Genomic variation and genetic structure profile of Iraqi barley accessions using ISSR and arbitrary functional gene-based molecular markers

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Research Article

Abstract

Few attempts have been made in Iraq to investigate the genetic variation in barley accessions using molecular markers. In this context, the objective of this study was to investigate the diversity of 59 accessions of barley using inter simple sequence repeat (ISSR), conserved DNA-derived polymorphism (CDDP), and start codon targeted (SCoT) markers. A total of 391 amplified polymorphic bands were generated using 45 ISSR, 9 CDDP, and 12 SCoT primers that produced 255, 35, and 101 polymorphic bands, respectively. The average values of gene diversity were 0.77, 0.67, and 0.81 for ISSR, CDDP, and SCoT markers, respectively. The mean values of polymorphism information content for ISSR, CDDP, and SCoT markers were 0.74, 0.63, and 0.80 respectively. The discrimination power of the three approaches for assessing allelic diversity in barley accessions was as follows: SCoT > ISSR > CDDP. The barley accessions were classified and clustered into two main groups. Molecular variance analysis revealed 15, 9, and 14% variability among populations with ISSR, CDDP, and SCoT markers, respectively. The mantel test results revealed that the three molecular marker matrices had significant positive relationships. The SCoT markers have the potential to be useful tools for selecting appropriate parents for breeding program.

Introduction

World Food Security announced serious concern about the growing world population. According to recent estimates, the world population will reach 9.8 billion or more, and meeting the needs of this estimate of the global population will likely require a doubling in food production. It is critical to improve the productivity of not only food crops, but also crops used for livestock fodder to meet the food demands of the near future (Qaim 2020). For this purpose, in different plant species, inclusive genome and population structure explorations of genetic diversity have received considerable attention. To adapt to different environmental conditions, including fluctuation in climate conditions, the genetic diversity in a given species allows plants to overcome these obstacles in an orderly manner. The genetic variation in a population of a plant species, containing landraces, cultivars, as well as wild individuals, is an essential resource for sustainable agricultural practices and increasing food production (Latutrie et al. 2019). For that reason, to discover useful genes in crops, the assessment of genetic diversities in the available genetic resources is the main step in conducting the plant research project.

Barley (*Hordeum vulgare L.*) is considered the oldest and most important cereal crop cultivated by humans, and is the fourth largest cereal plant after maize, wheat, and rice worldwide. In general, the genome of barley is a diploid that contains 14 chromosomes. This crop requires low cultivation inputs, including water demand, fertilizer, and toleration of abiotic stress (Baum et al. 2015). From this point, the distribution and cultivation of barley are largely perceived in regions of arid and semi-arid rainfall. It is used as human food as the feed of livestock as malt industries. During the evolution from growing landraces to cultivation, barley is the suitable model crop to study phenotypic differences and genome diversity (Brantestam et al. 2007). Many researchers have established significant contributions through traditional techniques to barley improvement in a breeding program that depends on phenotypic traits, and many researchers apply it (Hadado et al. 2009; Hagenblad et al. 2019). However, complex traits like the quality of seeds, yield, and abiotic stress tolerance are not easy tasks. For detecting variation in barley plants, these conventional methods are unfavorable, and these traits only represent a minor portion of the plant genome, and besides the environmental factors highly affect it (Govindaraj et al. 2015; Mzid et al. 2016).

One of the main tasks of breeding programs is the evaluation of genetic variation in the present collection of germplasm, as it leads to the selection of varieties and lines with better performance and higher diversity under particular conditions. Techniques of DNA markers, in particular those with polymerase chain reaction, have been established, and they are currently available, especially in studies on genetic diversity and genetic relationships between different plant species, and it provides valuable information (Agarwal et al. 2008; Lateef 2015).

Up to date, different molecular markers [restriction fragment length polymorphism (RFLP), random Amplified Polymorphic DNA (RAPD), simple sequence repeat (SSR), inter simple sequence repeat (ISSR), amplified fragment length polymorphisms (AFLP), and single nucleotide polymorphisms (SNPs)] have been used and widely applied to investigate genetic diversity and characterize germplasm (Brbaklić et al. 2021; Tahir 2014; Tahir and Omer 2017). The main drawbacks of these techniques are

the high cost of AFLP, as polyacrylamide gels are required for detecting polymorphism and need technical skill (Meudt and Clarke 2007). In the RAPD marker, no information on heterozygosity can be provided (Jiang 2013). For detecting polymorphism in the genome by using the SSR technique, it is essential to identify the flanking sequences and develop specific primers (Kaur et al. 2015). In addition, SNPs markers seem exciting, but it could be expensive by researchers with limited resources, as it is in most developing countries, and require specific equipment, like next-generation sequencing.

Among different types of molecular markers available for barley, ISSR markers have proven suitable markers inbreeding and genetic diversity studies due to high polymorphism and repeatability across the whole genome. This type of marker-based on polumerase chain reaction (PCR) amplification of repeated DNA nucleotides to target multiple locations in the genome. For their use, no previous genomic information is required, and small amounts of DNA are needed (Adhikari et al. 2017). Conserved DNA-derived polymorphisms (gene family sequences discernible in many copies within plant genomes) are powerful and cost-effective molecular approaches for accessing polymorphisms (variability) in plant species. However, there are other newly derived molecular marker systems, conserved DNA-derived polymorphism (CDDP), start codon targeted polymorphism (SCoT) markers, and CAAT box-derived polymorphism (CBDP) markers, which have curtailed conserved gene sequences and present at multiple sites within plant genomes, and hence provide various primer binding positions (Collard and Mackill 2009).

Worldwide, modern crop breeding has resulted in a rapid reduction in genetic diversity over time due to concentrated selections on targeted genes or quantitative trait loci. Consequently, knowing the current condition of genetic diversity and the extent of the possible genetic decline in Iraq's germplasm could be beneficial for barley breeding in terms of effectively improving important traits and correctly predicting genetic relatedness and diversity. Up to this point, there have been limited initiatives in Iraq to explore genetic variation in barley accessions using molecular markers. (Al_Hadeithi 2015; Al-Hadeithi 2016). In this context, the objective of this study was therefore to investigate the diversity of 59 accessions of barley plants collected from different geographical regions in Iraq using ISSR, CDDP, and SCoT markers.

Materials And Methods

Plant material and DNA isolation

A total of 59 barley accessions were considered in the present work, which originated in almost all research centres in Iraq (Table 1). These accessions are the most widely cultivated in Iraq. The complete genomic DNA was extracted from young leaves (2-week old seedlings), which were grown in greenhouse plants from each accession according to the cetyltrimethylammonium bromide (CTAB) protocol (Tahir 2015). The quality of DNA was estimated and examined by using a 1.5% agarose gel. Then, DNA samples were diluted to about 50 ng/μL using ddH2O, and the extracted genomic DNA was saved in the freezer (-20oC) until used in a polymerase chain reaction.

ISSR, CDDP, and SCoT assay

In the present investigation, 45 ISSR primers were selected mostly from the set of Biotechnology Laboratory, University of British Columbia, Canada (Table 2). Nine CDDP and 12 SCoT primers were also tested in our study (Tables 3 and 4). PCR reactions were performed in a quantity of 25 μL. The reaction mixture contained 4 μL of isolated DNA from each sample, 10 μL master mixes (GoTaq® Green Master Mix, Promega, USA), 2 μL of used primer, then the final volume of 25 μL completed with de-ionized water.

The PCR was carried out in accordance with this procedure. : 1 cycle of 94ºC for 10 min, followed by 35 cycles of 1 min denaturing step at 94°C, 1 min annealing temperatures which ranged between (42–60°C) depending on the different ISSR, CDDP, and SCot primer sequences, and 2 min extension at 72°C. Finally, the post-extension was set up at 72°C for 7 min. The amplification reaction products were detected and separated by 1.5% agarose gels (1xTBE buffer) stained with ethidium bromide and visualized under UV light.

Scoring and statistical data analysis

After amplification of the fragments, the scorable bands were manually coded by recording 0 and 1 for the absence and presence of bands, respectively. To calculate the similarity coefficient of Jaccard, the scored data matrices were subjected to statistical analysis using the XLSTAT 2016 computer software. To perform cluster analysis between accessions, the Jaccard coefficient was converted into a dissimilarity matrix using the unweighted pair-group technique with arithmetic averages (UPGMA). The binary data (0 and 1) was converted to A and T to create the dendrogram tree using CLC Sequence Viewer version 8. Polymorphism information content (PIC) allele frequency and gene diversity were calculated using Power Marker version 3.25 software to measure the efficiency of the markers and distinguish between accessions. To infer the genetic structure and clarify the numbers of sub-populations for the population structure, a model analysis was conducted with the help of the software STRUCTURE, version 2.3.4 (Pritchard et al. 2000). The numbers of supposed populations (K) were set from one to ten, and the analysis was repeated three times. The burn-in and MCMC were fixed to 50,000 each for each category, and iterations were set down to 5000. The run with the maximum likelihood was engaged to set accessions into populations.

Table 1
Code, name of 59 barley accessions included in this investigation.

Accession Code	Origin	Accession Name	Accession Code	Origin	Accession Name
V1	South of Iraq	Shoaa	V31	Middle of Iraq	Scio/3
V2	South of Iraq	Boraak	V32	Middle of Iraq	Victoria
V3	South of Iraq	Radical	V33	Middle of Iraq	Black-Bhoos-B
V4	South of Iraq	Arivat	V34	Middle of Iraq	Irani
V5	South of Iraq	16 HB	V35	Middle of Iraq	A1
V6	South of Iraq	Furat 9	V36	Middle of Iraq	MORA
V7	South of Iraq	Al-warka	V37	Middle of Iraq	ABN
V8	South of Iraq	Numar	V38	Middle of Iraq	Arabi aswad
V9	South of Iraq	Al-amal	V39	Middle of Iraq	Clipper
V10	South of Iraq	Rafidain-1	V40	Middle of Iraq	Bhoos-H1
V11	South of Iraq	Al-khayr	V41	Middle of Iraq	BN2R
V12	South of Iraq	BN6	V42	Middle of Iraq	BA4
V13	South of Iraq	IBAA-99	V43	North of Iraq	Qala-1
V14	North of Iraq	Saydsadiq	V44	North of Iraq	Black-kalar
V15	Middle of Iraq	Bhoos-244	V45	North of Iraq	White-kalar
V16	Middle of Iraq	IBAA-265	V46	North of Iraq	Black-Akre
V17	North of Iraq	White-Akre	V47	North of Iraq	Black-Garmiyan
V18	North of Iraq	Black-Bhoos Akre	V48	North of Iraq	Black-Chiman
V19	North of Iraq	Black-Zaxo	V49	North of Iraq	Ukranian-Zarayan
V20	North of Iraq	White-Zaxo	V50	North of Iraq	White-Zarayan
V21	South of Iraq	Bhoos-912	V51	North of Iraq	Abrash
V22	North of Iraq	White-Halabja	V52	North of Iraq	Bujayl 1-Shaqlawa
V23	South of Iraq	Samr	V53	North of Iraq	Bujayl 2-Shaqlawa
V24	South of Iraq	GOB	V54	North of Iraq	Bujayl 3-Shaqlawa
V25	South of Iraq	Abiad	V55	South of Iraq	Rehaan
V26	South of Iraq	CANELA	V56	South of Iraq	Sameer
V27	South of Iraq	MSEL	V57	South of Iraq	Warka-B12
V28	South of Iraq	Acsad strain	V58	South of Iraq	Al-Hazzar
V29	South of Iraq	Acsad-14	V59	South of Iraq	IBAA-995
V30	South of Iraq	Gk-Omega			

Results And Discussion

Polymorphism parameters of ISSR markers

All ISSR primers produced scorable and well-defined amplification products, and showed polymorphisms among the 59 analyzed barley accessions (Table 2). The 45 ISSR primers used in this study generated 255 scorable polymorphic bands. The numbers of amplified bands detected in our study ranged between 1 and 11 for the ISSR markers UBC-813 and ISSR-9, respectively. The major allele frequency reached from 0.10 to 0.81, with 0.36 as an average allele per marker. Major alleles with the highest frequency (81%) were observed for the ISCS20 marker. The gene diversity values were observed in the variety of 0.32–0.96 with an average value of 0.77 per ISSR marker. PIC revealed complete consent of discriminating power of ISSR primers, suggesting a high efficiency of this DNA marker to discover genetic diversity among barley accession used in this investigation. Our results showed that the PIC values ranged from 0.96 (ISSR-8 and UBC-823) to 0.29 (ISCS20) with a mean of 0.74 (Table 2). This represents the positive capability of ISSR-8 and UBC-823 primers to assess genotyping in barley germplasm, and therefore provides a useful tool for analyzing population genetics on diverse plants and recognizing population. In the current study, 26 ISSR markers had PIC values larger than the average PIC value (0.74), which could be helpful for trait mapping and tagging studies in Iraqi barley accessions. The ability to determine genetic differences among different genotypes may be more directly related to the number of polymorphisms identified with each marker technique employed in diversity research. In a previous experiment conducted by a group of researchers, Yongcui et al. (2005) studied 60 barley accessions using two molecular marker techniques (RAMP and ISSR). For ISSR in their investigation, the PIC value ranged from (0.20 to 0.93) with an average of 0.676.

Table 2

Sequences, annealing temperature, and polymorphism information parameters of the ISSR markers used in this investigation.

ISSR markers	Sequences (5−3)	Annealing temperature (°C)	Allele number	No. of polymorphism	Major allele frequency	Gene diversity	PIC
UBC-808	AGAGAG AGAGAGAGAGC	50.00	11.00	5.00	0.24	0.86	0.84
UBC-811	GAGAGAGAGAGAGAGAC	50.00	6.00	3.00	0.42	0.66	0.60
UBC-812	GAGAGAGAGAGAGAGAA	50.40	33.00	8.00	0.12	0.95	0.95
UBC-818	CACACACACACACACAG	52.80	22.00	9.00	0.36	0.84	0.83
UBC-823	TCTCTCTCTCTCTCTCC	50.00	40.00	9.00	0.10	0.96	0.96
UBC-825	ACACAC ACACACACACT	50.00	15.00	6.00	0.34	0.84	0.82
UBC-826	ACACACACACACACACC	50.00	22.00	9.00	0.15	0.92	0.91
UBC-841	GAGAGAGAGAGAGAGACTC	50.00	12.00	4.00	0.66	0.55	0.53
UBC-888	CGTCGTCGTCACACACACACACA	52.00	29.00	6.00	0.12	0.95	0.94
UBC-891	ACTACTACTTGTGTGTGTGTGTG	52.00	38.00	8.00	0.20	0.94	0.93
UBC-847	CACACACACACACACAGC	50.00	27.00	7.00	0.10	0.95	0.94
UBC-813	CTCTCTCTCTCTCTCTT	50.00	2.00	1.00	0.63	0.47	0.36
UBC-810	GAGAGAGAGAGAGAGAT	50.00	21.00	6.00	0.24	0.89	0.88
UBC-814	CTCTCTCTCTCTCTCTA	50.00	10.00	5.00	0.36	0.79	0.76
UBC-815	CTCTCTCTCTCTCTCTG	50.00	21.00	8.00	0.17	0.92	0.91
UBC-822	TCTCTCTCTCTCTCTCA	50.00	28.00	6.00	0.12	0.94	0.94
UBC-834	AGAGAGAGAGAGAGAGGT	50.00	9.00	5.00	0.34	0.80	0.76
UBC-845	CTCTCTCTCTCTCTCTTG	50.00	23.00	9.00	0.24	0.89	0.88
ISSR-1	AGACACACACACACACAT	50.00	7.00	3.00	0.32	0.76	0.73
ISSR-6	GCCTCCTCCTCCTCCTCC	50.00	13.00	5.00	0.22	0.85	0.84
ISSR-7	AGATCCTCCTCCTCCTCC	50.00	12.00	6.00	0.54	0.68	0.67
ISSR-8	ATCACACACACACACACA	50.00	32.00	9.00	0.08	0.96	0.96
ISSR-9	CACACACACACACACATG	50.00	27.00	11.00	0.14	0.93	0.93

ISSR markers	Sequences (5−3)	Annealing temperature (°C)	Allele number	No. of polymorphism	Major allele frequency	Gene diversity	PIC
ISSR-10	GAGAGAGAGAGAGAGAGG	50.00	26.00	10.00	0.20	0.93	0.92
ISSR-12	AGAGAGAGAGAGAGAGCT	50.00	8.00	6.00	0.56	0.63	0.58
ISSR-14	GAGAGAGAGAGAGAGAGC	50.00	9.00	5.00	0.46	0.75	0.73
ISSR-16	ACACACACACACACACGA	50.00	7.00	3.00	0.46	0.69	0.65
ISSR-18	TGTGTGTGTGTGTGTGGG	50.00	18.00	8.00	0.24	0.86	0.84
UBC-846	CACACACACACACACAAT	50.00	34.00	10.00	0.20	0.93	0.93
UBC-849	GTGTGTGTGTGTGTGTGA	50.00	20.00	8.00	0.34	0.84	0.83
UBC-852	GATAGATAGACAGACA	48.00	4.00	2.00	0.53	0.59	0.51
UBC-856	ACACACACACACACYA	42.00	3.00	2.00	0.49	0.53	0.42
UBC-881	GGGTGGGGTGGGGTG	54.00	31.00	8.00	0.15	0.94	0.94
HB-10	GAGAGAGAGAGACC	50.00	21.00	7.00	0.25	0.88	0.87
HB-11	GTGTGTGTGTGTCC	50.00	12.00	6.00	0.49	0.73	0.71
HB-15	GTGGTGGTGGC	50.00	8.00	4.00	0.53	0.66	0.62
ISSR-AGC6G	AGCAGCAGCAGCAGCAGCG	55.00	3.00	2.00	0.58	0.51	0.41
PCP-3	GTGCGTGCGTGCGTGC	60.00	16.00	5.00	0.36	0.82	0.81
ISCS20	DHBCGACGACGACGACGA	60.00	5.00	3.00	0.81	0.32	0.29
ISCS21	BDBACAACAACAACAACA	55.00	8.00	4.00	0.73	0.45	0.43
ISSR-CCA	DDBCCACCACCACCACCA	55.00	6.00	4.00	0.71	0.47	0.45
HB-12	CACCACCACGC	48.50	5.00	3.00	0.68	0.50	0.47
B-13	CAACAACAACAACAA	44.70	5.00	3.00	0.64	0.54	0.50
ISSR-CA	BDBCACACACACACA	50.00	9.00	4.00	0.31	0.80	0.77
Mean			16.32	5.80	0.36	0.77	0.74
Total				255.00			

Polymorphism information of CDDP markers

Genome-conserved sections in various plant species have helped to develop molecular markers, such as SCoT and CDDP. These markers use longer primers and higher annealing temperatures, making them more reliable, reproducible, and simple to create than other arbitrary markers like RAPD or DAF. Furthermore, they focus on gene domains, making them preferred to random markers in QTL mapping applications. To study genetic diversity among 59 barley accessions, nine CDDP primers were tested. All primers produced scorable fragments. Across all barley accessions, 35 polymorphic bands with an average of 3.89 bands per primer were generated (Table 3). The maximum and minimum number of polymorphic bands were obtained by

MADS-1, WRKY-R3, and ERF2 (6 bands) and MYB1, ERF1, and KNOX1 (2 bands), respectively. The frequency of the main allele ranged from 0.34 to 0.75, with a mean value of 0.48. ERF1 had the highest frequency of major alleles in the barley accessions, with a 0.75 frequency. The gene diversity in the barley accessions collection with an average of 0.67 and ranged from 0.42 (ERF1) to 0.82 (WRKYF1). PIC values for nine primers ranged from 0.39 (ERF1) to 0.80 (WRKYF1) with an average value of 0.63 per primer. The PIC values detected in the CDDP primers were ranked in descending order as WRKY-F1 > ERF2 and WRKY-R3 > MYB2 > KNOX1 > ABP1-1 > MYB1 > MADS-1 > ERF1. WRKY-F1 is a transcription factor with developmental and physiological roles in plants (Xie et al. 2005).

The average polymorphic allele (3.89) found in this study was lower than the 4.60 alleles found in barley previously reported by Ahmed et al. (2021) using 10 CDDP primers across 82 barley genotypes. The average PIC value (0.63) in this study was high compared to another study finding documented by Ahmed et al. (2021), who mentioned a PIC mean value of 0.37.

Table 3
Primer information and diversity parameters for the CDDP markers used in this investigation.

CDDP markers	Sequence of primer (5–3)	Annealing temperature (°C)	No. of polymorphism	Major allele frequency	Gene diversity	PIC
ABP1-1	ACSCCSATCCACCGC	50.00	3.00	0.44	0.67	0.61
WRKYF-1	TGGCGSAAGTACGGCCAG	50.00	4.00	0.34	0.82	0.80
MYB1	GGCAAGGGCTGCCGC	50.00	2.00	0.54	0.60	0.54
MYB2	GGCAAGGGCTGCCGG	50.00	4.00	0.44	0.74	0.71
ERF1	CACTACCCCGGSCTSCG	50.00	2.00	0.75	0.42	0.39
ERF2	GCSGAGATCCGSGACCC	50.00	6.00	0.39	0.77	0.74
KNOX1	AAGGGSAAGCTSCCSAAG	50.00	2.00	0.39	0.68	0.62
MADS-1	ATGGGCCGSGGCAAGGTGC	50.00	6.00	0.63	0.55	0.51
WRKY-R3	GCASGTGTGCTCGCC	50.00	6.00	0.42	0.76	0.74
Mean			3.89	0.48	0.67	0.63
Total			35.00			

Polymorphism analysis of SCoT markers

For the genetic diversity analysis in 59 barley accessions, twelve SCoT markers were used. A total of 101 scorable and sharp polymorphic bands were generated across all barley accessions. The minimum and maximum number of polymorphic bands were obtained by SCoT12 (3 bands) and SCoT32 (15 bands), respectively. The average gene diversity was 0.81, with the SCoT3 primer having the lowest (0.48) and the SCoT16 and SCoT32 primers having the highest (0.97). PIC values for twelve SCoT primers ranged from 0.46 (SCoT3) to 0.97 (SCoT16 and SCoT32) with a mean value of 0.80 per primer (Table 4). The PIC values found in the SCoT primers were ranked in descending order as follows: SCoT16 and 32 > SCoT6 and 29 > SCoT23 > SCoT22 > SCoT7 > SCoT13 > SCoT2 > SCoT36 > SCoT12 > SCoT3. Recently, the relationship between 82 Iranian barley accessions was determined using 10 SCoT markers byAhmed et al. (2021), who scored 54 polymorphic bands. The PIC value for the used marker was ranged between 0.23 (SCoT11) to 0.43 (SCoT28), with an average value of 0.33 per primer. Lately, the relationship between 48 *Aegilops triuncialis* accessions was determined using 14 SCoT markers byKhodaee et al. (2021), who scored 147 polymorphic bands. The PIC value for the chosen marker ranged from 0.14 (SCoT3) to 0.42 SCoT14, with a mean of 0.26 per primer, which was less than the PIC value in our study.Pour-Aboughadareh et al.(2018) similarly evaluated the molecular genetic diversity and relationships among some *Triticum* and *Aegilops* species by using 15 ScoT markers. In total, 164 polymorphic bands were detected in their investigation, and the PIC value was ranged from (0.41) to (0.50) with an average of 0.48 per primer.

Table 4
List of Scot primers used and the information obtained in the barley tested.

SCoT markers	Sequences (5–3)	Annealing temperature (°C)	No. of polymorphism	Major allele frequency	Gene diversity	PIC
SCoT2	CAACAATGGCTACCACCC	50.70	4.00	0.53	0.68	0.66
SCoT3	CAACAATGGCTACCACCG	51.30	7.00	0.71	0.48	0.46
SCoT6	CAACAATGGCTACCACGC	52.10	8.00	0.24	0.89	0.89
SCoT7	CAACAATGGCTACCACGG	51.30	9.00	0.22	0.88	0.87
SCoT12	ACGACATGGCGACCAACG	55.90	3.00	0.46	0.66	0.59
SCoT13	ACGACATGGCGACCATCG	55.40	8.00	0.31	0.86	0.85
SCoT16	ACCATGGCTACCACCGAC	54.10	13.00	0.10	0.97	0.97
SCoT22	AACCATGGCTACCACCAC	51.90	6.00	0.19	0.89	0.88
SCoT23	CACCATGGCTACCACCAG	52.40	8.00	0.17	0.90	0.89
SCoT29	CCATGGCTACCACCGGCC	57.90	14.00	0.27	0.90	0.90
SCoT32	CCATGGCTACCACCGCAC	55.90	15.00	0.05	0.97	0.97
SCoT36	GCAACAATGGCTACCACC	51.50	6.00	0.44	0.69	0.65
Mean			8.42	0.31	0.81	0.80
Total			101.00			

The allelic abundance of accessions in plants is a measure of their genetic variation resource, often exploited by informative molecular markers that identify populations for screening, breeding, and conservation (Igwe et al. 2021; Vinceti et al. 2013). The allelic count and frequency ranges produced in this research were significant, confirming the informative nature of this collection of primers in barley accessions. The major allele frequency in three markers was in the following order: SCoT (0.31) < ISSR (0.36) < CDDP (0.48), demonstrating the usefulness of SCoT and ISSR markers for determining the allelic diversity of this important crop. The gene diversity reported was arranged in the following order: SCoT (0.81) > ISSR (0.77) > CDDP (0.67), proving the utility of SCoT and ISSR markers in estimating the allelic diversity of barley accessions. The PIC provided proficiency of marker systems, which helped determine the potential and value of the primers used in the process of fingerprinting (Serrote et al. 2020). In the present investigation, the SCoT marker technique revealed a higher mean of PIC (0.80) than ISSR (0.74) and CDDP (0.63), which explains the precise application of the SCoT technique in the assessment of accessions diversity. Based on the above results, the discrimination power of the three approaches for assessing allelic diversity in barley accessions was as follows: SCoT > ISSR > CDDP, exhibiting that SCoT functional gene-based markers were both informative and effective at assessing genetic diversity. In addition to SCoT and ISSR performance, CDDP markers demonstrated a poor ability to differentiate barley accessions. Finally, it was suggested that genetic analyses based on SCoT and ISSR markers would be extremely useful for crop improvement programs, including QTL mapping, genetic diversity estimation, linking maps, and genotype identification, as SCoT markers were derived from the functional region of the genome.

Clustering and population structure analysis of barley accessions

Cluster analysis of different barley accessions

Multivariate statistical approaches are critical in studying genetic diversity. Cluster analysis, one of the multivariate statistical techniques, separates individuals into graphs based on intervals. A marker profile data aims to maintain a genetic relationship between genotypes under investigation, using distance measures that express accessions' relationship. The dendrogram was

constructed based on 45 ISSR markers to better estimate the genetic distance among 59 Barley accessions (Fig. 1). The UPGMA method and Jaccard coefficient dissimilarity were performed for analyzing the ISSR markers data set. Two major clades within 7 subgroups at the dissimilarity threshold were indicated (Fig. 1A). The first clade included almost all barley accessions, which comprised 38 tested accessions. This group contains 5 subgroups including the barley accessions: Bhoos-912, BA4, BN6, Bhoos-244, BN2R, CANELA, ABN, A1, MORA, MSEL) grouped in the first subgroup, and the second subgroup consisted of 10 barley accessions named Al-warka, 16 HB, GOB, Numar, Furat 9, Boraak, Radical, Arivat, Rafidain-1, Al-khayr, which mostly spread and cultivated in South of Iraq. In addition, 13 barley accession clustered in the third subgroup, including Black-Bhoos-B, Arabi aswad, Victoria, Scio/3, Acsad strain, Acsad strain, Samr, Irani, White-Halabja, Abiad, White-Zaxo, Black-Zaxo, Black-Bhoos Akre, while the fourth subgroup had 4 barley accession: Al-amal, IBAA-99, IBAA-265, White-Akre. However, Bhoos-H1 was isolated in the fifth subgroup, demonstrating its genetic divergence from the other accessions. Whereas, in the second clade, two main sub-clusters were exhibited. The first sub-cluster comprised 5 accessions (Rehaan, Sameer, Warka-B12, Al-Hazzar, IBAA-995), which are mainly distributed over the South of Iraq, and the rest of the barley accessions were grouped in the second sub-cluster. The clustering pattern for ISSR data showed that barley accessions can be separated into two major groups according to geographical origin. Therefore, to enhance the appearance of heterosis, the breeder may use genetic distance data to make informed decisions about crossing accessions from distinct groups or subgroups for population development, or to promote the analysis of various parents to cross in hybrid combinations. The present outcome supports previous reports on the correlation between ISSR markers and eco-geographical distribution of the accessions (Etminan et al. 2016; Wang et al. 2009; Yongcui et al. 2005). Our results confirm the effectiveness of ISSR markers in evaluating the genetic diversity reported before by Wang et al. (2009), who scored 145 polymorphic bands using 11 ISSR primers.

Regarding the clustering patterns for CDDP primers, two main groups were optioned, but with different sub-clusters. In general, two main sub-clusters were determined in the first group. The first sub-cluster included only Ukranian-Zarayan accession, while the second sub-cluster in the same group separated the barley accessions to the two sets of arrangements. The first sub-sub-cluster was composed of three accessions (Black-Akre, Black-Garmiyan, Black-Chiman), which were cultivated by the farmer in the North of Iraq, and the remaining barley accessions were arranged in the second sub-sub-cluster based on genomic similarity. However, group 2 included only two barley accessions, namely Al-Hazzar and IBAA-995, indicating their genomic differences from the rest of the accessions (Fig. 1B). The number of groups detected by CDDP markers in this study was lower than in the reporting of Ahmed et al. (2021), who exhibited three clusters in 82 Iranian barley accessions. This could be due to the type of markers and the number of accessions used.

Clustering of barley accessions using the SCoT molecular dataset revealed two major classes (Fig. 1C). The first class included most barley accessions categorized into two different sub-clusters. One of them comprised three barley accessions, namely (A1, Arabi Aswad, and Clipper), while the other sub-cluster distributes the most barley accession into three sub-sub-clusters. The first sub-sub-cluster comprised four barley accessions (16 HB, Furat 9, Al-warka, and Black-kalar), which were mostly distributed and cultivated in the South of Iraq. The second sub-sub-cluster included five barley accessions (Black-Akre, Black-Garmiyan, Black-Chiman, White-Zarayan, and Bujayl 2-Shaqlawa). The farmer widely cultivated these barley accessions in North of Iraq, while forty-two barley accessions based on genomic dissimilarity were arranged in the third sub-sub-cluster in this particular group. However, the second class involved only five distinct barley accessions, namely Rehaan, Sameer, Warka, B12, Al-Hazzar, and IBAA-995, which originated from the South of Iraq. The number of clusters formed by the SCoT approach in this study was smaller than that previously reported by Ahmed et al. (2021).

Intriguingly, the general dendrogram constructed using the combined data of all molecular markers used in our investigation (ISSR, CDDP, and SCoT) (Fig. 1D) divided barley accessions into two major clusters. Almost all barley accessions were well distributed in the first cluster, which included fifty barley accessions. In this particular cluster, it is clear that some barley accessions based on genetic distance were grouped, especially for the accessions of Arabi aswad and Clipper, while the rest of the forty-eight accessions were grouped in six sub-sub-clusters. Conversely, the second cluster consisted of nine barley accessions, namely Black-Akre, Black-Garmiyan, Black-Chiman, Bujayl2-Shaqlawa, Rehaan, Sameer, Warka-B12, Al-Hazzar, IBAA-995. Accordingly, our finding supported the available suggestion that many molecular techniques could either be applied

individually or in combination with other molecular marker techniques to find reliable information about genetic relationships and assess the genetic variation, which would support strategies for effective collection of barley germplasm and knowing their conservation. Insufficient genetic differentiation could imply a high level of gene flow. Stimulatingly, taking a close look at the dendrogram using two different marker systems, a similar pattern of alignments for most barley accessions can be found. Similarly, a group of researchers, Naceur et al. (2012), worked on 31 barley accessions to reveal genetic distance, and obtained 9 classes demonstrating wide diversity among studied accession. This is probably due to the collection of barley germplasm from three countries (Egypt, Algeria, Tunisia). Whereas, compared to our previous work, seven clusters were obtained using the SSRs marker technique (Tahir et al. 2020). This type of molecular technique was now widely used by another research groups to determine the genetic diversity of plant species (Liu et al. 2020; Saidi et al. 2018; Talebi et al. 2018). For many plants, all three markers have been effectively used to determine genetic relationships and diversity. In addition, DNA analysis using three methods has proved to be an inexpensive and efficient way to provide molecular data for evaluating genetic differences (Amom and Nongdam 2017; Hajibarat et al. 2015; Khodaee et al. 2021; Tiwari et al. 2016). It has been noted the larger the distance between accessions, the greater the likelihood of accumulating wider genetic diversity, which also defines their places on clusters (Skroch and Nienhuis 1995).

Population structure profile in barley accessions

STRUCTURE software and the Bayesian statistical index were used to perform effective population structure assessment, reliable population grouping, and identification of mixed genotypes. Separate and combined data of all molecular markers ISSRs, CDDP, and SCoT were used to estimate the population structure of 59 barley accessions. To measure the level of genetic stratification in a multi-locus data set, the program STRUCTURE has become one of the most commonly used programs. However, this program has its limitation, as it cannot interfere with the number of clusters (k) that best fit the data set (Pritchard et al. 2000). To solve this limitation, the STRUCTURE harvester, a web-based program, has been generated for quickly analyzing and summarizing the data outcome from the STRUCTURE program. Therefore, detecting numbers of K groups that best fit the population will be selected (Evanno et al. 2005). In this investigation, to evaluate the likely number of inferred populations, the likelihood of K equals 1 to 10, with each K-value replicated three times (Earl 2012).

The optimal value of k is 2 in all three used markers (Figs. 2 and 3). The STRUCTURE outcomes suggested the population could be divided into two main populations in all case scenarios. The grouping was mildly following the geographic background of the barley accessions. Although two populations may be practical for our panel, based on the size of the population and the difference in numbers of barley accessions representing three main areas in Iraq from which they were collected. The optimal value of k = 2 occasionally misrepresents the true structure in the population, and could mean that either the STRUCTURE program unsuccessfully identified the underlying genetic structure of the collection, or there is no definite population structure (Cullingham et al. 2020). Therefore, cluster analysis was performed, as mentioned previously, to truly understand the genetic structure of this population. Based on the results of these analyses, it was observed that there are no differences between the two analyses and completely match the cluster analysis obtained by the molecular markers data set. This meets our expectations better, as it was observed that the accessions from the neighboring locations were mostly clustered together compared with the populations derived by STRUCTURE.

Remarkably, the result from STRUCTURE Harvester for all molecular markers demonstrated that (k) value had the maximum peak at K = 2, inferring that the probable number of genetic clusters in the population incorporates all individuals from 59 accessions with the highest likelihood (Figs. 2 and 3). This was observed when the mean of the log of posterior probability was graphed, demonstrating that two populations can be observed, which were visualized in two distinct colors (Green and Red). Based on the membership fractions, the accessions with a probability of 80% or above were assigned to matching populations. with others characterized as an admixture, and indicating the purity of tested materials. The combination of the two mentioned colors represents barley accessions in which they possess different genetic structures.

Concerning the separate and combined analysis of structure for all marker data set conducted in this investigation, the first population indicated in red color including individuals of pure genetic make-up (with the probability of ≥ 80%), which

comprised 16 (Bujayl 2-Shaqlawa, Sameer, Black-Akre, Black-Garmiyan, Black-Chiman, Warka-B12, IBAA-995, Bujayl 3-Shaqlawa, Rehaan, Bujayl 1-Shaqlawa, Ukranian-Zarayan, Al-Hazzar, Abrash, Shoaa, Gk-Omega, and Qala-1), 28 (Boraak, Bhoos-244, Scio/3, Acsad strain, Al-warka, IBAA-99, Numar, Radical, IBAA-265, Victoria, Rafidain-1, Al-amal, Irani, White-Akre, 16 HB, Samr, Arivat, Black-Bhoos Akre, Black-Bhoos-B, Saydsadiq, Bhoos-H1, Furat 9, BN6, Gk-Omega, White-Halabja, Acsad-14, Black-Zaxo, and Bhoos-912) 12 (Sameer, A1, Clipper, Warka-B12, Black-Garmiyan, Black-Chiman, Arabi aswad, Rehaan, Al-Hazzar, IBAA-995, White-Zarayan, and Bujayl 2-Shaqlawa), and 15 (Sameer, Bujayl 2-Shaqlawa, Black-Akre, Black-Garmiyan, Warka-B12, IBAA-995, Rehaan, Al-Hazzar, Bujayl 3-Shaqlawa, Bujayl 1-Shaqlawa, Abrash, Black-Chiman, White-Zarayan, and Gk-Omega) barley accessions for ISSR, CDDP, SCoT, and combined molecular dataset, respectively. Furthermore, the second population, depicted in green, included barley accessions with a pure genetic background (with the probability of ≥ 80%). This distinct population basically contained 22 (CANELA, Scio/3, Acsad-14, Bhoos-244, IBAA-265, Victoria, ABN, Acsad strain, Bhoos-H1, Boraak, Al-amal, Bhoos-912, MSEL, Irani, BN2R, IBAA-99, White-Halabja, Rafidain-1, Black-Zaxo, Arabi aswad, Black-Bhoos-B, BA4, A1, Abiad, Black-Bhoos Akre, Al-khayr, BN6, MORA, White-Zaxo, and Numar), 26 (Ukranian-Zarayan, White-Zarayan, Al-Hazzar, Black-Garmiyan, Black-Chiman, Rehaan, Abrash, Bujayl 1-Shaqlawa, GOB, Warka-B12, Qala-1, Black-Akre, ABN, Bujayl 3-Shaqlawa, Black-kalar, IBAA-995, BA4, White-Zaxo, MORA, Bujayl 2-Shaqlawa, White-kalar, BN2R, Abiad, MSEL, Sameer, and CANELA), 38 (Bhoos-244, White-Akre, White-Halabja, Abiad, Qala-1, Black-Bhoos-B, IBAA-265, White-Zaxo, CANELA, MSEL, Scio/3, Victoria, Ukranian-Zarayan, Irani, BA4, Al-khayr, Saydsadiq, Black-Bhoos Akre, Black-Zaxo, Boraak, Samr, White-kalar, ABN, MORA, BN2R, Bujayl 1-Shaqlawa, Acsad strain, Rafidain-1, Bhoos-912, Numar, Al-amal, GOB, Shoaa, Radical, Bujayl 3-Shaqlawa, IBAA-99, Bhoos-H1, and Arivat), and 29 (Bhoos-244, IBAA-265, CANELA, Scio/3, Victoria, Al-amal, White-Halabja, Acsad strain, Boraak, MSEL, ABN, Rafidain-1, Acsad-14, IBAA-99, Bhoos-912, Black-Zaxo, Black Bhoos-B, Irani, Bhoos-H1, Al-khayr, BN2R, BA4, Abiad, Black-Bhoos Akre, BN6, MORA, Radical, White-Zaxo, and Numar) barley accessions in ISSR, CDDP, SCoT as well as all markers data, respectively, while, the remaining barley accessions (with the probability of < 80%) are considered admixture of genome from other populations. Interestingly, in all cases, the combination of two populations in numbers of individuals was much higher than in the admixture form, which showed the uniformity of tested accessions. This finding confirms that the lemma and palea remain tightly closed during the period of pollen release in barley. This phenomenon is known as cleistogamy (Nair et al. 2010). However, admixture probably occurred due to breeding lines developed through random mating by the breeders, for it is a specific trait improvement (Hernandez et al. 2020).

Genetic differences within and among populations

The analysis of molecular variance (AMOVA) based on the results achieved from the molecular data from three different marker systems was suggested that 15,14, 9, and 14% of total variation were among the three populations (North, Middle, and South of Iraq) in which the sample was grown and collected (Table 1) based on the molecular data set from ISSR, CDDP, SCoT and the combined data of all three marker systems, respectively, while the analysis was revealed the variation was much higher within the studied individuals, which were 85, 86, and 91% depending on the data obtained from ISSR, CDDP, and SCoT, respectively (Table 5), whereas, the collective data from all three marker sets propose 86% of total differences were inside the population. These outcomes suggest that barley accessions from Iraq shared a common ancestry and are highly admixed, with high variation within populations from all marker systems in which this investigation was conducted. This result revealed a distinct genetic base for the 59 barley accessions. The AMOVA results confirmed clustering and structure analyses. The partitioning of molecular variance reported that the greatest divergence was detected among individuals in the same population. Genetic diversity within and between populations improves the selection of populations that account for the vast majority of extant variations. If genetic diversity is largely found within a population, it means fewer populations are needed to conserve and sustain the differences in accessions or populations. Conversely, if genetic diversity across populations is preserved, a greater number of preserved across populations, more populations should be emphasized for maintenance and utilization (Igwe et al. 2021).

Table 5
Analysis of molecular variance (AMOVA) showing genetic diversity in the Iraqi barley accessions.

Method	Source	df	SS	MS	Est. Var.	%	P-value
ISSR data	Among Pops	2	320.99	160.49	6.59	15**	0.001
	Within Pops	56	2012.1	35.93	35.93	85**	
	Total	58	2333.09		42.52	100	
CDDP data	Among Pops	2	36.76	18.38	0.74	14**	0.001
	Within Pops	56	247.55	4.42	4.42	86**	
	Total	58	284.31		5.16	100	
SCoT data	Among Pops	2	73.72	36.86	1.29	9**	0.001
	Within Pops	56	703.13	12.56	12.56	91**	
	Total	58	776.85		13.84	100	
ISSR, CDDP, and SCoT data	Among Pops	2	431.51	215.76	8.61	14**	0.001
	Within Pops	56	2962.78	52.91	52.91	86**	
	Total	58	3394.29		61.52	100%	

Df: degree of freedom, SS: sum of aquare, MS: mean os square, Est. Var: estimated variance, p-value: probability value

Correlation analysis between genetic dissimilarity achieved by three sets of markers data

To compare the genetic distance matrices produced by three marker systems (ISSRs, CDDP, and SCoT), the mantel test was performed. However, this analysis has its limitation, especially in the case of using two different markers (Vieira et al. 2007). In the case of our investigation, 45 ISSR, 9 CDDP, and 12 SCoT primers were conducted. Remarkably, mantel test correlation values revealed a positive significant correlation between and among all three different marker systems in which clusters with a general dendrogram were present (Table 6). The highest mantel value was observed between ISSR and CDDP markers, while the minimum was displayed between ISSR and SCoT markers. This demonstrates the novelty of current work and the possibility of composing reference collections of tested barley accessions using the information attained from genetic profiles tested by three different molecular methods. Natural selection could also clarify the appropriate relationship between the diverse patterns of these markers in the regions exacerbated by ISSR, CDDP, and SCoT markers. These findings are consistent with previous research of Ahmed et al. (2021), who detected significant associations between CDDP and SCoT markers.

Table 6
Mantel coefficient values for correlation between clusters
obtained by three different molecular markers.

Markers	ISSR	CDDP	SCoT	Pooled markers data
ISSR	-	0.49**	0.37**	
CDDP		-	0.36**	0.25**
SCot			-	

Conclusion

In any plant genetic resource conservation program, the main goal is to obtain the highest possible level of genetic diversity. The three different marker systems revealed a comprehensive pattern of genetic diversity among the barley accessions collected. Our findings revealed a high level of genetic diversity among barley accessions. From all the above analyses, it is possible to conclude that selecting an ideal primer with high information content from all studies would improve the efficiency of future studies. SCoT and ISSR primers were considered more effective primers to distinguish between barley accessions. Besides, the CDDP markers could be used to determine the genetic variations among tested accessions. Dendrogram and structural analysis of accessions with different genomic statutes indicated considerable accessions grouping. Furthermore, these results could allow future insights into barley breeding programs, and thus the crossing between more genetically distant individuals increases the chance of segregation in their offspring. Consequently, the SCoT-selected primers could be effective tools for selecting desirable hybrids for enhanced breeding and germplasm preservation. Assessment of SCoT marker linkages with significant agronomic variables in barley can develop marker-assisted selection strategies using these functionally gene-based molecular markers. Specific alleles/bands for various gene-based markers may also be used to clone and design competitive allele-specific PCR (KASP) markers in barley.

Declarations

Author contributions

DL and NT performed the experiments and analyzed the data. NT and KM planned the experiments and wrote the manuscript.

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Disclosure statement

The authors declare no conflict of interest.

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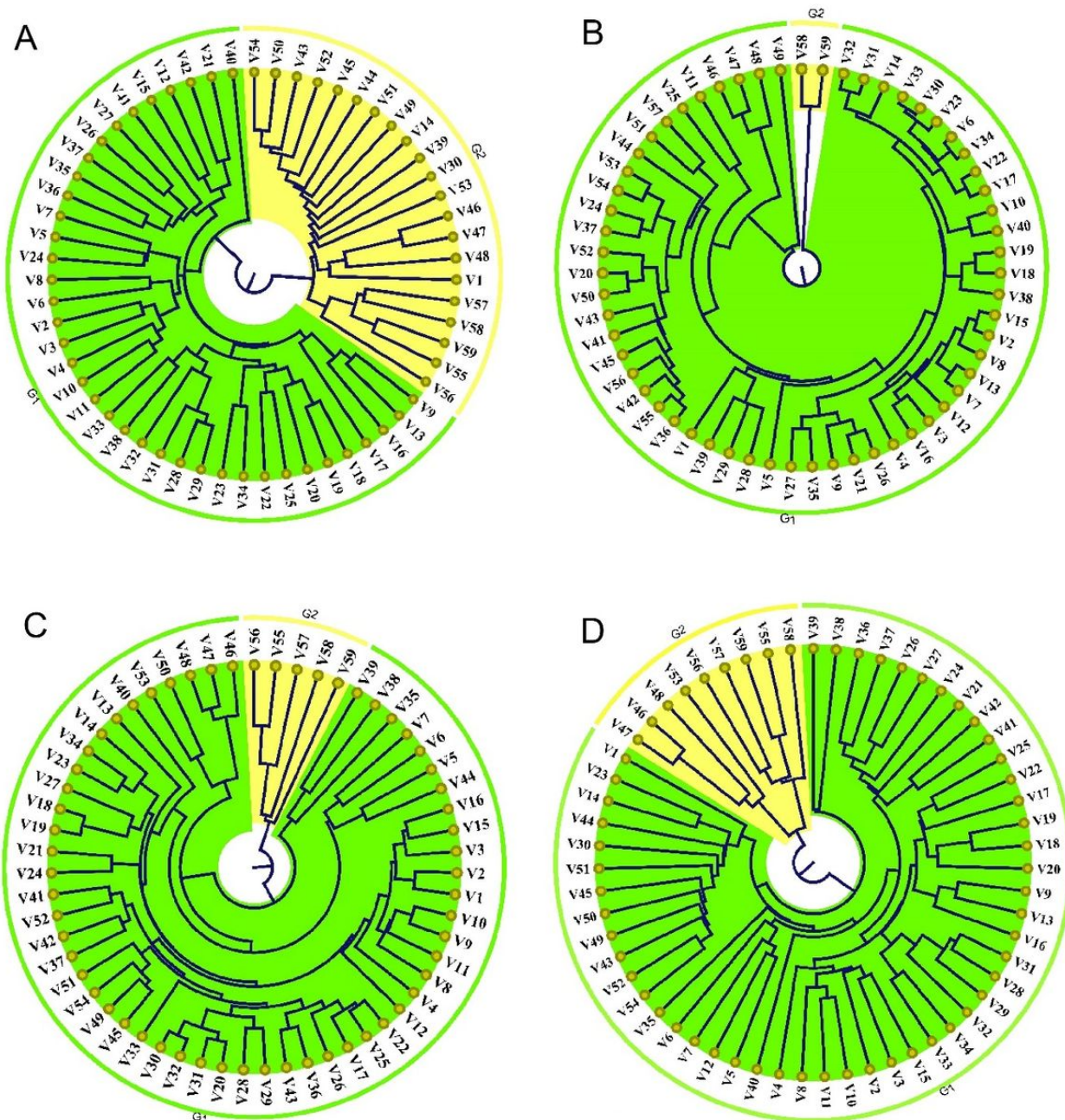
Figures

Figure 1

Dendrogram of 59 barley accessions constructed by following unweighted pair-group average methods, that distributed the accessions on 2 major groups with different sub-clusters. A: based on cluster analysis of ISSR markers data set. B: based on cluster analysis of CDDP markers data set. C: based on cluster analysis of SCoT markers data set. D: based on cluster analysis of ISSR, CDDP and SCoT markers data set. The whole name of the accessions is described in Tables 1.
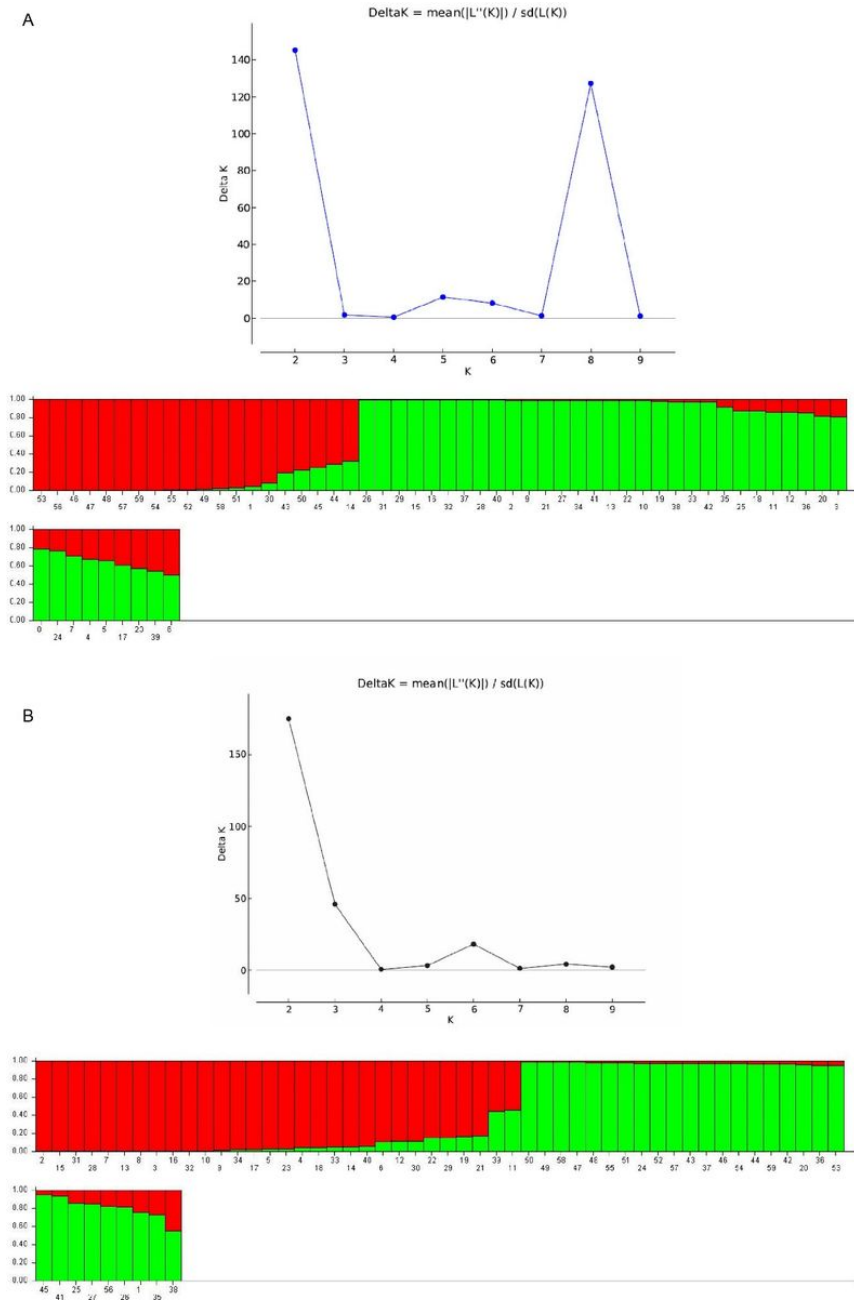
Figure 2

Determination of the optimal value of K and population structure profile of 59 barley accessions. A: based on the ISSR marker data set. B: based on the CDDP marker data set. The numbers represent individual codes in the horizontal axis, and each color represents a sub-population. The full name of the accessions is defined in Tables 1.
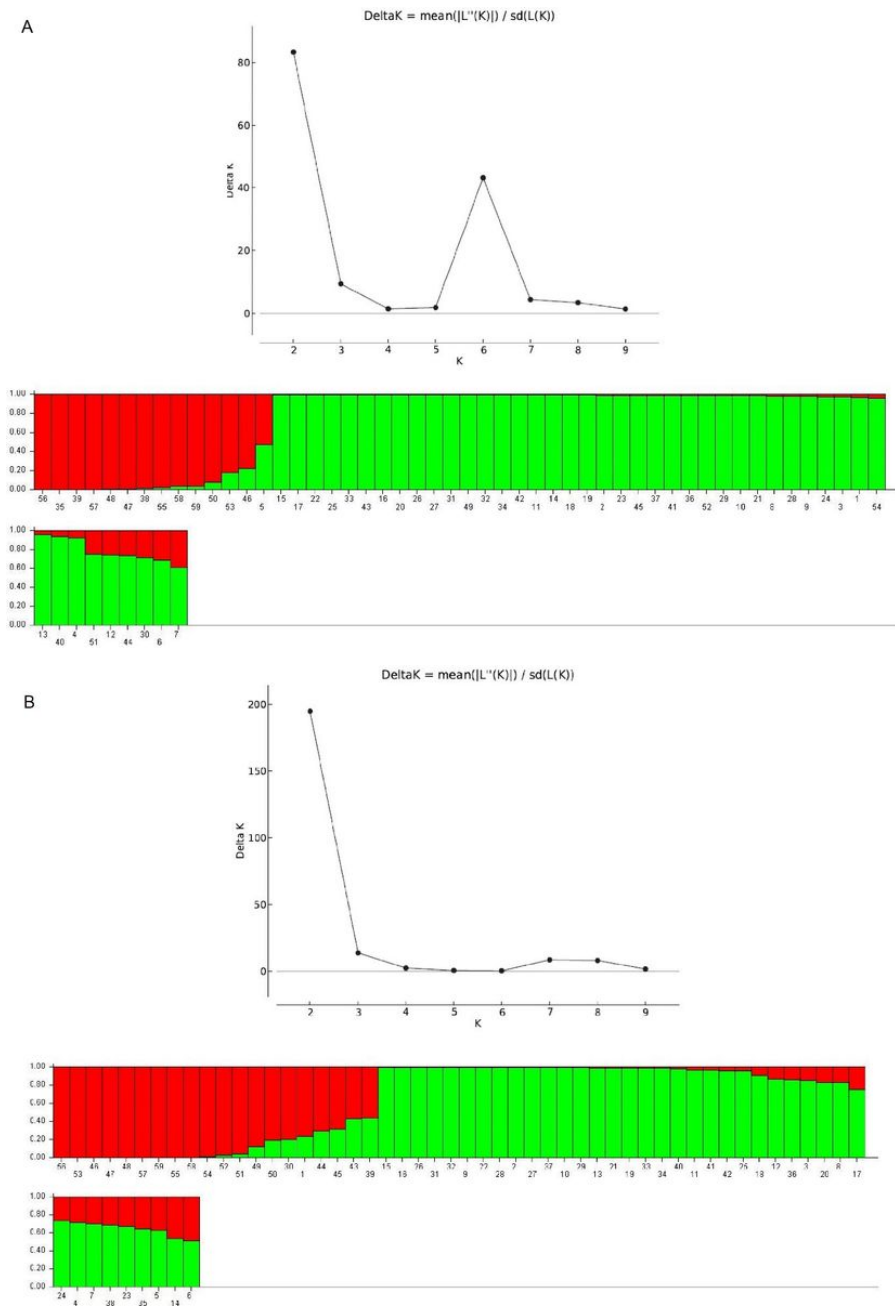
Figure 3

Describing the optimal value of K and population structure of 59 barley accessions. A: based on the SCoT marker data set. B: based on the ISSR, CDDP, and SCoT markers data set. The numbers define accession codes on the horizontal axis, while each color represents a sub-population. The complete name of the accessions is designated in Tables 1.