The Journal of Animal & Plant Sciences, 27(5): 2017, Page: 1582-1587
ISSN: 1018-7081

EVALUATION OF GENETIC DIVERSITY IN A MINI CORE COLLECTION OF IRANIAN DURUM WHEAT GERMPLASMS

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ABSTRACT

Durum wheat is important cereal that is widely cultivated all over the world. In this study, genetic diversity in a mini core collection of the 43 durum wheat genotypes (including 25 breeding lines and 18 landraces) was investigated using fifteen inter-simple sequence repeat markers (ISSR). Based on the results, high levels of polymorphism (98.70%) were observed, which indicated that ISSR was efficient technique to detect the genetic variation of this collection. Analysis of molecular variance (AMOVA) revealed high genetic variability (90%) within breeding lines and landrace genotypes. Comparing the genetic diversity of breeding lines and landraces, based on genetic variation parameters, revealed that the effective number of alleles (*Ne*), Nei's gene diversity (*H*) and Shannon's information index (*I*) in the landrace genotypes is higher than breeding lines. Cluster analysis classified all individuals in five groups. Also, inter-population differentiation (*Gst*) estimated on the basis of these primers represented that, 93% of the total genetic variability refer to variation within two sets of genotypes. Overall, our results revealed a high level of genetic variation among a mini core collection of durum wheat, especially Iranian landraces, which can be of interest for conservation and future breeding programs.

Keywords: Durum wheat; genetic variation; landrace genotypes; ISSR marker.

INTRODUCTION

Durum wheat (*Triticum turgidum* L. subsp. *durum*), as one of the important cereal, is the only tetraploid species (2n=4x=28) of commercial wheat that is widely cultivated in Mediterranean climates (Pour-Siahbidi *et al*., 2013). In the future years, the world need for wheat will be greater than in the present days. Hence, in response to this challenge, breeders must increase the yield and simultaneously reduce the unfavorite impact of environment factors on the agricultural production systems (Todorovska *et al*., 2015).

Molecular marker techniques provide valuable information in crop breeding, especially in studies on genetic variability and relationships between different crop species. PCR-based DNA markers are used to characterize mostly neutral, unique, and moderately repetitive sequences of the genome (Lagudah and Hallorn, 1988). Among typical marker systems, ISSR is the most common marker based on polymerase chain reaction, and due to high annealing temperature and extended sequence in comparison to other techniques can produce more reproducible and reliable band patterns. and is also suitable for detecting genetic polymorphisms in different crops (Moradkhani *et al*., 2012). Furthermore ISSR markers, in most of plants, are more in demand because they are known to be repeatable, highly polymorphic, as well as very reproducible and highly informative (Borne and Branchard, 2001)

Genetic erosion in cultivated wheat collection provides a good motivation for investigating about genetic diversity in old varieties, and also determines the potential of wheat germplasm for improving efficiency and hence their use for breeding, which eventually may result in improved food production (Mardi *et al*., 2011; Moradkhani *et al*., 2012). In the present study, we used two sets of different durum wheat genotypes consisting of breeding lines as well as Iranian landraces. The main goals of this study were (i) to determine the genetic variability in mini core collection of durum wheat genotypes using ISSR markers, and (ii) to evaluate the informativeness of the used markers for detecting molecular variation.

MATERIALS AND METHODS

Plant material and DNA extraction: A total of 43 durum genotypes including 25 breeding lines and 18 landrace genotypes were considered in the current study (Table 1). After germination and growth, the total genomic DNA was extracted from young leaves of glasshouse-grown plants according to the CTAB protocol (Doyle and Doyle, 1987).

ISSR amplification and statistical analysis: After optimizing PCR, fifteen ISSR primers were selected from the set of Biotechnology Laboratory, University of British Columbia, Canada. The PCRs were performed in the reaction mixture 20 µL volume, with 10 µL master mix 2XPCR (ready-to-use PCR master mix 2X), 6 µL ddH_2O, 2 µL of isolated DNA from each sample and 2 µL of each primer. Amplification was run at 94 ºC for 4 min, followed by 30 cycles of denaturation at 94 ºC for 30 seconds, primer annealing at 45 ºC for 45 seconds and primer elongation at 72 ºC for 2 min. The final extension was 7 min at 72 ºC. The amplification reaction products were detected by 1.5% agarose gels stained with Ethidium Bromide under UV light.

Data analysis: To measure the informativeness of the ISSR markers, polymorphism information content (*PIC*), resolving power (*Rp*) and marker index (*MI*) parameters were calculated according to formula suggested by Anderson *et al*. (1993), Prevost and Wilkinson (1999) and Varshney *et al*. (2007), respectively.

To the distribution of the genetic variation within and among populations (breeding line and landrace genotype sets), analysis of molecular variance (AMOVA) was calculated using GenAlEx software (Peakall and Smouse, 2006). To characterize genetic variation, some of parameters including Shannon's information index (*I*), Nei's gene diversity (*H*), the observed (*Na*) and effective number (*Ne*) of alleles, gene flow (*Nm*) and inter-population differentiation (*Gst*) were estimated by POPGENE 1.31 software (Yeh *et al*., 1999). Cluster analysis based on Jaccard's similarity coefficients matrix was constructed using DARwin software (Perrier *et al.,* 2003).

Table 1. Code, name and pedigree of 43 durum wheat landraces and breeding lines.

No	Code/ name/pedigree	Type	No	Code/ name/pedigree	Type
1	46198	BL	23	M142045	BL
2	46172	BL	24	M142069	BL
3	46112	BL	25	M142070	BL
4	46078	BL	26	KC_643	LR
5	46061	BL	27	KC_659	LR
6	SORA/2*PLATA_12/3/SORA/2*PLATA_12 //SOMAT_3/4/AJAI	BL	28	KC.911	LR
7	SOMAT_4/INTER_8/4/GODRIN/GUTROS //DUKEM/3/THKNE	BL	29	KC.981	LR
8	NUS/SULA//5*NUS/4/SULA/RBCE_2/ 3/HUI//CIT71/CII*2/5/AR	BL	30	KC.1033	LR
9	CNDO/VEE//CELTA/3/PATA_2/6/ ARAM_7//CREX/ALLA/5/ENTE/MEXI	BL	31	KC.1047	LR
10	USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/ YAV_1/6/ARDENTE/7/HUI/YAV79/8/	BL	32	KC_2887	LR
11	AJAIA_12/F3LOCAL(SEL.ETHIO.135.85)// PLATA_13/3/PLATA_6/GREEN	BL	33	KC_3296	LR
12	TOPDY_18/FOCHA_1//ALTAR 84/3/AJAIA_12/F3LOCAL (SEL.ETHIO.135.85)//PLATA_13/4/SOMAT_3/GREEN_22	BL	34	KC_3399	LR
13	RASCON_37/4/MAGH72/RUFO//ALG86/ RU/3/PLATA_16/5/PORTO_3*2/6/ARMENT //SRN_3/NIGRIS_4/3/CANELO_9.1	BL	35	KC_3632	LR
14	M84859	BL	36	TN_12598	LR
15	M141979	BL	37	KC_678	LR
16	M141982	BL	38	KC.874	LR
17	M141994	BL	39	KC.963	LR
18	M141995	BL	40	KC_1298	LR
19	M142005	BL	41	KC_1648	LR
20	M142017	BL	42	KC_3296	LR
21	M142025	BL	43	TN_12736	LR
22	M142038	BL			

BL and LR indicate breeding line and landrace genotype, respectively.

RESULTS

Fifteen primers were selected to evaluate the genetic diversity between and within mini collection of durum wheat cultivated in Iran. A total of 155 bands were produced, out of which 149 (96.13%) were polymorphic. The number of polymorphic bands ranged from 7 (ISSR-9) to 15 (ISSR-25) with an average of 10.2. The *Rp* values ranged from 10.04 (ISSR-23) to 21.34 (ISSR-25). The highest value of *MI* was observed for ISSR-25 (6.15), while the lowest value observed for ISSR-11 (1.28). The *PIC* values ranged from 0.14 (ISSR-11) to 0.49 (ISSR-3, ISSR-5, ISSR-23 and ISSR-25) with a mean of 0.41 (Table 2). The results of AMOVA analysis

showed that, 10 and 90% of total variance refer to between and within populations, respectively (Table 3). Thus, this estimative of partitioning genetic variance revealed higher variability within breeding lines and landrace genotypes.

Table 2. Inter Simple Sequence Repeat (ISSR) primers and their amplification results generated in 43 genotypes of durum wheat.

Primer	Sequence (5′→3′)	*TAB*	*NPB*	*PPB*	*PIC*	*Rp*	*MI*
ISSR-1	$DBDA(AC)_7$	13	11	84.61	0.41	18.51	4.51
ISSR-3	$(GGAT)_4$	10	10	100	0.49	10.74	4.97
ISSR-4	$(AG)_8YT$	9	9	100	0.42	12.55	3.79
ISSR-5	$(AC)_8C$	11	11	100	0.49	11.67	5.47
ISSR-6	$(GA)_8RC$	12	12	100	0.37	17.95	4.52
ISSR-7	$(CT)_8C$	11	11	100	0.49	12.51	5.39
ISSR-9	$(CA)_8C$	8	7	87.5	0.40	11.53	2.81
ISSR-11	$(AC)_8YA$	9	9	100	0.14	16.60	1.28
ISSR-12	$(GT)_8YG$	9	9	100	0.47	11.02	4.27
ISSR-13	$(GA)_8YC$	10	8	80	0.43	13.62	3.47
ISSR-14	$(AG)_8T$	11	11	100	0.43	15.06	4.74
ISSR-15	$(AC)_8YC$	11	7	63.63	0.27	11.72	2.99
ISSR-23	$(CT)_8RC$	9	8	88.88	0.49	10.04	3.94
ISSR-25	$(CA)_8RG$	15	15	100	0.41	21.34	6.15
ISSR-28	$(TC)_8G$	11	11	100	0.49	12.27	5.42
Mean		10.33	10.20	93.61	0.41	13.81	4.25

Note: *TAB* total amplified bands, *NPB* number of polymorphic bands, *PPB* percentage of polymorphic bands, *PIC* polymorphism information content, *MI* marker index, *Rp* resolving power
Note: B = (non A), D = (non C), Y= (C, T) and R= (A, G)

Table 3. Analysis of molecular variance (AMOVA) based on ISSR for durum wheat genotypes.

Source of variation	*df*	MS	VC	TV (%)
Among Pop.	1	67.735	2.244	10
Within Pop.	41	20.774	20.774	90
Total	42		23.018	100

Note: *SS* sum of squares, *MS* mean squares, *VC* variance components, *TV* total variance

Summary of the genetic diversity indices in two sets of durum wheat genotypes is shown in Table 4. The average *Na* value was 1.96±0.19; the maximum (1.87±0.32) and minimum (1.78±0.41) ones being for breeding lines and landraces, respectively. The *Ne* value was invariably less than the *Na* value for each set, presenting a distinction in the range of 1.52±0.33 (in breeding lines set) to 1.55±0.38 (in landraces set), with a mean of 1.56±0.32. The mean Nei's gene diversity (*H*) value was 0.33±0.14, so that the highest and lowest values related to landraces (0.33±0.19) and breeding lines (0.30±0.16), respectively. Also, the mean of Shannon's information index (*I*) was 0.49±0.18; the maximum (0.44±0.27) and minimum (0.41±0.22) ones being for landraces and breeding lines, respectively. In addition, *Gst* determined on the basis of the ISSR markers was 0.07, indicating that 7% of the total genetic variability was among two sets of materials and 93% of the total genetic variability was within two sets of genotypes. Also, the *Nm* value among two sets of genotypes was 6.51.

Table 4. Summary of genetic variations between two sets of durum wheat genotypes as revealed through ISSR analysis.

Set	Observed no. of alleles (*Na*)	Effective no. of alleles (*Ne*)	Nei's genetic diversity (*H*)	Shannon's in formation index (*I*)	Percentage of polymorphic loci (*PPB*)	Inter-set differentiation (*Gst*)	Estima te of gene flow (*Nm*)
Breeding lines	1.87±0.32	1.52±0.33	0.30±0.16	0.41±0.22	87.74	0.07	6.51
Landraces	1.78±0.41	1.55±0.38	0.33±0.19	0.44±0.27	78.71		
Total	1.96±0.19	1.56±0.32	0.33±0.14	0.49±0.18	96.13		

In order to study genetic relationships among different genotypes, cluster analysis based on Jaccard's similarity coefficients matrix and UPGMA algorithm was done. As shown in Fig. 1, the 43 durum wheats were classified into two main groups (GI and GII). The first group derived into two sub-clusters. The first sub-cluster (SubI) consisted of 37 individuals, so that SubI-a with 24 individuals of breeding lines and landraces generated a distinguished cluster. Eleven breeding lines also placed in separate sub-cluster (SubI-b). The second main sub-cluster (SubII) included of two landraces and one breeding line. The second main group (GII) also consisted of three breeding lines.

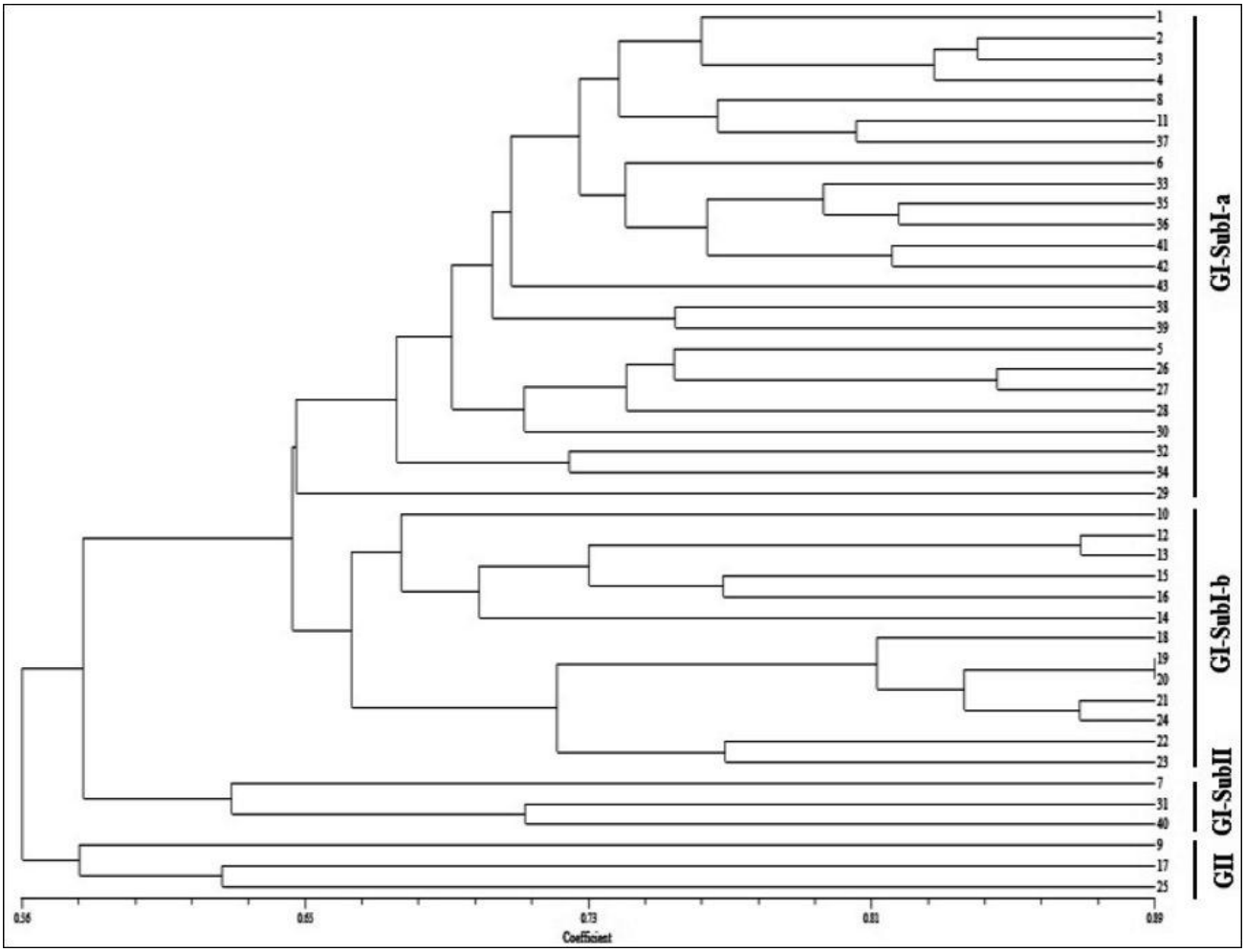

Fig. 1. Dendrogram of 43 durum wheat genotypes produced by Jaccard's similarity coefficient using UPGMA clustering method based on ISSR data (for explanation of accessions codes, see Table 1).

DISCUSSION

Germplasm evaluation helps to identify diverse genotypes, which can be utilized by breeding for creating the desired variation. Morphological characters may be affected by environmental conditions. Therefore, the use of morphological characters for classification may result in discrepancies (Abdel Kalil *et al*., 2014). In contrast, molecular characterization is now the favored means to quantify variation within plant genetic resources. Efficiency of a molecular marker system depends on the amount of polymorphism can discover among the set of individuals under investigation. ISSR marker has been proved to be useful in population genetic studies because of its high reproducibility and great power for the detection of polymorphism (Galvan *et al*., 2003; Cao *et al*., 2006). In the present study, we evaluated a mini collection of durum wheat including 25 breeding lines and 18 Iranian landraces, which are widely cultivated in various areas in Iran. Our results showed that there was high genetic variation among tested genotypes. These findings are consistent with results from previous study carried out on Iranian durum wheat (Mardi *et al*., 2011). In this investigation, the efficiency of ISSR marker was estimated through parameters such as polymorphism information content (*PIC*), marker index (*MI*) and resolving power (*Rp*). Parameters such as *PIC* have been used usually for evaluating the informative potential of ISSR markers in different germplasm and cultivated genotypes (Patra *et al*., 2008; Tatikonda *et al*., 2009; Gomes *et al*., 2009; Grativol *et al*., 2011; Moradkhani *et al.,* 2015; Yousefi *et al*., 2015; Pour-Aboughadareh *et al.,* 2017). According to the results of data analysis the *PIC* value ranged from 0.14 (ISSR-11) to 0.49 (ISSR-3, ISSR-5, ISSR-7, ISSR-23 and ISSR-25) with an average of 0.41. Thus, the high values of *PIC* for the ISSR primers could be attributed to evaluation of genetic diversity. In this study, the average *PIC* value per primer (0.41) was comparable to that found by Sofalian *et al*. (2009) in evaluation of genetic variation of Iranian wheat germplasm using ISSR markers. The marker index (*MI*), which can be considered to be a common measure of efficiency in discovering polymorphism (Yousefi *et al*., 2015), varied between 1.28 and 6.15 (with a mean 4.25) in different ISSR primers (Table 2). On the other hand, an important property of a suitable marker system is the capacity to distinguish among different accessions. Resolving power parameter described by Prevost and Wilkinson (1999) as a degree of the discriminatory power of ISSR molecular markers. Our study revealed the *Rp* values of primers ranged from 10.04 for ISSR-23 to 21.34 for ISSR-25 with an average of 13.81 for all primers. Furthermore, primer ISSR-25 was able to distinguish most of the 43 durum wheat genotypes tested. Consequently, our results were in conformity with the previous studies (Prevost and Wilkinson, 1999; Yousefi *et al*., 2015) that *MI* and *Rp* can be proposed as the best indictors for selecting most informative primers. Comparing the genetic variation of breeding lines and landraces, based on genetic parameters, showed that effective number of alleles (*Ne*), Nei's gene diversity (*H*) and Shannon's Information index (*I*) in landraces was higher than in breeding lines. Previously, the high level of genetic variation in Iranian durum wheat had reported been by Mardi *et al*. (2011) and El-Assal and Gaber (2012). The estimation of the genetic variance components was performed by AMOVA. The results of AMOVA revealed that variance within populations was higher than among them. Clustering of genotypes produced two main groups. So this dendrogram demonstrated a good level of genetic diversity within Iranian landraces. Furthermore, previously the results of AMOVA supported genetic variation within durum wheat germplasm.

Conclusion: The present study confirmed ISSR marker is an efficient tool to estimate the genetic variation of Iranian landraces and breeding lines. Additionally, the large polymorphic fragment percentage and number of polymorphic bands obtained in our study indicated the power of this marker in fingerprinting and diversity analyses. Our results revealed a high level of genetic diversity among a mini core collection of durum wheat, especially Iranian landraces, which can be of interest for conservation and future breeding programs.

Acknowledgements: The authors are grateful for financial support of Kermanshah Branch, Islamic Azad University. Also, the authors acknowledge and appreciate the comments of anonymous reviewers of the Journal of Animal and Plant Science.

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