCCTCCTACAGT	Arabnezhad *et al.* (2012)
PDAAG 1011-Reverse	GTCACGCCTTTCATTCCTTC	Arabnezhad *et al.* (2012)
PDAAG 1013-Forward	CCAAAACTCTGTTTTCTCTTTGG	Arabnezhad *et al.* (2012)
PDAAG 1013-Reverse	CCTGCATGAACTGAACTAGCC	Arabnezhad *et al.* (2012)
PDAAG 1014-Forward	TCGTGCATTTAGAACGTTGA	Arabnezhad *et al.* (2012)
PDAAG 1014-Reverse	GAGCACGACTTACGAGTTC	Arabnezhad *et al.* (2012)
PDAAG 1015-Forward	CTTGGTCGCTGCTTAAAATG	Arabnezhad *et al.* (2012)
PDAAG 1015-Reverse	TGGGAACAGGAGACCATCA	Arabnezhad *et al.* (2012)
PDAAG 1016-Forward	TCTCAAGCCTCTCAGGTTGC	Arabnezhad *et al.* (2012)
PDAAG 1016-Reverse	CCTAGTCGATGCTGTTGTTCC	Arabnezhad *et al.* (2012)
PDAAG 1017-Forward	GCTGCGAGGAGAGATTTCAT	Arabnezhad *et al.* (2012)
PDAAG 1017-Reverse	GGGAAAAATCTAAATGAACAGGTG	Arabnezhad *et al.* (2012)
PDAAG 1018-Forward	TGTCTGCTGCCATTCTGTTC	Arabnezhad *et al.* (2012)
PDAAG 1018-Reverse	CTGACCATGGACCACCTACC	Arabnezhad *et al.* (2012)
PDAAG 1019-Forward	ATTTCTTTCCCCCACGTTTC	Arabnezhad *et al.* (2012)
PDAAG 1019-Reverse	CCAGGTGACACTGCATTCC	Arabnezhad *et al.* (2012)
PDAAG 1020-Forward	CGCTCATAAATTAGGGCATTG	Arabnezhad *et al.* (2012)
PDAAG 1020-Reverse	CCCTAGGTGATGAAGGACCAC	Arabnezhad *et al.* (2012)
PDAAG 1021-Forward	GGAGAGAAACGGAACAAGAAG	Arabnezhad *et al.* (2012)
PDAAG 1021-Reverse	AGCGTCCAAGAACAAGGTATG	Arabnezhad *et al.* (2012)
PDAAG 1022-Forward	TTCGGAGAATTGGATCCTTG	Arabnezhad *et al.* (2012)
PDAAG 1022-Reverse	GTTTGGTCGGCTGAGATGTG	Arabnezhad *et al.* (2012)
PDAAG 1023-Forward	AGACGCTCACCTTGGAACTT	Arabnezhad *et al.* (2012)
PDAAG 1023-Reverse	ACCCCGCTCATGAATTAGG	Arabnezhad *et al.* (2012)
PDAAG 1024-Forward	CTTCTCCACTGGCATCTTCC	Arabnezhad *et al.* (2012)
PDAAG 1024-Reverse	CACCCGTTGGGCATCTTA	Arabnezhad *et al.* (2012)
PDAAG 1025-Forward	ATCCCGTCCTCTCTTTCCA	Arabnezhad *et al.* (2012)
PDAAG 1025-Reverse	CATGCATACATATACGCAAAGAA	Arabnezhad *et al.* (2012)
KSU-PDL 2-Forward	TTGGAGTAGGAGACGACAATA	Al-Faifi *et al.* (2016)
KSU-PDL 2-Reverse	GGGAGTGAGAGGGATATGTAG	Al-Faifi *et al.* (2016)
KSU-PDL 4-Forward	CAACATAAGGAAAAATGATGC	Al-Faifi *et al.* (2016)
KSU-PDL 4-Reverse	TGCATCACTCTGGGTATAAAT	Al-Faifi *et al.* (2016)
KSU-PDL 6-Forward	GCTTTTGCAAATAACAACATC	Al-Faifi *et al.* (2016)
KSU-PDL 6-Reverse	CATGGAAAAGGCTCCTATC	Al-Faifi *et al.* (2016)
KSU-PDL 18-Forward	TGTGGTCTATCCATTTTGTGT	Al-Faifi *et al.* (2016)
KSU-PDL 18-Reverse	GTCATGCAGTTCTCAAAGAAA	Al-Faifi *et al.* (2016)
KSU-PDL 21-Forward	GCTACTCCTTCTTCTTCTCCTT	Al-Faifi *et al.* (2016)
KSU-PDL 21-Reverse	TGATGATTGGTTGAGATTAAGA	Al-Faifi *et al.* (2016)
KSU-PDL 29-Forward	AGCACATGGCAGTTACTCTAC	Al-Faifi *et al.* (2016)
KSU-PDL 29-Reverse	AACAACAACAATCAGTCCAAA	Al-Faifi *et al.* (2016)
KSU-PDL 42-Forward	GACCGTACAGTCACATGATTT	Al-Faifi *et al.* (2016)
KSU-PDL 42-Reverse	TAGGAGAGAGAGAGGGTTTTG	Al-Faifi *et al.* (2016)
KSU-PDL 58-Forward	GAGAAGAGAAAGGGAGAGAGA	Al-Faifi *et al.* (2016)
KSU-PDL 58-Reverse	GCCCTTCTTAATCAACAAAAT	Al-Faifi *et al.* (2016)
KSU-PDL 64-Forward	ACTCTTGTGGGACTCCTTTAC	Al-Faifi *et al.* (2016)
KSU-PDL 64-Reverse	CCTAAATGTGCTTTCCTTCTT	Al-Faifi *et al.* (2016)
KSU-PDL 76-Forward	TTGGAGTAGGAGACGACAATA	Al-Faifi *et al.* (2016)
KSU-PDL 76-Reverse	AGAGAGAGATGGGGAAGAAG	Al-Faifi *et al.* (2016)

probable prediction for the number of sub-populations for both ISSRs and SSRs (Fig. 3A and Fig. 4A). ISSRs based structure analysis depicted that bar plot has been configured into three different colors i.e. red, blue and green (Fig. 3C). The highest contribution was recorded from red color. So, similar depiction was found in neighbor joining tree (Fig. 3B). Structure analysis on the basis of SSRs exhibited that bar plot has been separated into three different colors i.e.

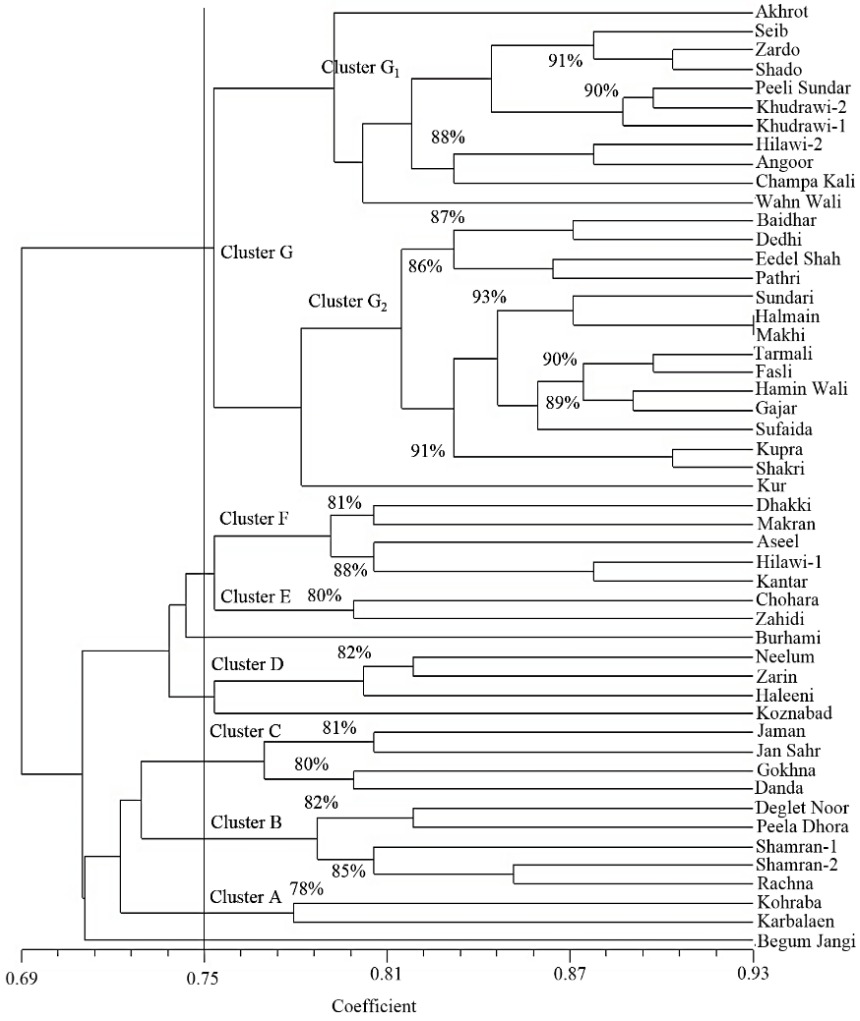

Fig. 1: Dendrogram showing genetic relationship among fifty date palm genotypes based on ISSR markers

red, blue and green (Fig. 4C). The highest contribution was recorded from green color. So, similar depiction was found in neighbor joining tree (Fig. 4B).

Markers discriminating catalog

A total of 30 SSRs and 30 ISSRs were used for fingerprinting in collected date palm genotypes. From 30 ISSRs, two ISSRs (UBC-811 and UBC-840) were monomorphic and the other 28 were polymorphic and polymorphism was shown (Fig. 5). From 30 SSRs, only primer PDAAG-1010 was polymorphic, 21 were monomorphic and eight were non-amplified (Table 4). The range of allele size for ISSRs varied from 260 to 1600 bps. The highest *PIC* (0.394) and *Dj* (0.722) was obtained through UBC-808, while the lowest *PIC* (0.113) and *Dj* (0.559) was obtained through UBC-817 as compared to all other primers. Moreover, the highest *Cj* (0.882) was calculated in UBC-817, while the lowest *Cj* (0.598) as compared to all other ISSRs primers (Table 5). *PIC*, *Dj* and *Cj* for PDAAG-1010 are listed in Table 5.

Comparison of ISSRs and SSR markers systems

ISSRs showed the highest number of assay unit (30) than SSRs (22). The maximum number of polymorphic bands (141) and number of polymorphic bands/ assay (4.7) were revealed from ISSRs; while the minimum polymorphic bands (4.00) and number of polymorphic bands/ assay (0.13) were revealed from SSRs. Number of monomorphic bands were lower in ISSRs (12) than SSRs (22). Greater number of loci (153), number of loci/ assay unit (51), effective multiplex ration (4.7) and markers index (1.32) were revealed by ISSRs as compared to SSRs. Expected heterozygosity was greater for SSRs (0.51) than ISSRs (0.28) as listed in Table 6.

Discussion

The addition of new genotypes in the gene pool can cause

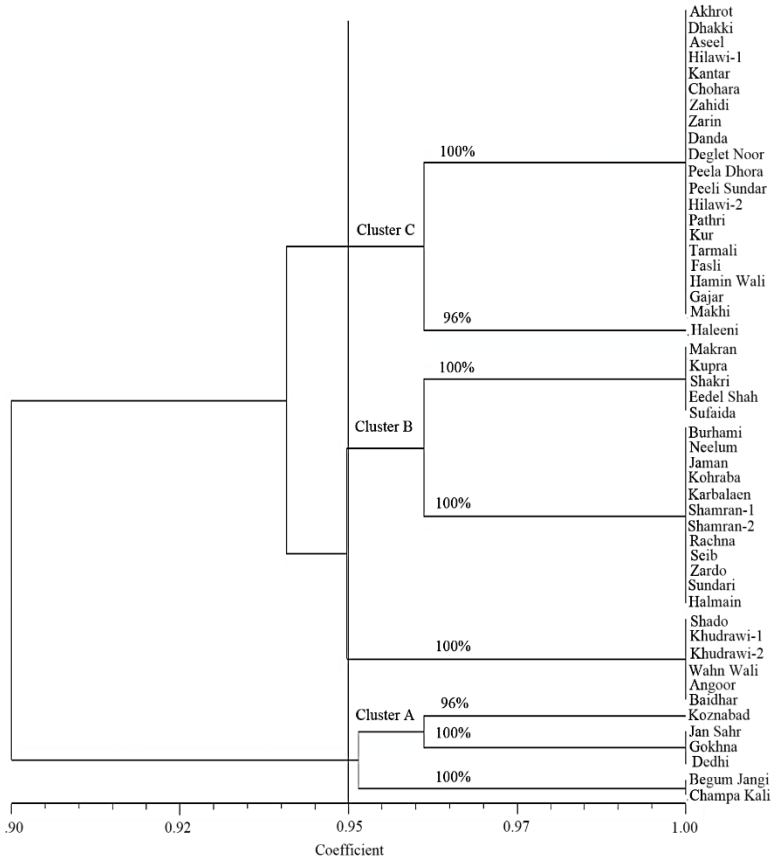

Fig. 2: Dendrogram showing genetic relationship among fifty date palm genotypes based on SSR markers

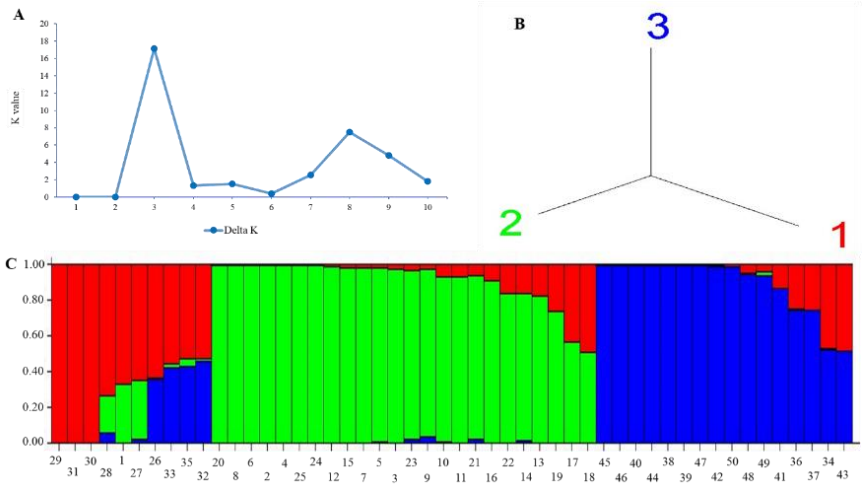

Fig. 3: Population structure analysis showing genetic relationship among fifty date palm genotypes based on ISSR markers; A = best K value graph, B = neighbor joinng tree and C = bar plot

complication to distinguish the difference among germplasm only using morphological and biochemical markers. Morphological characteristics, biochemical properties and pedigree information are traditional ways of germplasm identification. These identification resources are greatly influenced through environmental fluctuations, cultural practices, nutritional aspects and numerous other management practices (Teng *et al.* 2002; Anjum *et al.* 2018). In addition, farmers name their genotypes on the basis of genotypes location, fruit color, taste and shape since

Table 4: Amplification of SSRs for evaluation of genetic diversity in date palm genotypes

Marker name	Annealing temperature (°C)	Range of allele size	Amplification of SSRs
PDAG 1001	54	800	Monomorphic
PDAG 1002	52	80	Monomorphic
PDAG 1003	55	250	Monomorphic
PDAG 1005	54	300	Monomorphic
PDAG 1008	56	280	Monomorphic
PDAG 1010	54	200-250	Polymorphic
PDAG 1011	58	-	Non-amplified
PDAG 1013	55	250	Monomorphic
PDAG 1014	52	200	Monomorphic
PDAG 1015	55	150	Monomorphic
PDAG 1016	55	400	Monomorphic
PDAG 1017	54	-	Non-amplified
PDAG 1018	52	60	Monomorphic
PDAG 1019	55	200	Monomorphic
PDAG 1020	56	150	Monomorphic
PDAG 1021	54	170	Monomorphic
PDAG 1022	58	200	Monomorphic
PDAG 1023	54	-	Non-amplified
PDAG 1024	56	-	Non-amplified
PDAG 1025	56	230	Monomorphic
KSU-PDL 2	50	-	Non-amplified
KSU-PDL 4	54	150	Monomorphic
KSU-PDL 6	54	100	Monomorphic
KSU-PDL 18	54	70	Monomorphic
KSU-PDL 21	54	-	Non-amplified
KSU-PDL 29	52	400	Monomorphic
KSU-PDL 42	53	0	Non-amplified
KSU-PDL 58	50	0	Non-amplified
KSU-PDL 64	54	150	Monomorphic
KSU-PDL 76	52	150	Monomorphic

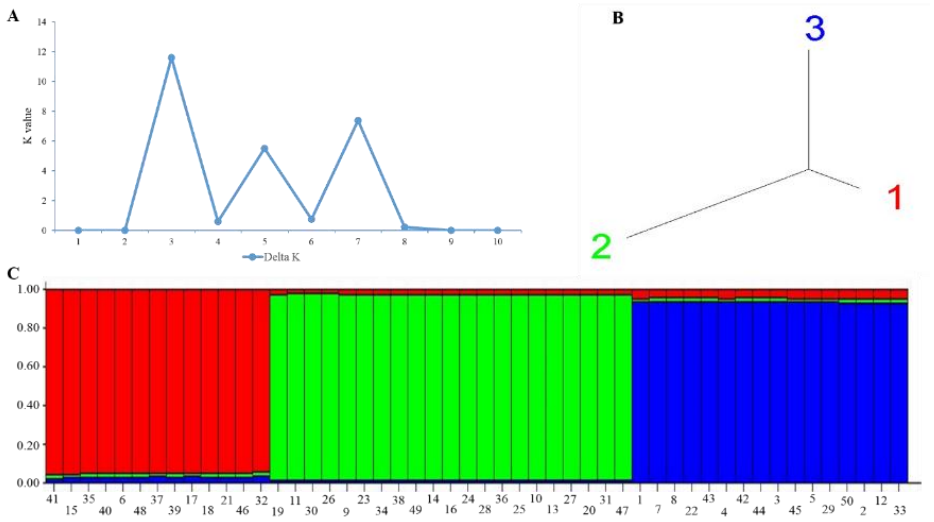

Fig. 4: Structure analysis showing genetic relationship among fifty date palm genotypes based on SSR markers; **A** = best K value graph; **B** = neighbor joinng tree and **C** = bar plot

the ancient time (Sharif *et al.* 2019). Hence, misnaming of genotypes is a big problem in classification of date palm genotypes. Therefore, present study encourages the use of different molecular markers for identification and authentication of date palm genotypes, as genetic make-up of genotypes is not influenced due to climatic conditions and external impact (Ahmad *et al.* 2019). Among molecular markers, SSRs and ISSRs are reliable for DNA fingerprinting. The current study successfully evaluated the genetic diversity/ fingerprinting and population structure of fifty date palm genotypes and tried to resolve the misnaming of genotypes in nomenclature.

ISSRs and SSRs based dendrograms exhibited variation in total number of main clusters, sub clusters and location of genotypes within clusters. Hence, current differences might be due to different markers behavior

Table 5: Markers discriminating indices of ISSRs and SSRS

Marker name	Range of allele size (bp)	Number of loci	Polymorphic bands	*PIC*	*Cj*	*Dj*
UBC-808	300 - 1050	7	7	0.394	0.598	0.722
UBC-809	400 - 650	4	3	0.329	0.664	0.668
UBC-810	350 - 1200	8	8	0.329	0.664	0.696
UBC-812	300 - 1100	4	4	0.384	0.609	0.697
UBC-813	500 - 1000	4	4	0.387	0.606	0.607
UBC-814	550 - 900	2	2	0.210	0.786	0.604
UBC-815	300 - 1500	7	7	0.168	0.829	0.586
UBC-816	700 - 1450	6	6	0.203	0.792	0.683
UBC-817	1100 - 1150	2	1	0.113	0.882	0.559
UBC-818	370 - 1200	10	9	0.359	0.634	0.610
UBC-819	800 - 1500	3	2	0.228	0.767	0.606
UBC-820	400 - 1000	8	8	0.215	0.781	0.574
UBC-822	550 - 750	3	2	0.221	0.775	0.605
UBC-823	550 - 770	4	3	0.145	0.852	0.570
UBC-825	300 - 1200	5	5	0.265	0.730	0.656
UBC-826	770 - 1350	6	6	0.353	0.640	0.646
UBC-827	400 - 1600	8	7	0.371	0.621	0.675
UBC-828	450 - 1250	6	6	0.314	0.649	0.666
UBC-829	550 - 1300	5	5	0.325	0.669	0.655
UBC-834	450 - 1200	5	5	0.304	0.690	0.649
UBC-836	300 - 900	4	4	0.292	0.702	0.675
UBC-841	450 - 1100	6	5	0.321	0.672	0.664
UBC-842	450 - 1400	3	2	0.319	0.775	0.613
UBC-845	350 - 1000	9	9	0.306	0.688	0.624
UBC-846	260 - 800	4	3	0.243	0.752	0.624
UBC-847	600 - 1500	6	5	0.372	0.620	0.624
UBC-848	300 - 700	6	5	0.205	0.791	0.624
UBC-850	400 - 1100	4	3	0.208	0.788	0.624
PDAAG-1010	200-250	4	4	0.510	0.746	0.677

PIC= Polymorphic information contents, *Cj* = Confusion probability, *Dj*= Discriminating power, bp= Base pair

Table 6: Indices for the comparison of ISSRs and SSRs

Indices	Abbreviations	Markers system	
		ISSRs	SSRs
Number of assay unit	*U*	30.00	22.00
Number of polymorphic bands	n_p	141.00	4.00
Number of monomorphic bands	n_{np}	12.00	22.00
Number of polymorphic bands/ assay	n_p/U	4.70	0.13
Number of loci	*L*	153.00	26.00
Number of loci/ assay unit	N_u	5.10	1.18
Expected heterozygosity of polymorphic loci	H_{ep}	0.28	0.51
Fraction of polymorphic bands	β	0.92	0.15
Effective multiplex ratio	*E*	4.70	0.18
Markers index	*MI*	1.32	0.09

because different markers identify different distinctive regions of DNA variation within the genome (Ashraf *et al.* 2016). Regarding the ISSRs, cluster analysis and similarity matrix determined the highest genetic similarity between Halmain and Makhi (93%) than all other genotypes. Halmain and Makhi, Zardo and Shado, Peeli Sundar and Khudrawi-2, Tarmali and Fasli, and Kupra and Shakri genotypes were close to each other showing similar genetic make-up. Similarly, the highest genetic similarity through ISSRs was recoded in previous findings (Karim *et al.* 2010; Mirbahar *et al.* 2016). Cluster G is admixture of genotypes of two different regions which is due to germplasm exchange, ecological differences and distinctive adoptive behavior of genotypes (Hamza *et al.* 2012; Naeem *et al.* 2018). Cluster analysis of ISSRs revealed that two genotypes Begum Jangi and Burhami remain independent and did not cluster with any other genotypes in the current study. These two genotypes are highly divergent due to different and unique genetic background. The highest polymorphism and genetic diversity was found in these two genotypes. The greater genetic variation in these genotypes revealed that these were diverse clones and introduced long years ago as a cultivar (Ahmad *et al.* 2019). Regarding the SSRs, cluster analysis and similarity matrix revealed the highest genetic similarity among the genotypes of Jhang and Bahawalpur regions. All clusters (A, B and C) showed the mixture of genotypes of two different locations. So, this similarity among these genotypes was due to exchange of germplasm, different adaptive conditions of environment (Elshibli and Korpelainen 2008). Moreover, the highest genetic similarity has already been reported among date palm genotypes collected from different geographical

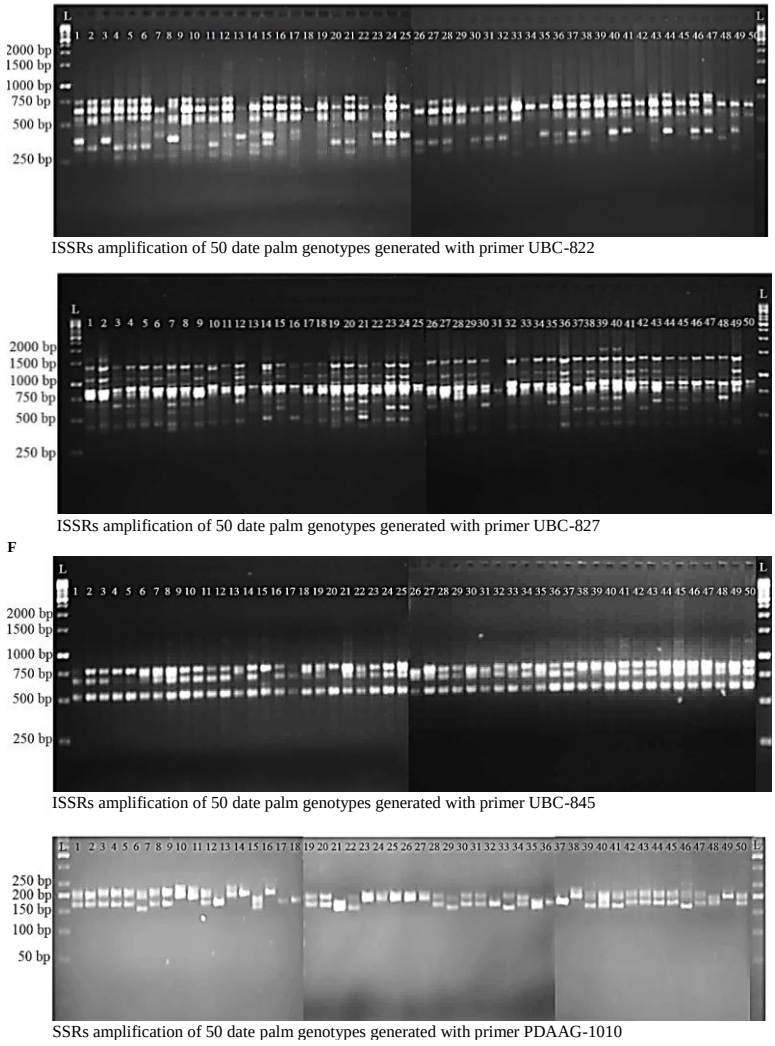

Fig. 5: ISSR and SSRs amplification of 50 date palm genotypes

regions (Elmeer *et al.* 2011; Azouzi *et al.* 2015). Current study is under conformity of earlier work because they examined that cluster analysis significantly discriminated the genotypes of different countries *i.e.,* North African and Middle Eastern through SSRs (Arabnezhad *et al.* 2012).

Genetic divergence, allelic admixture and evolutionary relationship can be evaluated through population structure analysis developed from different molecular markers (Naeem *et al.* 2018). Population structure analysis of ISSRs showed the existence of three main groups *i.e.,* red, blue and green in the studied population. Red color group had the highest allelic admixture as compared to other two groups. Bar plot and neighbor joining tree indicated the presence of three main groups *i.e.,* red, blue and green in the studied population. Green color group shared the maximum allelic admixture than other two groups. Structure analyses proved complex genetic structures and strong relationship within some genotypes present in the studied genotypes. Allelic admixture is because of local adaptation of foreign genotypes. The introduction of exotic germplasm within the country is very common (Naeem *et al.* 2018). Allelic mixtures resulting in the introduction of new genetic linkages into a population increase heterozygosity (Azouzi *et al.* 2015). The results of structure analysis confirmed the results of genotype clustering on the basis of similarity matrix. Recently, Chaluvadi *et al.* (2014) evaluated allelic admixture and close affinity among date palm genotypes using structure analysis.

Different markers indices *i.e.*, *PIC*, *Cj* and *Dj* are suitable tools for determination of efficiency of a molecular marker. All these indices vary and depend on application nature of molecular markers (Naeem *et al.* 2018). The highest polymorphism was recorded in ISSRs due to dominant nature and higher number of loci as compared to SSRs (Hamza *et al.* 2013). Application of primers for ISSRs and SSRs was same; however, 28 ISSRs and only one SSR showed polymorphism. So, SSRs give less polymorphism because of its conserved nature and continuous selection of

genotypes. ISSRs revealed higher level of genetic diversity in date palm genotypes than SSRs. Previous studies confirmed that ISSRs revealed the highest polymorphism due to many loci which is effective for evaluation of genetic diversity in date palm genotypes (Karim *et al.* 2010; Ashraf *et al.* 2016). Concerning the ISSRs, UBC-808 had the highest *PIC* and *Dj*, while lower *Cj* among all the studied primers. Therefore, UBC-808 had excellent potential for discrimination among studied germplasm. UBC-817 had poor potential to evaluate genetic diversity among the studied genotypes because of higher *Cj* and lower *PIC* and *Dj v*alues. *PIC* and *Dj* are directly proportional to each other, while inversely proportional with *Cj*. Previous finding confirmed that excellent primer for allelic variation is that which had higher *PIC* and *Dj* and lower *Cj* (Naeem *et al.* 2018; Ahmad *et al.* 2019).

Comparison of two markers systems on the basis of discriminating efficiency revealed that expected heterozygosity of SSRs was higher than ISSRs markers system, indicating higher allelic variability among date palm genotypes (Belaj *et al.* 2003). The highest markers index and effective multiplex ratio showed the distinctive nature of ISSRs markers system (Ashraf *et al.* 2016).

Conclusion

The studied date palm germplasm has very high genetic similarity. The population structure analysis indicated the complex genetic structures of date palm genotypes with high level of allelic admixture. Therefore, selection of suitable markers and markers system is imperative for characterization of germplasm. Selection of a molecular marker or set of markers with in a markers system by considering *PIC*, *Dj* and *Cj* values could yield encouraging results for genotypic characterization. While comparing the two markers systems *i.e.* ISSRs and SSRs regarding their efficiency to reveal the difference among date palm genotypes, ISSRs could be more suitable markers due to higher value of effective multiplex ration (*E*) and markers index (*MI*).

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