www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article/Araştırma makalesi
DOI: 10.46309/biodicon.2021.818510
14/1 (2021) 35-39

Determination of genetic divergence in some bread wheat varieties by IRAP and ISSR analyses

Zekiye Budak BAŞÇİFTÇİ [1], Murat OLGUN [1], Serap SUNAR [*2], Nalan Yildirim DOĞAN [3], N. Gözde AYTER [1], Guleray AGAR [4]
ORCID: 0000-0002-4034-2537; 0000-0001-6981-4545; 0000-0002-2011-1117; 0000-0002-5344-5367; 0000-0002-5121-4303; 0000-0002-8445-5082

[1] Eskişehir Osmangazi University, Faculty of Agriculture, Eskişehir, Turkey.
[2] Erzincan University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Erzincan 24000, Turkey.
[3] Department of Biology, Faculty of Science, Erzincan University, Erzincan, Turkey
[4] Department of Biology, Faculty of Science, Ataturk University, Erzurum, Turkey

Abstract

In this study, it was aimed to detect the differences of genotypes through the measurement of genetic distance in bread wheat *(Triticum aestivum* L.) genotypes. The genetic diversity of 12 bread wheat genotypes (Bozkır, Harmankaya 99, Altay 2000, Yıldırım, Bezostaja 1, Ahmetağa, Müfitbey, Aldane, Es 26, Alperbey, Atay 85 ad Eraybey) was examined through ISSR and IRAP techniques. As fast molecular techniques, ISSR and IRAP methods can be effectively included in breeding programs both for genetic exploration and evaluation and for the protection of elite breeding and production materials. 12 genotypes were classified into two main clusters in the dendrogram produced by using the ISSR and IRAP markers. While Cluster I included Bozkır, Harmankaya 99, Altay 2000 and Yıldırım, cluster II included Bezostaja 1, Ahmetağa, Müfitbey, Aldane, Es 26, Alperbey, Atay 85 and Eraybey. Furthermore, the results obtained in this study indicated that ISSR and IRAP methods were effective for the definition of bread wheat genotypes.

Keywords: bread wheat genotypes, genetic difference, IRAP, ISSR

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Bazı ekmeklik buğday çeşitlerinin IRAP ve ISSR analizleri ile genetik farklılığının belirlenmesi

Özet

Bu çalışmada ekmeklik buğday (*Triticum aestivum* L.) genotiplerinde genetik uzaklık ölçümü yapılarak genotip farklılıklarının tespiti amaçlanmıştır. 12 ekmeklik buğday genotipinin (Bozkır, Harmankaya 99, Altay 2000, Yıldırım, Bezostaja 1, Ahmetağa, Müfitbey, Aldane, Es 26, Alperbey, Atay 85 ad Eraybey) genetik çeşitliliği ISSR ve IRAP teknikleri ile incelenmiştir. ISSR ve IRAP yöntemleri, hem genetik inceleme ve değerlendirme hem de elit yetiştirme ve üretim materyallerinin korunması için ıslah programlarına etkin bir şekilde dahil edilebilir. ISSR ve IRAP belirteçleri kullanılarak üretilen dendrogramda 12 genotip iki ana küme halinde sınıflanmıştır. İlk küme Bozkır, Harmankaya 99, Altay 2000 ve Yıldırım'ı içerirken, ikinci küme Bezostaja 1, Ahmetağa, Müfitbey, Aldane, Es 26, Alperbey, Atay 85 ve Eraybey'i içermektedir. Ayrıca bu çalışmadan elde edilen sonuçlar ISSR ve IRAP yöntemlerinin ekmeklik buğday genotiplerinin tanımlanmasında etkin olduğunu göstermiştir.

Anahtar kelimeler: ekmeklik buğday genotipleri, genetik farklılık, IRAP, ISSR

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +904462245344; Fax.: +904462245343; E-mail: ssunar@erzincan.edu.tr
© Copyright 2021 by Biological Diversity and Conservation Received: 02.11.2020; Published: 15.04.2021 BioDiCon. 936-1120

1. Introduction

The request for grain exports is also growing because of increased prosperity and the increase in world population. Nevertheless, the requirement for feed grains and the request for future energy production are in tendency to enhance domestic demand. To get more products from the existing regions is the best way to acquire the desired increase in production[1].

In the world, wheat is the main source of nutrient. It is predicted that there would be 773 million ton of production in 2020/21 (FAO 2020/21). For nutritional requirements, it is necessary that increased population should increase in wheat production. the adverse effect of drought and high temperatureon a crop can be minimized by avoiding stress at the most sen-sitive stages of crop development such as reproductive andgrain-filling periods [2, 3]. Genotype x environment interactions (G x E), which alter the rankings of genotypes from site to site and from season to season, have restricted the rate of genetic advance for the areas in such environments [4,5,1,6]. New wheat cultivars are improved by breeders with the aim of increasing total production. Besides, the yield performances of these new cultivars are tested in different places. The success of a new wheat variety is based on its yield and adaptation potential in those places [7].

Genetic diversity has an essential role in the success of crop breeding programs and new cultivar improvement. Knowledge of genetic diversity has been successfully employed for efficient germplasm management and utilization, genetic fingerprinting and genotype selection, novel biotechnological methods have been efficiently employed in genotypic differences of genotypes [8, 9, 10]. In this study, it was aimed to detect the differences of genotypes through the measurement of genetic distance in bread wheat genotypes and to show the usability of chromosome mapping for different aims such as breeding programs, elite seed productions [11, 12].

2. Materials and methods

This study was conducted at Osmangazi University Agriculture College Eskişehir experimental station during the 2015-2016 crop growing season(36º 56º North, 30º 32º East, 788 m altitude). 12 bread wheat genotypes (Bozkır, Harmankaya 99, Altay 2000, Yıldırım, Bezostaja 1, Ahmetağa, Müfitbey, Aldane, Es 26, Alperbey, Atay 85 ad Eraybey) were used in this study. The precipitation in 2015-2016 and long term years (1965-2016) was 342.1 mm and 322.6 mm, respectively. Moreover, minimum, maximum and average temperatures were found to be -3.8° C, 32.4 °C , 8.4 °C; -9.4 °C , 28.4 °C , 7.9 °C for prolonged years in years 2015-2016. The soil properties in the experimental area were 301.7 mmol/kg in P_2O_5, 357.4 mmol/kg in K_2O, 1.05 % in $CaCO_3$, and 2.02% in organic matter, 6.04 in pH, and 1.89 dS/m in electrical conductivity. We conducted experiments with three replications in random block design. Bread wheat genotypes were planted on the 15th of September with 475 seed/ m^2 rate. The plot had dimensions of 6 m / 1.2 m (7.2 m^2). Sixty kg N ha^{-1} (½ at sowing period and ½ at tillering period) and 60 kg ha−1 P_2O_5 (at sowing) were administered. In this study, superphosphate (46% P_2O_5) and ammonium sulfate (21% N) were used as fertilizer. Genotypes were collected on the 10th of July, and the seeds of the genotypes were kept for analysis.

1.1. Isolation of gDNA

The Qiagen DNA extraction kit (Qiagen, Hilden, Germany) was used to get the extract of genomic DNA (gDNA) from the powdered leaf materials, by following the manufacturer's directives. gDNA samples loading into agarose gel 1% (w/v) in 0.5xTBE (Tris-Borate- EDTA) buffer at 70 V was performed for 150 minutes. In order to check the quality and quantity of the amplified DNA products, a spectrophotometer (Qiagen, Qiaxpert Instrument, Germany) was used. Pure of sample DNA was used in ISSR and IRAP marker techniques.

1.2. IRAP amplification

As it is seen in Table 1, 10 primers had been used for the generation of IRAP profiles. A Bio-Rad thermocycler (Bio-Rad Laboratories Inc., Hercules, CA, USA) was used to perform PCR amplifications. The reaction mixtures (20 µl) were fixed as described below: 45 ng of g DNA, 1× buffer (10 mM Tris–HCl, 50 mM KCl, pH=8.3), 1.5 mM of magnesium chloride, 0.2 µM of each dNTP, 0.5 U of Taq polymerase (Cinagen Co., Iran) and 10 pmol of each primer were added.

The PCR program for the IRAP was as the following: an initial denaturation 5 min at the temperature of 94°C, 35 cycles of 45s at the temperature of 95°C, 40s at the temperature of 55°C to 63°C (Table 1), 2 min at the temperature of 72°C, followed by a final extension of 5 min at the temperature of 72°C.

1.3. ISSR amplification

26 oligonucleotide primers were tested for ISSR amplifications, among which 12 primers were selected and used for future studies (Table 1). The PCR mixture (25µl) was prepared as the following: 40 ng of template DNA, 10x

buffer, 200 Mm of each of the four dNTPs, 1 U of Taq DNA polymerase, 0.5 mM of primer and 1.5 mM $MgCl_2$. Amplification was performed in a thermal cycler programmed for an initial denaturation at a temperature of 94°C for the period of 5 min followed with the following 35 cycles of 45 s at the temperature of 94ºC, 1 min at the annealing temperature and 1 min at the temperature of 72°C, ends with a final extension stage of 7 min at the temperature of 72ºC.

1.4. Electrophoresis

The PCR products (27ml) were blended with 6X gel loading buffer (3ml) and exposed to agarose. The electrophoresis was then applied to separate them via 1.5% agarose gel (1.5% w/v) in 0.5xTBE buffer (Tris-Borate-EDTA) with was performed 70 V for the period of 150 min. Ethidium bromide solution (2 µl Etbr/100ml 1xTBE buffer) was used to stain the gel for 40 min, and The Bio Doc Image Analysis System with Uvi-soft analysis package (Cambridge, UK) was used to determine the amplified DNA products.

1.5. Analysis

The TotalLab TL120 computer software program was used to evaluate the ISSR and IRAP bands. The scoring of PCR products was conducted as the presence (1) and absence (0) of band for all genotypes, and their analysis was performed. After the Jaccard (1908) similarity index was calculated using the data, the construction of a dendrogram was conducted based upon the relevant matrix with the UPGMA (unweighted pair group method using arithmetic average) technique..

3. Results

1.1. IRAP and ISSR analysis

The results of our IRAP and ISSR analysis are presented in Table 1. Ten IRAP primers revealed specific and stable results in *wheat* genome (Table 1).

A total of 92 bands, 86.9% of which were polymorphic, were produced by ten IRAP primers. The percentage of polymorphic bands that were produced by each primer ranged from 66.6% to 100%. While the primer Copiar provided the highest number of bands (14), the Sukkula primer provided the lowest number of bands (6). The band sizes of the primers used were 15-25 bp.

Table1. Details of banding pattern revealed through IRAP and ISSR primers. (R = A, G; Y= C, T)

Mar-kers	Primer/primer Combination	Sequence (5'–3')	Length of amplified bands	No of bands	No polymorphic bands	Polymorphism ratio (%)
IRAP	Nikita	CGCATTTGTTCAAGCCTAAACC	500- 1900	12	10	83.3
	LTR6150	CTGGTTCGGCCCATGTCTATGTATCCACACATGTA	250- 1700	9	8	88.8
	3' LTR	TGTTTCCCATGCGACGTTCCCCAACA	750- 2300	8	8	100
	Sukkula	GATAGGGTCGCATCTTGGGCGTGAC	500- 1700	6	4	66.6
	Copiar	TTG AAC CCC TTT TGA TGT AT	300- 2000	14	12	85.7
	Stowaway	CTTATATTTAGGAACGGAGGGAGT	250- 1500	8	7	87.5
	5' LTR1	TTGCCTCTAGGGCATATTTCCAACA	500- 2400	9	7	77.7
	5' LTR2	ATCATTCCCTCTAGGGCATAATTC	250- 1900	8	8	100
	WLTR2105	ACTCCATAGATGGATCTTGGTGA	600- 1800	7	6	85.7
	LTR6149	CTCGCTCGCCCACTACATCAACCGCGTTTATT	400- 2200	11	10	90.9
	Total		250- 2400	92	80	86.9
ISSR	ISR1	$(CA)_8$ G	500- 1900	10	8	80
	ISR2	$(AC)_8$ AT	300- 2600	14	12	85.7
	ISR3	$(AC)_8$G	750- 2100	8	7	87.5
	ISR4	$(AG)_8$GC	300- 1800	6	4	66.6
	ISR5	$(AG)_8$YA	400- 2400	9	8	88.8
	ISR6	$(AG)_8$TA	500- 1900	8	6	75
	ISR7	$(GA)_8$YT	300- 2750	12	10	83.3
	ISR8	$(AG)_8$YT	500- 1600	5	4	80
	ISR9	(CT)8T	600- 2000	13	11	84.6
	ISR10	(GT)8C	750- 1500	8	8	100
	ISR11	(GA)8T	500- 1200	12	11	91.6
	ISR12	(GA)8C	300- 1800	10	8	80
	Total		300- 2750	115	97	84.3

Twelve ISSR primers combinations were utilized for the analysis of genetic diversity in 12 wheat genotypes. As it can be seen in the table, a total of 115 scorable amplification products, 97 of which were polymorphic bands,

ranged from 300 to 2750 bp. Ratio polymorphism was found to be 84.3%.While the highest number of bands was acquired from primer ISR2 (14bands), the lowest number of bands was acquired from ISR8 (5 bands). A combination of data from the IRAP and ISSR markers were used for the construction of the UPGMA dendrogram (Figure 1). 12 wheat genotypes were classified into 2 main clusters. While Cluster I included Bozkır, Harmankaya 99, Altay 2000, Yıldırım, , cluster II included Bezostaja -1, Ahmetağa, Müfitbey, Aldane, ES-26, Alperbey, Atay 85, Eraybey. While the greatest similarity was found between genotype Bozkır and Harmankaya 99 (0.113), the greatest dissimilarity was found between Bozkır and Eraybey (0. 892).

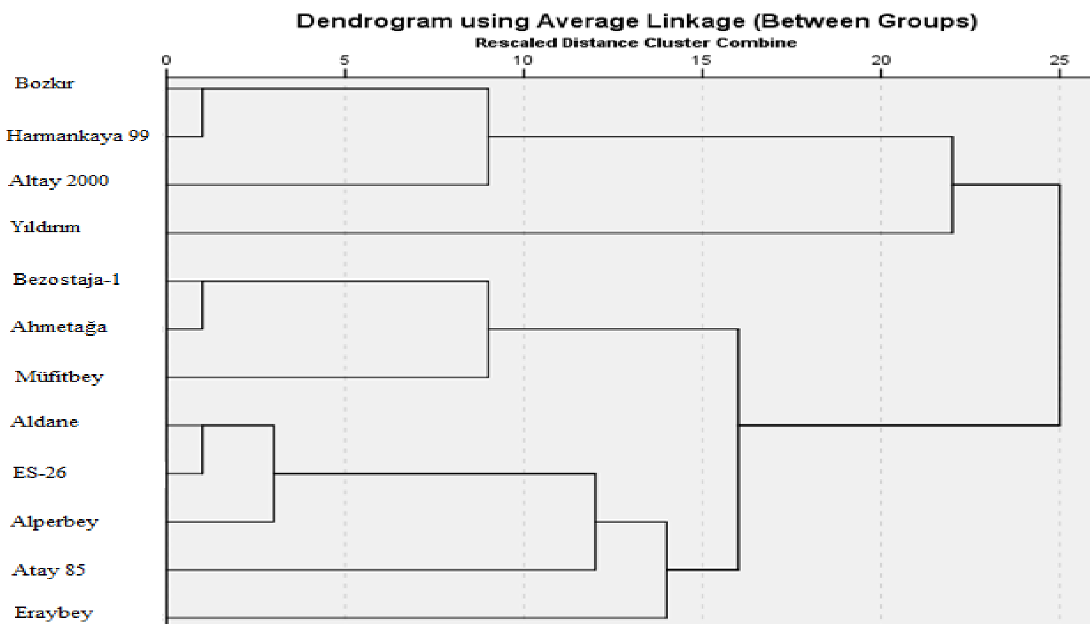

Figure 1. UPGMA clustering for 12 wheat genotypes based on IRAP and ISSR markers.

4. Conclusions and discussion

The genotypes used in the study were improved as a result of long breeding studies, and each of them had different genetic characteristics/genetic capacity. Furthermore, each of them had a different capability to respond to biotic and abiotic stresses. These varieties are utilized as witness/control varieties in breeding studies and other scientific studies. Furthermore, these genotypes are also used as witness/control genotypes in breeding programs and other scientific studies. For instance, some genotypes have more resistance to yellow rust compared to others. Another one is more resistant to drought. Working with genotypes having different genetic characteristics/capacities refers to working with a wider genetic variation. Thus, to reveal their genetic similarity and the demonstration of this phenomena will help genetic progress in future breeding programs.

Retrotransposon-based molecular markers containing IRAP and REMAP have been used to examine genetic diversity in bread wheat (*Triticum aestivum* L.). Retrotransposon sequences, between plant families can be easily used across species lines, among intimately related genera, and even sometimes between plant families [13, 14].

ISSR profiles were utilized to examine intra-specific genetic diversity analysis in many bread wheat genotypes [15]. They were successfully utilized for the prediction of genetic diversity in main crops such as maize [16], wheat [17], rice [18], barley [19] and cotton [20] It was reported by Carvalho et al. [21] that ISSR markers provided great potential for differentiating closely related wheat cultivars and botanical varieties and allowed for the determination of higher levels of polymorphism.

According to the similarity matrix (Fig. 1) Bozkır, Harmankaya 99; Bezostaja 1, Ahmetağa and Müfitbey; Aldane, Es 26, Alperbey and Atay 85 were determined to be close related varieties; furthermore, distant related varieties were Bozkır and Eraybey. Wide differences in genetic characteristics in bread wheat genotypes may enable to develop novel promising genotypes in germplasms of crop programs. The results indicated that IRAP and ISSR analyses were useful for the differentiation of the bread wheat genotypes tested in this study.

Wheat, which is the most consumed, cultured and most preferred plant of the world, will continue to be the plant that mostly occupies the agenda on production and breeding programs in the future.

To meet the food needs, that will rapidly increase in the future, can be achieved with the development of plants that are highly efficient, high quality, stable and resistant to stress. Such a goal is only achieved by effective and successfully implemented breeding programs with vast genotypic variation.

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