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Assessment of genetic variation and population structure in Iraqi barley accessions using ISSR, CDDP, and SCoT markers

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Abstract: 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 44 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 ranked 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 might be useful tools for selecting appropriate parents for a breeding program.

Keywords: clustering; diversity; genetic differentiation; *Hordeum vulgare*; molecular markers

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 terms of world cereal production and is used for a variety of purposes, including as animal feed, as food for humans, and as a component in brewing (Cai et al. 2020). In general, the genome of barley is a diploid that contains 14 chromosomes. This crop requires low cultivation inputs, including water demand, fertilizer, and tolerating 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 in human nutrition, livestock feed, and the malt industry. During the transition from producing landraces to cultivation, barley was the best model crop for studying phenotypic differences and genomic diversity (Brantestam et al. 2007). Many researchers have established significant contributions through traditional techniques to barley improvement

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in a breeding program that depends on phenotypic traits, and many researchers apply it (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 unfavourable, and these traits only represent a minor portion of the plant genome, and besides, environmental factors highly affect it (Govindaraj et al. 2015; Mzid et al. 2016).

The selection of varieties and lines with superior performance and more diversity under certain conditions is one of the primary goals of breeding programs, and one of the primary duties of breeding programs is the evaluation of genetic variety in the existing collection of germplasm. Polymerase chain reaction (PCR)-based DNA marker techniques have been developed and are now widely available; these techniques have proven particularly beneficial in investigations of genetic diversity and genetic linkages across plant species (Agarwal et al. 2008; Lateef 2015).

Inter simple sequence repeat (ISSR) markers have proven to be suitable markers for inbreeding and genetic diversity research among the several types of molecular markers available for barley due to their high polymorphism and repeatability across the entire genome. This type of marker-based on polymerase 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, such as 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 are present at multiple sites within plant genomes, and hence provide various primer binding positions (Collard & Mackill 2009).

There have been limited initiatives in Iraq to explore genetic variation in barley accessions using molecular markers (Al-Hadeithi 2015, 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.

MATERIAL 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 S1 in Electronic Supplementary Material (ESM)). 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 2014, 2015; Tahir & Omer 2017). The quality of DNA was estimated and examined by using a 1.5% agarose gel. Then, after estimating the quality of DNA samples, a nanodrop spectrophotometer (Nano Plus, MAAN LAB AB, Sweden) was used and obtained samples were diluted according to the concentration of DNA. The extracted genomic DNA was saved in the freezer (–20 °C) until used in a polymerase chain reaction.

ISSR, CDDP, and SCoT assay. In the present investigation, 44 ISSR primers were tested. The universal primers (UBC) designed at Biotechnology Laboratory, University of British Columbia, Canada and used by most researchers (Table S2 in ESM). Nine CDDP and 12 SCoT primers were also tested in our study (Table S3 and S4 in ESM). PCR reactions were performed at an overall quantity of 25 µL. The reaction mixture contained 4 µL of genomic DNA from each sample, 10 µL master mix (GoTaq® Green Master Mix, Promega, USA), 2 µL (10 µM) of selected primers, and the final volume of 25 µL completed with de-ionized water (dH_2O). The PCR was performed following this protocol: denaturation (94 °C 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) and 2 min extension at 72 °C. Finally, post-extension was set up at 72 °C for 7 min. This protocol performed well in other studies using similar molecular marker systems (Ahmed et al. 2022; Aziz & Tahir 2022; Rasul et al. 2022). The amplification reaction products were detected and separated by 1.5% agarose gels (1× TBE buffer), stained with ethidium bromide. PCR products visualized under UV light using ENDURO™ GDS Touch Gel Documentation System (Labnet, USA).

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

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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 (Ver. 8). Polymorphism information content (PIC) allele frequency and gene diversity were calculated using Power Marker (Ver 3.25) software. Model analysis was performed using the software STRUCTURE (Ver. 2.3.4) (Pritchard et al. 2000).

RESULTS AND DISCUSSION

Polymorphism parameters of ISSR markers. All ISSR primers produced scorable and well-defined amplification products and showed polymorphisms among the 59 analysed barley accessions (Table S2 and Figure S1 in ESM). The 44 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 ranged from 0.10 to 0.81, with 0.36 as the average allele per marker. Major alleles with the highest frequency (81%) were observed for the ISCS20 marker. The gene diversity values ranged from 0.32–0.96, with an average value of 0.77 per ISSR marker. PIC revealed the complete consent of discriminating power of ISSR primers, suggesting a high efficiency of this DNA marker to discover genetic diversity among the 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 S2 in ESM). This demonstrates the positive ability of ISSR-8 and UBC-823 primers to assess genotyping in barley germplasm, and thus provides a useful tool for analysing population genetics on diverse plants and identifying populations. 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.

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 according to the results that these primers have produced in past studies (Ahmed et al. 2022; Aziz & Tahir 2022). All primers produced scorable fragments (Figure S2 in ESM). Across all barley accessions, 35 polymorphic bands with an average of 3.89 bands per primer were generated (Table S3 in ESM). 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 had 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.

Polymorphism analysis of SCoT markers. For the genetic diversity analysis of 59 barley accessions, twelve SCoT markers were used based on the performance of this marker in previous research (Gholamian et al. 2019; Rasul et al. 2022). A total of 101 scoreable and sharp polymorphic bands were generated across

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all barley accessions (Figure S3 in ESM). 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 S4 in ESM). 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 by Ahmed et al. (2021), who scored 54 polymorphic bands. The PIC value for the used marker ranged from 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 by Khodaee 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 ranged from 0.41 to 0.50, with an average of 0.48 per primer.

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 (Vinceti et al. 2013; Igwe et al. 2021). 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 for 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. These differences are perhaps due to the polymorphism in the different regions of the barley genome. ISSR markers target microsatellite-flanked regions of the genome, whereas CDDP and SCoT markers amplify conserved regions of functional genes. 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 accession 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 informative and effective at assessing genetic diversity. In addition to SCoT and ISSR performance, CDDP markers demonstrated a moderate 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. The consistency of the data should, theoretically, improve constructing populations as genome coverage increases. As a result, three different types of marker systems were yielding 391 bands. The high levels of polymorphism produced by diverse markers confirmed their usefulness in studies of genetic variability in Iraqi barley collections.

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 44 ISSR markers to better estimate the genetic distance among 59 Barley accessions (Figure 1). The UPGMA method and Jaccard coefficient dissimilarity were performed for analysing the ISSR markers data set. Two major clades within 7 subgroups at the dissimilarity threshold were indicated (Figure 1A). The first clade included almost all barley accessions, which comprised 38 tested accessions. This group contains 5 subgroups including the 10 barley accessions grouped in the first subgroup, and the second subgroup similarly consisted of 10 barley accessions,

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which are mostly spread and cultivated in the South of Iraq. In addition, 13 barley accession clustered in the third subgroup, while the fourth subgroup had 4 barley accession. However, V40 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, 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

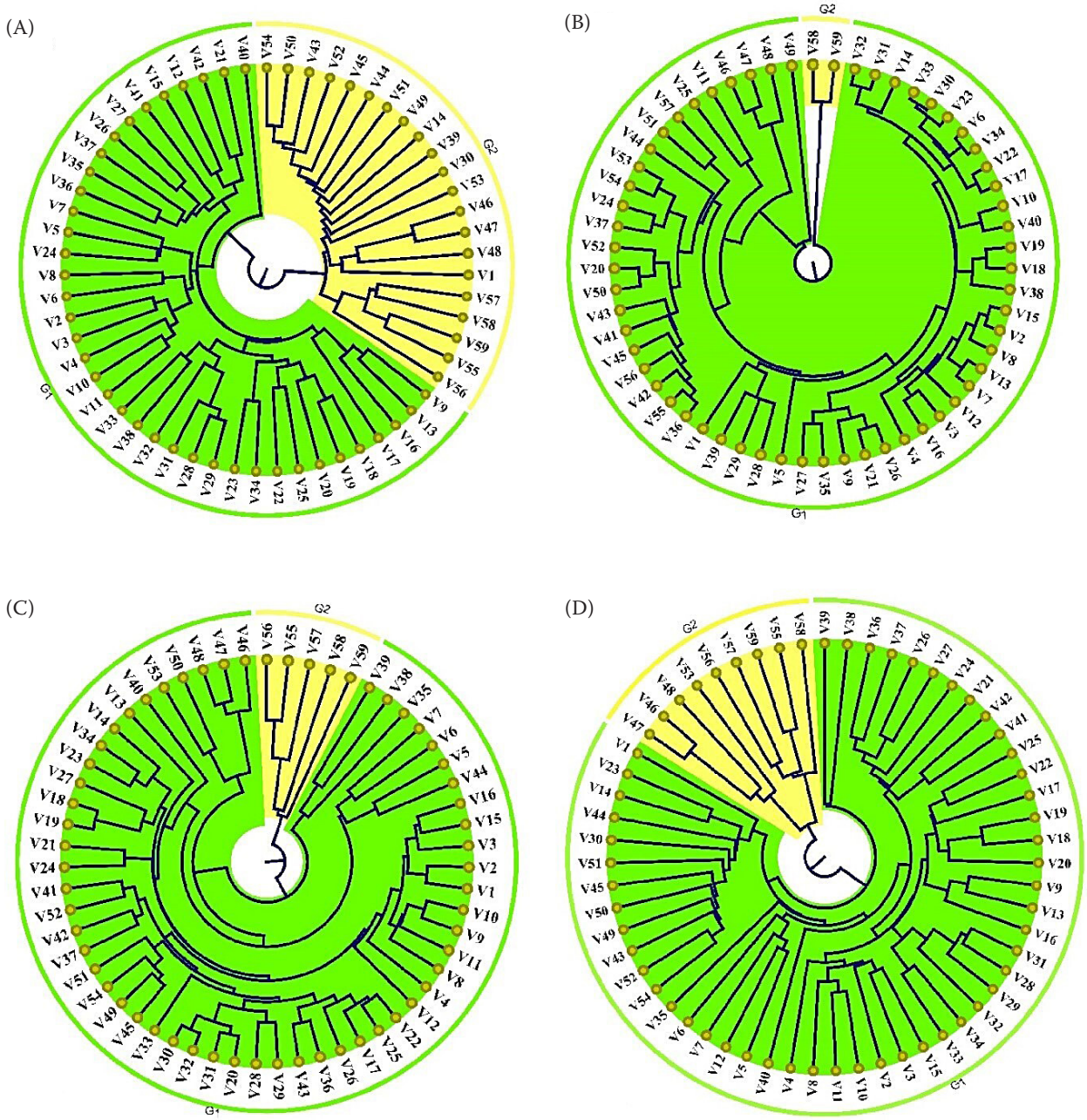

Figure 1. Dendrogram of 59 barley accessions constructed by following unweighted pair-group average methods, that distributed the accessions on 2 major groups with different sub-clusters: based-on cluster analysis of ISSR markers data set (A), based-on cluster analysis of CDDP markers data set (B), based-on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

ISSR – inter simple sequence repeat; CDDP – conserved DNA-derived polymorphism; SCoT – start codon targeted; the whole name of the accessions is described in Table S1 in ESM

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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 (Yongcui et al. 2005; Wang et al. 2009; Etminan et al. 2016). 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 V49 accession, while the second subcluster in the same group separated the barley accessions into the two sets of arrangements. The first sub-sub-cluster was composed of three accessions (V46, V47, and V48), which were cultivated by the farmer in the north of Iraq, and the remaining barley accessions were arranged in the second sub-subcluster based on genomic similarity. However, group 2 included only two barley accessions, namely V58 and V59, indicating their genomic differences from the rest of the accessions (Figure 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 (Figure 1C). The majority of barley accessions were grouped into two subclusters within the first class. One sub-cluster consisted of three barley accessions, whereas the other sub-cluster distributed the greatest number of barley accessions among the three sub-sub-clusters. The first sub-sub-cluster consisted of four barley accessions that were dispersed and grown mostly in the southern region of Iraq. Five barley accessions made up the second sub-sub-cluster. The farmer widely cultivated these barley accessions in the 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 V55, V56, V57, V58, and V59, 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).

Interestingly, the general dendrogram constructed using the combined data of all molecular markers used in our investigation (ISSR, CDDP, and SCoT) (Figure 1D) divided barley accessions into two major clusters. A total of fifty barley accessions were evenly dispersed throughout the first cluster. Some barley accessions were clearly clustered in this group based on genetic distance, especially the V38 and V39 accessions; the remaining forty-eight accessions were sorted into six sub-sub-clusters. Conversely, the second cluster consisted of nine barley accessions. 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. 2021). Other research groups have been using this molecular method extensively to assess the genetic variation among plant species (Saidi et al. 2018; Talebi et al. 2018; Liu et al. 2020). 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 (Tiwari et al. 2016; Amom & Nongdam 2017; Khodaee et al. 2021). 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 & Nienhuis 1995).

Population structure profile in barley accessions. STRUCTURE software and the Bayesian statistical

https://doi.org/10.17221/112/2022-CJGPB

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 analysing 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).

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). In order to get a better grasp on the underlying genetic structure of this population, it was necessary to do cluster analysis, as was described earlier. The cluster analysis produced from the molecular markers data set was found to be perfectly consistent with the outcomes of these investigations. This meets our expectations better, as it was observed that the accessions from the neighbouring 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 (Figures 2, 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 colours (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 colours 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 colour including individuals of pure genetic make-up (with the probability of ≥ 80%), which comprised 16, 28, 12, and 15 barley accessions for ISSR, CDDP, SCoT, and combined molecular dataset, respectively (Figures 2, 3). Furthermore, the second population, depicted in green, included barley accessions with a pure genetic background (with a probability of ≥ 80%). This distinct population basically contained 22, 26, and 29 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 the genome from other populations (Figures 2, 3). 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 study provides more evidence that the lemma and palea of barley remain securely closed throughout the time when pollen is being released. 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 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 S1 in ESM) based on the molecular data set from ISSR, CDDP, SCoT and the combined data of all three marker systems, respectively, while the analysis 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 S5 in ESM), 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

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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 grouping and structural analyses were shown accurately by the AMOVA results. According to the results of the partitioning of molecular variance study, the greatest amount of divergence was found

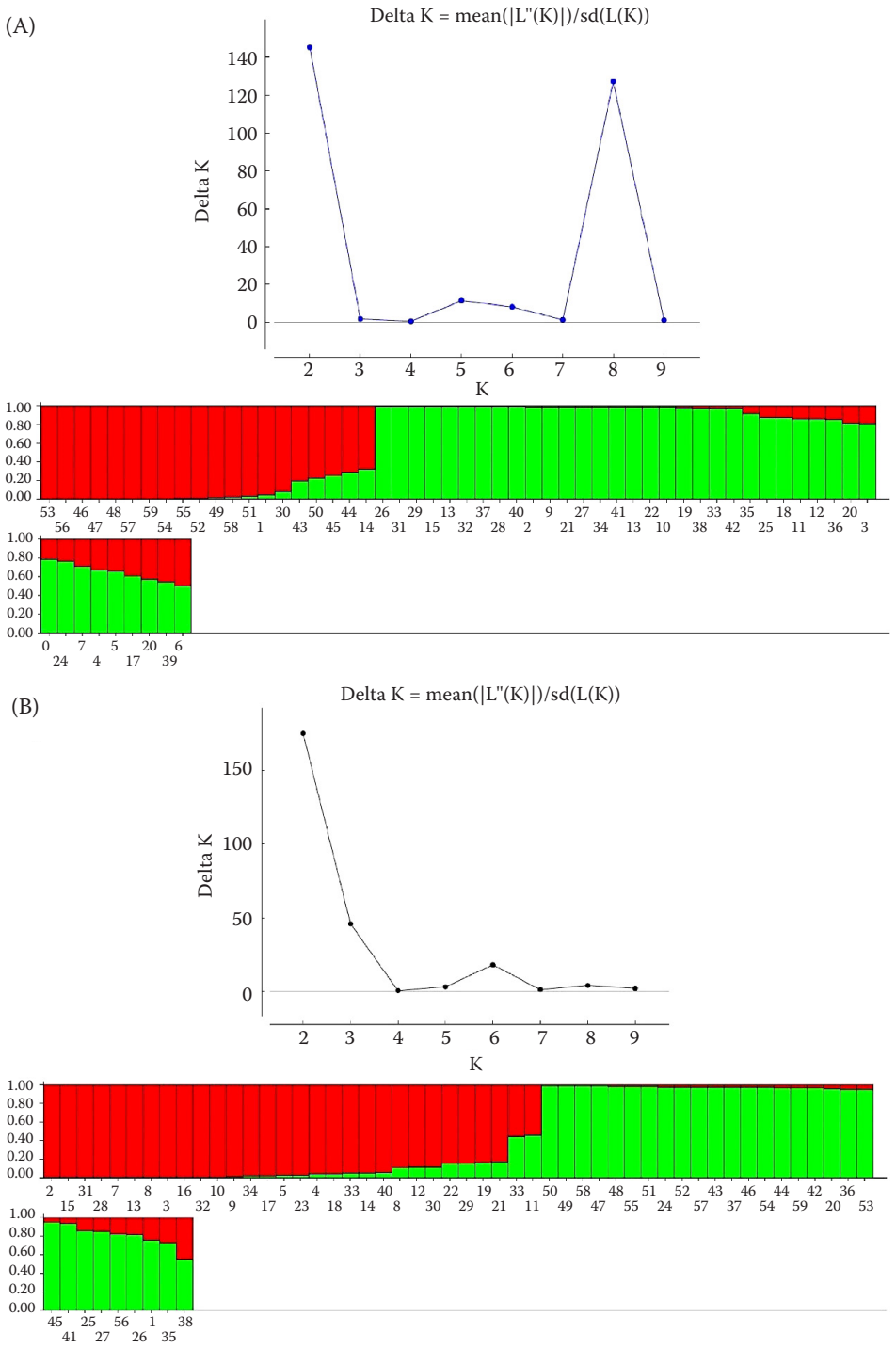

Figure 2. Determination of the optimal value of K and population structure profile of 59 barley accessions: based on the ISSR marker data set (A), based on the CDDP marker data set (B)

ISSR – inter simple sequence repeat; CDDP – conserved DNA-derived polymorphism; the numbers represent individual codes in the horizontal axis, and each colour represents a sub-population; the full name of the accessions is defined in Table S1 in ESM

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among individuals that belonged to the same group. Genetic diversity within and between populations improves the selection of populations that account for the vast majority of extant variations.

Three markers genetic dissimilarity correlation analysis. The Mantel test was used to compare the genetic distance matrices generated by three marker systems (ISSRs, CDDP, and SCoT). However, this tech-

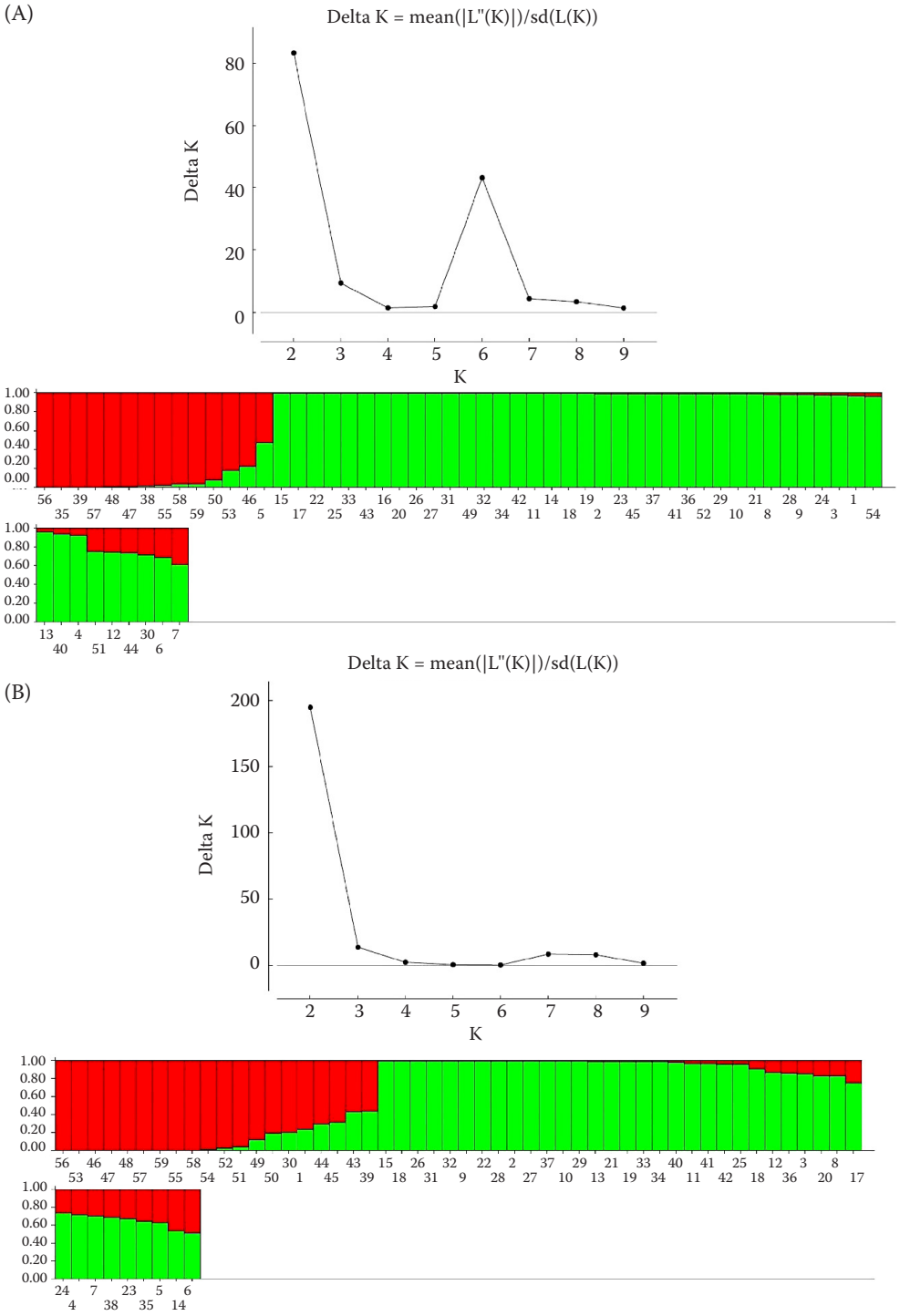

Figure 3. Describing the optimal value of K and population structure of 59 barley accessions: based on the SCoT marker data set (A), based on the ISSR, CDDP, and SCoT markers data set (B)
ISSR – inter simple sequence repeat; CDDP – conserved DNA-derived polymorphism; SCoT – start codon targeted; the numbers define accession codes on the horizontal axis, while each colour represents a sub-population; the complete name of the accessions is designated in Table S1 in ESM

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nique has limitations, particularly when employing two distinct markers (Vieira et al. 2007). In the case of our investigation, 44 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 S6 in ESM). The highest Mantel value was observed between ISSR and CDDP markers (0.49), while the minimum was displayed between CDDP and combined data markers (0.25). 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 the previous research of Ahmed et al. (2021), who detected significant associations between CDDP and SCoT markers.

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.

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