Genetic variability and population structure of Agropyron desertorum accessions from Iran based on ISSR assay

Hamid Hatami Maleki (hatamimaleki@yahoo.com)
University of Maragheh
Reza Mohammadi
ABRII: Agricultural Biotechnology Research Institute of Iran
Mousa Arshad
Islamic Azad University of Mahabad
Mina Hasanzadeh
University of Maragheh
Maryam Rafiee
Urmia University

Research Article

Keywords: Agropyron, Forage plant, Genetic diversity, Heterotic group

Posted Date: February 7th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2535655/v1

Version of Record: A version of this preprint was published at Genetic Resources and Crop Evolution on April 27th, 2023. See the published version at https://doi.org/10.1007/s10722-023-01579-8.

Abstract

The genus *Agropyron* as forage plant has several species which represented resistance against environmental stresses. DNA markers possessed a key role in assessment of plant germplasm and parental selection. In this regard, the genetic diversity of 34 *A. desertorum* accessions was studied using 28 ISSR primers. A total of 448 loci were amplified in *A. desertorum* genome that 402 (90%) out of them were polymorphic. The primer $(AG)_8YC$ produced the greatest number of polymorphic fragments while the primer $(AC)_8YA$ produced the lowest number. The number of effective alleles ranged between 1.26 and 1.57. The highest value of Shanon index was belonged to primer $(GA)_8T$ while the highest value of Nei index (0.33) was detected for primers $(GA)_8T$, $(TC)_8C$, and $(GACA)_4$. Primer $(GA)_8GCC$ with PIC = 0.40 was the best informative marker in evaluation of *A. desertorum* genetic diversity. Genetic similarity among studied accessions was between 0.45 (G03 and G17) and 0.80 (G01 and G11). Using Bayesian and WPGMA classification algorithms, the *A. desertorum* germplasm was divided into two major subgroups (Red and Green) consisted of 13 and 17 accessions and also admixture accessions. Late mature accessions were distinguished from early mature accessions and mostly localized in Green subgroup. The Red subgroup had more private allele as well as heterozygosity compared with Green subgroup and therefore had also promising selection potential. Totally, ISSR markers could distinguished early mature genotypes and this is significant for more forage yield. Likewise, the recognized heterotic groups could help breeders to select highly polymorphic genotypes in polycross breeding.

Introduction

The genus *Agropyron* from family Poaceae is a close relative of genus *Triticum* and it has got approximately 150 species with a wide distribution worldwide (Sakamoto 1964). Albeit, most species of this genus are autotetraploid but diploid, tetraploid and hexaploid species were also reported for it. Autotetraploid and diploid species are found in Europe and Asia meanwhile hexaploid species are rare in mentioned continents and have been seen mainly in Turkey, Iran and Georgia (Copete et al. 2018). *Agropyron* represented as excellent source of animal fodder and is a suitable habitat for domestic animals and wildlife. Plants of this genus are important in weed control, soil stabilization and watershed management and are a valuable gene pool for breeding bread wheat cultivars (Li et al. 2017; Absattar et al. 2018). Moreover, valuable characteristics such as tolerance to biotic and abiotic stresses including low temperature, salinity, drought, diseases and pests can be find among *Agropyron* plants and therefore, protecting the gene pool of this plant leading to improving pastures and increasing fodder production. (Arghavani et al. 2010). Regarding Bor (1970) reports, 19 out of 150 species of *Agropyron* can be find in country Iran. *Agropyron desertorum* is one of prevalence species in Iran and has high growth potential, high yield, good quality and high nutritional value. It also has a high resistance to drought and cold stresses and has the ability to stabilize sand and is generally adapted to slightly humid to dry weather conditions in the steppe or desert areas (Jafari et al. 2008). Due to mentioned important attributes, *A. desertorum* is suitable candidate for improving pastures in arid and semi-arid regions.

Genetic diversity is the adaptation mechanism of living organisms in response to environmental changes and so, low level of genetic diversity resulted in lower chance to conquer the environmental changes. The measurement of genetic diversity plays an important role in various aspects such as revealing patterns of genomic differentiation, allelic richness, selecting suitable parents, improving populations, reducing genetic vulnerability and increasing genotypic adaptation to different natural environments (Alam et al. 2015; Govindaraj et al. 2015). On the other hand, considering the importance of biodiversity development in agriculture, ecology, and conservation biology, germplasm evaluation and accurate identification of plant species is very important (Ayaz et al. 2020). DNA markers provide a robust estimation of genetic variability that could not obtain using phenotypic data alone. Opposite to exceeding implementation of phenotypic markers in plant germplasm assessment, they have some disadvantages such as lack of stability against environmental changes, time-consuming, depending on the plant growth stage, and their small number (Salgotra and Stewart 2020). Unlike phenotypic markers, genome based DNA markers are not affected by environmental changes and plant growth stages, and due to their large number in the genome, a large number of genetic characteristics can be evaluated in genotypes panel through relatively simple and cheap methods. Among DNA markers, ISSRs (Inter simple sequence repeats) are pieces of DNA with a size of about 100–3000 bp that is between two microsatellite regions in opposite directions (Hayward et al. 2015). ISSR marker system, is highly polymorphic, fast, and cost-effective which do not require any information about the genome sequence. Hence, ISSR markers could be more efficiently engaged in germplasm assessment, genome mapping, evolutionary biology, and taxonomic studies (Reddy et al. 2002). There are several studies about the usage of molecular markers in evaluation of genetic diversity of *Agropyron* species. Some studies were conducted to analyze genetic variability within and among *Agropyron* species. For instance, Che et al. (2015) was successfully implemented 29 polymorphic SSR markers to distinguished collected *Agropyron* species from China including *A. desertorum, A. mongolicum, A. michnoi, and A. cristatum*. In the other study, Absattar et al. (2018) evaluated three species of *Agropyron* (A. *desertorum*, *A. fragile*, *A. cristatum*) collected from Kazakhstan by using DArT technology and reported that of *A. desertorum* and A. fragile are similar with together meanwhile had dissimilarity with *A. cristatum*. Recently Han et al. (2021) used chloroplast genomic markers in assessment of five typical *Agropyron* species and reported A. mongolicum is closely related to *A. michnoi*, meanwhile others such as *A. desertorum* have a closer genetic relationship with the Triticum genus. Despite of reports about

genetic differentiation of *A. desertorum* from other species, there is narrow research about the genetic variability among *A. desertorum* accessions specially with ISSR marker system. About application of ISSR markers, Farshadfar et al. (2018) evaluated genetic variability among 13 *A. desertorum* accessions collected from several regions of Iran by 12 ISSR primers and classified them into four distinct groups. Regarding limited research works related with variability among *A. desertorum* accessions as well as application of molecular markers in identification of distant parental genotypes, this project was conducted to evaluate the genetic variability and population structure of several *A. desertorum* accessions from Iran.

Materials And Methods

Plant material, Genomic DNA extraction and ISSR assay

A suite of 33 *A. desertorum* domestic accessions accompanied with one foreign accessions from America were kindly supplied by gene bank of Agriculture and Natural Resources Research and Education Center of Isfahan province, Iran (Table 1). The code, origin and attributes of the studied accessions are presented in Table 1. Regarding accessions information (Table 1), some accessions have the same code but with differed numbers for example accessions 1000/262-1, 1000/262-2 and 1000/262-3 which showing originating from common plant. Since, *A. desertorum* is cross pollinated plant therefore each of such mentioned derived plants could be having differed attributes. Previous reports (Rahnemoun et al. 2018) showed that this examined *A. desertorum* germplasm represents discrepancy in date of maturity as important phenological trait. In the present work, seeds of each accessions are grown in a plastic pots at greenhouse state. Afterward achieving plants to sufficient growth stage, the leaves of plants in each pot were excised and mixed for genomic DNA extraction. Genomic DNA was extracted according to method defined by Doyle and Doyle (1987). The quality of the DNA was checked by running 1µl DNA in 0.8% (w/v) gels in 0.5X TBE buffer (45mM Tris base, 45mM boric acid, 1mM EDTA pH 8.0). Also, concentration of DNA samples was determined spectrophotometrically at 260 nm (BioPhotometer 6131; Eppendorf, Hamburg, Germany). Finally, DNA samples with a concentration of 20 ng/mol of genomic DNA were prepared for amplification in polymerase chain reaction.

Table 1
List of 34 *A. desertorum* accessions, maturity type, the subpopulation achieved based on Structure analysis, and subpopulation membership (Q-value) respectively

No.	Genbank Code[a]	Province/ Region	Q1	Q2	Subgroup	No.	Genbank Code	Province/ Region	Q1	Q2	Subgroup
G01	1000/262-1 EM	Isfahan/ Daran	0.888	0.112	Red	G18	1000-246 EM	Isfahan/ Daran	0.988	0.012	Red
G02	1000/352-1 EM	East Azerbaijan	0.991	0.009	Red	G19	1000/172-4 LM	Isfahan/ Chadegan	0.005	0.995	Green
G03	1000/11 EM	Isfahan	0.992	0.008	Red	G20	1000/21 LM	America	0.03	0.97	Green
G04	1000/190-1 EM	Isfahan	0.994	0.006	Red	G21	1000/88-3 LM	Isfahan	0.022	0.978	Green
G05	1000/172-1 EM	Isfahan	0.687	0.313	Mixed	G22	1000/188-2 EM	Tehran	0.086	0.914	Green
G06	1000/262-2 EM	Isfahan/ Daran	0.024	0.976	Green	G23	1000/334-2 EM	Chaharmahal and Bakhtiari / Borujen	0.076	0.924	Green
G07	1000/172-2 EM	Isfahan/ Chadegan	0.236	0.764	Green	G24	1000/334-3 EM	Chaharmahal and Bakhtiari/ Borujen	0.963	0.037	Red
G08	1000/424-1 LM	Chaharmahal and Bakhtiari / Borujen	0.087	0.913	Green	G25	1000/418 EM	Isfahan/ Daran	0.994	0.006	Red
G09	1000/107-1 LM	Isfahan	0.029	0.971	Green	G26	1000/352-2 EM	East Azerbaijan	0.966	0.034	Red
G10	1000/446 LM	Chaharmahal and Bakhtiari	0.011	0.989	Green	G27	1000/188-3 EM	Tehran	0.007	0.993	Green
G11	1000/262-3 LM	Isfahan/ Daran	0.007	0.993	Green	G28	1000/158-1 EM	Isfahan	0.054	0.946	Green
G12	1000/169 EM	Isfahan	0.965	0.035	Red	G29	1000/158-2 LM	Isfahan	0.009	0.991	Green
G13	1000/188-1 EM	Tehran	0.508	0.492	Mixed	G30	1000/107-2 EM	Isfahan	0.739	0.261	Red
G14	1000/172-3 EM	Isfahan/ Chadegan	0.967	0.033	Red	G31	1000/190-2 LM	Isfahan	0.009	0.991	Green
G15	1000/88-1 EM	Isfahan	0.99	0.01	Red	G32	1000/115 LM	Isfahan	0.009	0.991	Green
G16	1000/88-2 EM	Isfahan	0.093	0.907	Green	G33	1000/107-3 EM	Isfahan	0.94	0.06	Red
G17	1000/334-1 EM	Chaharmahal and Bakhtiari	0.993	0.007	Red	G34	1000/424-2 EM	Chaharmahal and Bakhtiari / Borujen	0.597	0.403	Mixed

[a] EM: early mature, LM: late mature

In this project, 28 ISSR primes (Table 2) were implemented in molecular diagnostics. For ISSR analysis, the polymerase chain reaction (PCR) was carried out in volume of 13 µl using Biometra UNO II thermo-cycler instrument. The reaction mixture containing 20 ng DNA, and 6 µL master mix (dNTP, $Mgcl_2$, Taq DNA polymerase), 5 µL ddH2O, and 1 µL primer. ISSR assay was done as following steps: an initial step of 4 min at 94°C, followed by 35 cycles of 94 ° C for 45 s, 52°C to 54°C for 45 s (Annealing temperature which varied based on primer type), 72 ° C

for 2 min, and 10 min at 72°C. The reaction products were mixed with an equal volume of formamide dye (98% formamide, 10mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol) and resolved in 2% (w/v) agarose gel (0.5X TBE). Then, they were stained with ethidium bromide (1.0µg ml-1) and photographed under UV light.

Table 2
ISSR primers name, sequence as well as marker performance characteristics

Primer name	Sequence(5 $\rightarrow$ 3)	Total band	polymorphic band	Number of monomorph band	Ne	H	I	PIC
ISSR1	)GA)$_9$C	18	14	4	1.38	0.25	0.39	0.31
ISSR2	(CT)$_9$G	22	20	2	1.37	0.23	0.37	0.30
ISSR3	GT (CAC) $_7$	17	16	1	1.51	0.31	0.47	0.36
ISSR4	(CA)$_8$RG	27	22	5	1.28	0.19	0.32	0.24
ISSR5	(GA)$_8$YT	17	17	-	1.34	0.23	0.38	0.33
ISSR6	(GA)$_8$YG	18	17	1	1.32	0.21	0.35	0.30
ISSR7	(AG)$_8$YC	27	25	2	1.37	0.25	0.40	0.33
ISSR8	(AG)$_8$T	15	14	1	1.51	0.30	0.46	0.37
ISSR9	(GA)$_8$T	15	15	-	1.54	0.33	0.50	0.36
ISSR10	(GA)$_8$A	12	11	1	1.26	0.19	0.32	0.29
ISSR11	CCC (GT)$_7$	16	13	3	1.32	0.21	0.34	0.22
ISSR12	(TC)$_8$C	16	15	1	1.56	0.33	0.49	0.39
ISSR13	(AG)$_8$CC	21	19	2	1.35	0.23	0.36	0.31
ISSR14	(GT)$_8$YA	13	10	3	1.34	0.22	0.34	0.26
ISSR15	(AC)$_8$YT	13	12	1	1.34	0.23	0.38	0.32
ISSR16	(AC)$_8$YA	7	6	1	1.35	0.23	0.37	0.27
ISSR17	(AC)$_8$YG	19	18	1	1.45	0.28	0.44	0.35
ISSR18	(GACA)$_4$	19	16	3	1.57	0.33	0.49	0.35
ISSR19	(GGAGA)$_3$	22	19	3	1.35	0.24	0.38	0.31
ISSR20	(GA)$_8$YA	17	17	-	1.46	0.28	0.44	0.33
ISSR21	(GA)$_8$RC	7	7	-	1.40	0.25	0.38	0.30
ISSR22	(AC)$_8$GCT	11	8	3	1.48	0.28	0.42	0.27
ISSR23	(AC)$_8$TG	21	19	2	1.38	0.26	0.42	0.34
ISSR24	(TCC)$_5$TG	12	10	2	1.39	0.24	0.38	0.30
ISSR25	(AC)$_8$GT	17	15	2	1.29	0.20	0.33	0.26
ISSR26	(GA)$_8$GCC	13	13	-	1.46	0.30	0.46	0.40
ISSR27	ACT ACG ACT (TG)$_7$	9	11	2	1.48	0.28	0.42	0.31
ISSR28	ACT CGT ACT (AG)$_7$	7	7	-	1.41	0.26	0.41	0.27
Mean	-	-16	14.5	2.09	1.40	0.25	0.40	0.31

Data analysis

The PCR amplification products were scored for the presence (1) or absence (0) of each band across all 34 *A. desertorum* accessions and then used to construct a binary data matrix. Then, several statistics such as effective number of alleles (ne), Shannon's Information Index (I)

(Shannon and Weaver 1949) and Nei's genetic diversity (h) (Nei 1973) were calculated with the aid of the POPGENE program version 1.31 (Yeh et al. 1999). Also, the PIC value which described as polymorphism information content was calculated for each primer following this formula PIC = 2ƒ (1 – ƒ). In which: ƒ= the frequency of present bands in the developing gel; and 1-ƒ= frequency of absent bands. In this study, Jaccard similarity coefficient were calculated between each pair-wise comparisons based on binary data matrix. Then using Jaccard similarity matrix, cluster analysis has been applied to classify studied *A. desertorum* germplasm in Darwin software (Perrier and Jacquemoud-Collet 2006). To inferred population structure among studied *A. desertorum* accessions a model-based Bayesian approach in the software STRUCTURE 2.3.4 (Novembre 2016) was used. In this regard, 10 independent runs were performed with the setting the number of sub populations (K) from 1 to 10, burn in time and MCMC (Markov Chain Monte Carlo) replication number both to 500,000, and a model for admixture and correlated allele frequencies. Delta K (ΔK) based on the second order rate of change in the likelihood (Evanno et al. 2005) was used to represent the K value. Inferred ancestry estimates of individuals (Q matrix) were derived for the selected population (Pritchard et al. 2000). For more clarifying the genetic relationship among accessions the adegenet package in R software (Jumbart et al. 2010) was implemented. Discriminant analysis of principal components (DAPC) was used for testing genetic relationships between groups. It uses K-means clustering which decomposes the total variance of a variable into within-group and between-group components. The best number of subpopulations shows the lowest associated Bayesian Information Criterion.

Results

ISSR makers produced in A. desertorum

In this project, fingerprinting of 34 *A. desertorum* accessions was done using 28 ISSR primers and a total of 448 loci were amplified by 28 ISSR primers (Table 2). Sample of banding pattern produced by ISSR2 primer ((CT)9G) is shown in Fig. 1a. Results showed that, 402 (90%) out of 448 scored bands were polymorphic and the number of polymorphic bands for each primer varied from 6 to 25 (Table 2). The ISSR7 primer ($(AG)_8$YC) produced the greatest number of polymorphic fragments (25 bands), while the ISSR16 primer ($(AC)_8$YA) produced the lowest number (6 bands) (Table 2). The mean value of number of bands as well as polymorphic loci were 16 and 14.5 respectively (Table 2). The range of Ne value for a given primer across all accessions was 1.26–1.57, with an average of 1.40 (Table 2). Based on Nei and Shanon indices the existence of genetic variability within *A. desertorum* was proved. The highest value of H (0.33) was detected in ISSR9 ($(GA)_8$T), ISSR12 ($(TC)_8$C), and ISSR18 ($(GACA)_4$) with average of 0.25 (Table 2). Regarding Table 2, the highest value of I was belonged to ISSR9 ($(GA)_8$T) while the lowest value of it (0.32) was calculated for ISSR4 ($(CA)_8$RG) and ISSR10 ($(GA)_8$A). The PIC value was calculated to assess the marker performance in evaluation of genetic diversity. The range of PIC was varied between 0.22 for ISSR11 primer (CCC $(GT)_7$) to 0.40 for ISSR26 primer ($(GA)_8$GCC) with an average value of 0.31 (Table 2).

Molecular variation and relationship among A. desertorum accessions

The genetic similarity among studied accessions was calculated by Jaccard's pair-wise similarity coefficient. The lowest amount of similarity (0.45) was seen between G03 and G17, whereas the highest amount of similarity (0.80) was seen between G01 and G11 (Table 3). The mean value of Jaccard's similarity coefficient was 0.64. Optimum number of subpopulations in the studied *A. desertorum* collection was estimated to be two (K = 2) (Fig. 1b). Via Bayesian classification algorithm, the studied *A. desertorum* germplasm was divided into two major subgroups (Fig. 1b). The group membership coefficient (Q values) related for each accessions were depicted in Table 1. In this regard, accessions G05, G13 and G34 out of studied accessions recognized with admixture genome (Table 1).

A phylogenetic tree (Fig. 1c) was constructed based on the shared-allele distance between individuals, using the WPGMA algorithm in Darwin (Li et al. 2010). The first (Red) and second (Green) groups consisted of 13 and 17 accessions of *A. desertorum* respectively, and the third group encompassed the 4 remaining accessions which had to somewhat a mixed genome. Analysis of amplified loci through two inferred subpopulations (Fig. 1d) revealed some discrepancies between these subpopulations so that the number of amplified loci across Red and Green subpopulations were 416 and 414 loci respectively. Our results showed the existence of 29 and 27 private loci related with Red and Green subgroups (Fig. 1d). Likewise, the heterozygosity of Red subpopulation was higher that Green subgroup. Density plot of individuals along the first discriminant function also indicated the presence of two groups based on Bayesian information criterion (BIC) (Fig. 2a). High genetic diversity was observed in green group. Green and Red colored peaks are completely distinct and there is just partial overlap. Graphs comparing distribution of the original accessions classification to sub-populations (Fig. 2b) showed that the accessions were subdivided into two subgroups.

Discussion

Multi-locus amplification as well as no prior information about genomic sequence, made ISSR markers as reliable marker system for fingerprinting, diversity analysis and genome mapping (Godwin et al. 1997). In this study, 28 ISSR primers were used for genetic diversity

analysis of 34 *A. desertorum* accessions and the efficacy of ISSR markers in clarifying *A. desertorum* variation was detected. Our results presented a total of 448 DNA fragments with an average of 16 bands per primer which majority of them was polymorph. Paralleled with our findings, Mohammadi et al. (2020) was used 10 ISSR primer in genetic diversity analysis of 48 accessions of *Festuca ovina* L. and reported 189 loci which all of them was polymorph. Similarly, this researchers (Mohammadi et al. 2023) utilized ISSR marker for assessment of 46 grass genotypes belonged to nine species collected from different regions of Iran and Hungary and reported 28 amplified loci per ISSR primer which 100% of them was polymorph.

Regarding our observed allelic polymorphism for ISSR markers and comparing to above mentioned findings, it can be expected that ISSR marker system can act as a powerful tool in identifying and separating different *Agropyron* accessions.

Overview of presented genetic diversity statistics including H, I and PIC values depicted that ISSR primers ISSR26 ($(GA)_8GCC$) with highest value of PIC and ISSR9 ($(GA)_8T$) with highest values of H and I had significant role in differentiation of studied *A. desertorum* accessions from each other. The PIC value imply on the power of differentiation related to each marker which resulted by its polymorphic alleles so, the highest value of this item present the efficacy of a marker in distinguishing genotypes. As reported by Serrote et al. (2019), for dominant marker like ISSR the PIC value ranged between 0 to 0.5 and informativeness of a marker can be recognized by PIC fluctuation and markers with value between 0.3 to 0.4 calculated as highly informativeness. In this regard, 20 ISSR primers out of studied primers are highly informative. Our results pertaining to Jaccard`s similarity coefficient among studied accessions showed the existence of genetic variability among studied accessions. On the other hand, we found rigorous intra species genetic variability for *A. desertorum* which is in accommodation with findings of Che et al. (2015). In detailed, Che et al. (2015) collected 103 *Agropyron* accessions (18 *A. desertorum*, 12 *A. mongolicum*, 4 *A. michnoi*, and 69 *A. cristatum*) from northern China and emphasized on the existence and magnitude of within species variation so that 88% of variation was seen within each species. This within species variability which found for *A. desertorum* accessions could be applied in its breeding programs through selection and hybridization of superior genotypes.

Regarding existence of different allelic frequencies, a population can be divided into different subgroups and possessed a structure (Novembre 2016). In the present work, the studied *A. desertorum* germplasm divided into two subpopulations and each studied accessions had own membership coefficient (Q value) which comparing by threshold value of 0.7 (Spataro et al. 2011) depicted its subgroup. Here, four accessions were admixture accessions that can be consequence of gene flow phenomenon (Frankham et al. 2002). Factors such as pollen transmission by wind and human activities, as well as migration or shared ancestor are involved in gen flow. Oppositely to population structure analysis, the WPGMA analyses illustrated the separation clearly and divided the accessions under study into three groups which that of admixture accessions was located in distinguished group. This finding also confirmed by DAPC illustration which verify the existence of two heterotic groups in the studied *A. desertorum* germplasm with some overlaps which could be due to existence of some admixture accessions (Razi et al. 2020).

The important point in presented classification is that the majority of *A. desertorum* accessions which located in Green subgroup, are late mature meanwhile the Red subgroup involved most of early mature accessions. Separation of early and late mature genotypes of *Agropyron* is momentous because there is negative correlation between phenological characters like flowering time followed by date of earing with forage yield and early mature plants not only have desired forage yield also have vital role in spring grazing. Early mature *A. desertorum* plants can complete their life cycle fast and therefore, could applied in dry areas. Late mature plant of *A. desertorum* is also important in cold regions. As resulted, the Red subpopulation had more private allele than Green subpopulation which imply on promising selection potential in Red subgroup compared with Green subgroup. The existence of private allele in studied accessions showing their evolutionary significance (Wu et al. 2020). On the other hand, all of studied accession has notable conservation value except for the accessions from Red subgroup with a higher proportion.

Conclusion

The ISSR marker system could efficiently clarify intra species genetic variability as well as population structure in the genus *Agropyron*. There is significant genetic diversity among *A. desertorum* accessions collected from Iran which can implemented in it`s future breeding programs. In this study, primer $(GA)_8GCC$ followed by primer $(TC)_8C$ with PIC value of 0.40 and 0.39 are informative primers that can proposed for genetic diversity analysis of any *A. desertorum* accessions. By application of ISSR markers, it is possible to differentiate two type of Agropyron including early mature and late mature accessions which is important from point of forage yield. In this project, two heterotic groups were identified by ISSR markers that could help breeders to select highly polymorphic genotypes of *A. desertorum* as polycross parents to achieve more heterosis in polycross breeding.

Declarations

Acknowledgment

The authors thank University of Maragheh and Agricultural Biotechnology Research Institute of Iran for lab facilities.

Funding

"This work was supported by university of Maragheh and Author Hamid Hatami Maleki has received research support from University of Maragheh "

Competing Interests

"The authors have no relevant financial or non-financial interests to disclose."

Author Contributions

"All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Reza Mohammadi and Hamid Hatami Maleki], [Mina Hasanzadeh, Mousa Arshad, Maryam Rafiee] and [Hamid hatami Maleki]. The first draft of the manuscript was written by [Hami Hatami Maleki] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript."

Data Availability

"The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request."

References

1. Absattar T, Absattarova A, Fillipova N, Otemissova A, Shavrukov Y (2018) Diversity array technology (DArT) 56K analysis, confirmed by SNP markers, distinguishes one crested wheatgrass Agropyron species from two others found in Kazakhstan. Mol Breed 38:37
2. Alam MA, Juraimi AS, Rafii MY, Hamid AA, Arolu IW, Abdul Latif M (2015) Genetic diversity analysis among collected purslane (*Portulaca oleracea* L.) accessions using ISSR markers. CR Biol 338:1–11
3. Arghavani A, Asghari A, Shokrpour M, Mohammaddost C (2010) Genetic diversity in ecotypes of two Agropyron Species using RAPD Markers. Res J Environ Sci 4:50–56
4. Ayaz A, Zaman W, Saqib S, Ullah F, Mahmood T (2020) Phylogeny and diversity of lamiaceae based on rps14 gene in Pakistan. Genetika 52:435–452
5. Bor NL (1970) Gramineae. In: Rechinger KH (ed) Flora Iranica. Akademische Druk-u, vol 70. Verlagsanstalt, Graz, Austria, pp 571–573
6. Che Y, Yang Y, Yang X, Li X, Li L (2015) Phylogenetic relationship and diversity among Agropyron Gaertn. germplasm using SSRs markers. Plant Syst Evol 301:163–170
7. Copete A, Moreno R, Cabrera A (2018) Characterization of a world collection of *Agropyron cristatum* accessions. Genet Resour Crop Evol 65:1455–1469
8. Doyle J, Doyle J (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15
9. Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620
10. Farshadfar M, Nowrozi R, Safari H, Shirvani H, Amjadian M (2018) Assessment of genetic diversity in Agropyron desertorum accessions using ISSR molecular marker. Future of Food: J Food Agric Society 6:20–29
11. Frankham R, Briscoe DA, Ballou JD (2002) Introduction to Conservation Genetics. Cambridge University Press
12. Godwin ID, Aitken EA, Smith LW (1997) Application of inter simple sequence repeat (ISSR) markers to plant genetics. Electrophoresis 18:1524–1528
13. Govindaraj M, Vetriventhan M, Srinivasan M (2015) Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. Genet Res Int 2015: 431487

14. Han H, Qiu R, Liu Y, Zhou X, Gao C, Pang Y, Zhao Y (2022) Analysis of chloroplast genomes provides insights into the evolution of Agropyron. Front Genet 13:832809
15. Hayward AC, Tollenaere R, Dalton-Morgan J, Batley J (2015) Molecular marker applications in plants. Methods Mol Biol 1245:13–27
16. Jafari AA, Seydemohammadi AR, Abdi N, Madah AH (2008) Seed and hay production in 31 genotypes of desert wheatgrass (*Agropyron desertorum*) using drought tolerance indices. Iran J Range Desert Res 15:114–128
17. Jafari AA, Setavarz H, Alizadeh MA (2006) Genetic variation for and correlations among seed yield and seed components in tall fescue. J New Seeds 8:47–65
18. Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11:94
19. Lewontin R (1972) Testing the theory of natural selection. Nature 236:181–182
20. Li H, Jiang B, Wang J, Lu Y, Zhang J, Pan C, Yang X, Li X, Liu W, Li L (2017) Appling of novel powdery mildew resistance genes from *Agropyron cristatum* chromosome 2P'. Theor Appl Genet 130:109–121
21. Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321–3323
22. Novembre J (2016) Pritchard, Stephens, and Donnelly on population structure. Genetics 204:391–393
23. Perrier X, Jacquemoud-Collet JP (2006) DARwin software https://darwin.cirad.fr/
24. Wu Q, Zang F, Ma Y, Zheng Y, Zang D (2020) Analysis of genetic diversity and population structure in endangered *Populus wulianensis* based on 18 newly developed EST-SSR markers. Global Ecol Conserv 24:e01329
25. Rahnemoun B, Hatami Maleki H, Mohammadi R (2018) Genetic variability in different accessions of Agropyron based on morphological traits. Modares J Biotechnol 9:517–523
26. Razi M, Amiri ME, Darvishzadeh R, Doulati Baneh H, Alipour H, Martínez-Gómez P (2020) Assessment of genetic diversity of cultivated and wild Iranian grape germplasm using retrotransposon-microsatellite amplified polymorphism (REMAP) markers and pomological traits. Mol Biol Rep 47:7593–7606
27. Reddy MP, Sarla N, Siddiq EA (2002) Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica 128:9–17
28. Mohammadi R, Panahi B, Amiri S (2020) ISSR Based Study of Fine Fescue (*Festuca ovina* L.) Highlighted the genetic diversity of Iranian accessions. Cytol Genet 54:257–263
29. Mohammadi R, Amiri S, Montakhabi Kalajahi V (2022) ISSR markers efficiency to assess cool-season grass species genetic diversity and phylogenetic relationships. Proc Natl Acad Sci India Sect B Biol Sci 92:691–699
30. Serrote CML, Reiniger LRS, Silva KB, Rabaiolli SMDS, Stefanel CM (2019) Determining the polymorphism information content of a molecular marker.Gene5;144175.
31. Sakamoto S (1964) Cytogenetical problems in *Agropyron* hybrids. Seiken Jiho 16:38–47
32. Salgotra RK, Stewart CN (2020) Functional markers for precision plant breeding. Int J Mol Sci 21:4792
33. Spataro G, Tiranti B, Arcaleni P, Bellucci E, Attene G, Papa R, Zeuli PS, Negri V (2011) Genetic diversity and structure of a worldwide collection of *Phaseolus coccineus* L. Theor Appl Genet 122:1281–1291
34. Yeh FC, Yang RC, Boyle T (1999) POPGENE version 1.31, Microsoft Window-based freeware for population genetic analysis. University of Alberta and Centre for International Forestry Research

Tables

Table 3 is available in the Supplementary Files section.

Figures

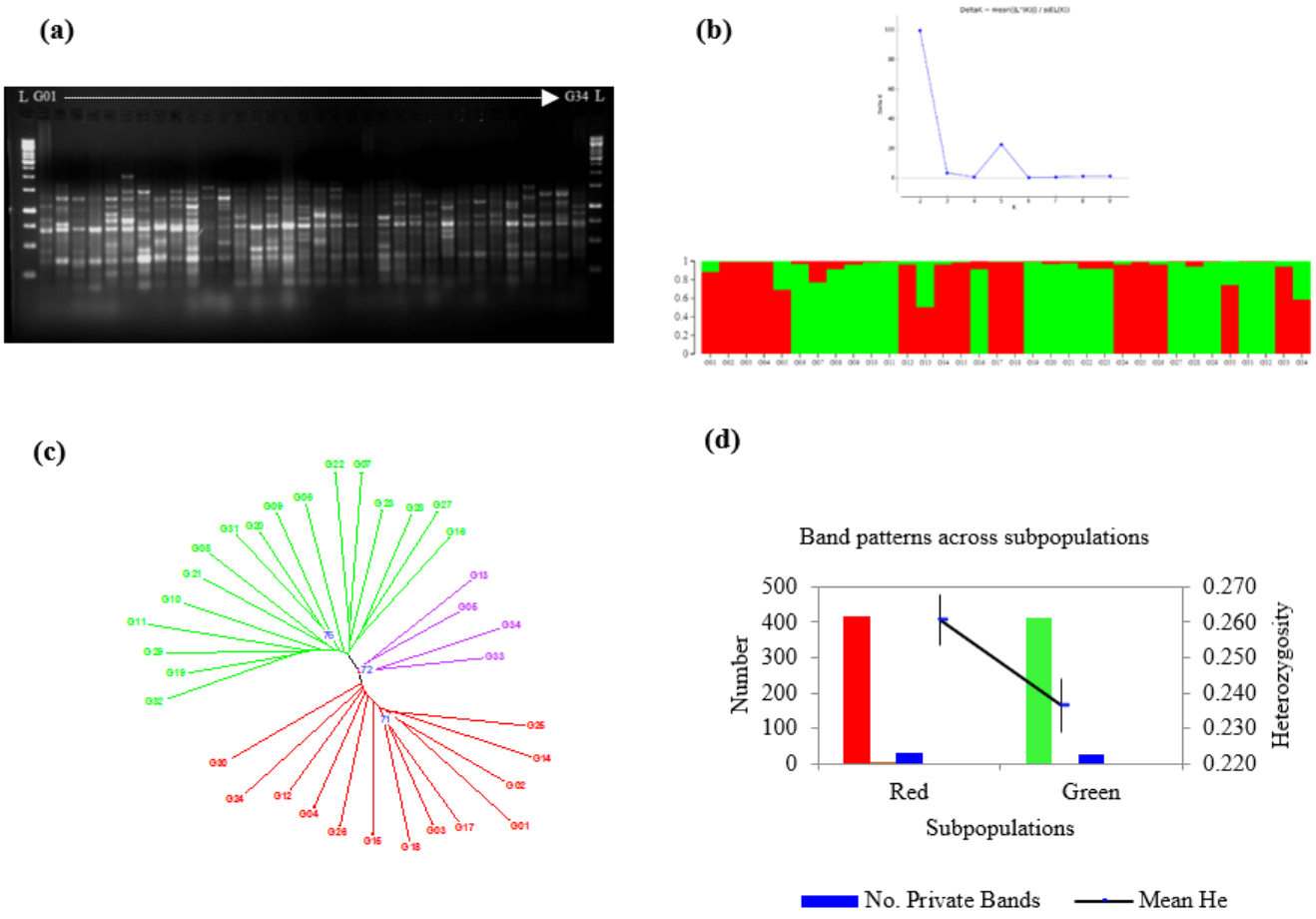

Figure 1

a: ISSR fingerprints of *A. desertorum* accessions using the ISSR2 primer ((CT)9G). M is 1 kb DNA ladder, **b**: *A. desertorum* genomic structure identified with ISSR markers in STRUCTURE software applying Bayesian method. At K = 2, the highest ΔK value was observed, **c**: A phylogenetic tree based on the shared-allele distance between individuals using the WPGMA algorithm, **d**: banding pattern of ISSR markers across two populations.

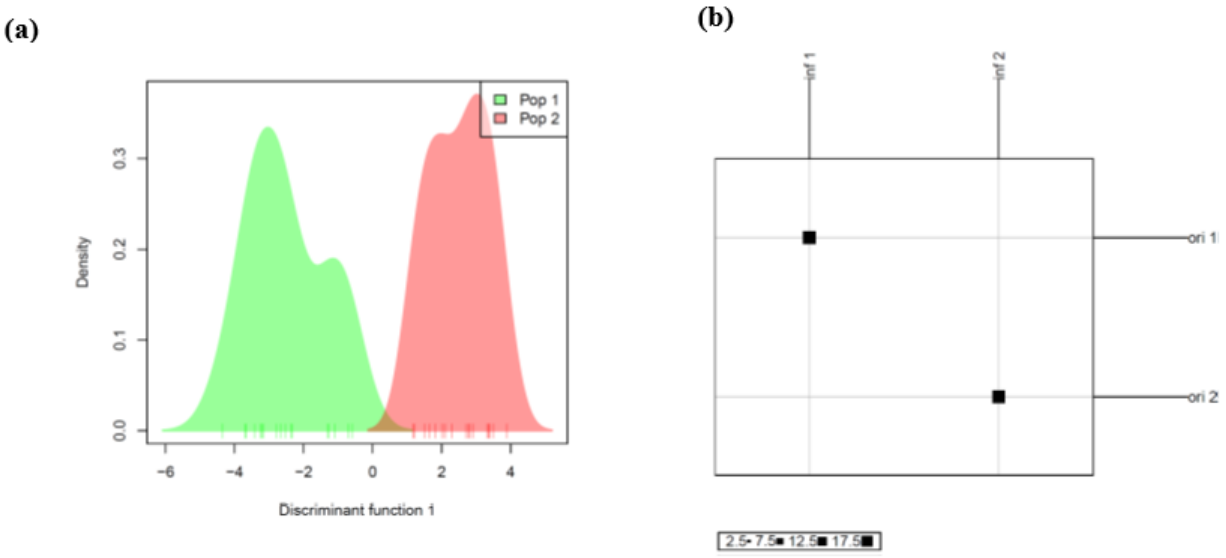

Figure 2

a: Density plot of individuals along the first discriminant function from the discriminant analysis of principal components for early mature and late mature accessions. **b:** Table graphs comparing distribution of the original accessions classification to the subpopulations using discriminant analysis of principal components with K = 2; K: Number of subpopulations. Ori 1: early mature; Ori 2: late mature

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table3.docx