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Molecular Characterization of Some Triticale Cultivars in Turkey

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ABSTRACT

Triticale has gained importance as an alternative crop to solve the nutritional problems of the rapidly increasing world population. Triticale gains the yield potential of durum wheat and adaptation of rye to cold, drought and marginal soil conditions in itself. It is also known that triticale is resistant to many diseases and pests. In this study, it was aimed to determine the genetic characterization of some spring and facultative triticale varieties registered in Turkey with the ISSR-PCR technique which is one of the molecular marker methods. Polymorphic and monomorphic band images were formed from 16 primers as a result of analysis of 20 primers in 5 registered triticale isolates, and 71.1% polymorphism was obtained. Four primers did not show any amplification in the triticale cultivars. Triticale cultivars were mainly divided into three groups. Alperbey and Tacettinbey which were in the first group, differed by 28% and Mehmetbey and Ayşehanım in the second group differed by 37%. In addition, EgeYıldızı in the third group differed 48% from Alperbey, 45% from Tacettinbey, 49% from Mehmetbey and 38% from Aysehanım. The genetic distance values between cultivars of triticale ranged from 0.509 to 0.712. These results obtained from ISSR-PCR method will provide significant contributions to the triticale breeding programs.

Keywords: Triticale, molecular characterization, polymerase chain reaction, PCR, ISSR , Inter-Simple Sequence Repeat

Introduction

Cereals have the most acreage and production among the cultivated plants in the world. Cereals provide approximately 50% of the annual protein consumed by humans and animals (Çölkesen, 1994). Cereals and their products have an important place in human nutrition in the world. Wheat, corn, rice, barley, and rye are the most produced cereals in the world. The food supply and nutrition has been the most important issue throughout history for humanity.

Increasing the productivity in agriculture is obligatory because agricultural land and the natural sources are limited although the world population is increasing. For this reason, the scientists have looked for the solution to develop new plants, species and varieties which can survive under extreme conditions and can give high yield even under stress conditions. The first important research for this aim was made in Scotland in 1875 to obtain a cereal which can be grown in different geographical and climatic conditions, can withstand cold, drought and acidic soils, has also the yield and quality potential of wheat. Triticale *(X. Triticosecale* Wittmack) was developed as a result of these researches (Müntzing, 1979).

Triticale which possesses the yield potential of durum wheat and adaptation of rye to cold, drought and marginal soil conditions, has gained importance as an alternative crop to solve the nutritional problems

of the rapidly increasing world population. . It is also known that triticale is resistant to many diseases and pests (Varghese *et al*., 1996). New varieties have been continued to develop for improving the properties of triticale. Since the genetic basis of amphidiploid triticale does not have a very wide variation, sometimes varieties developed rely on a similar genetic basis, which limits genetic advance. Molecular markers are one of the most reliable methods used in the characterization of genotypes and varieties in recent years.

Genetic diversity is the main source for breeding programs. Conducting the breeding studies as based on a broad genetic basis will ensure maintaining genetic diversity during to long-term as well as adaptation to changeable environmental conditions (Dirik H.,1997). In addition, increasing the genetic diversity is important to develop cultivars resistant to plant diseases and pests (Hajjar *et al*., 2008). However, it is essential to choose the most effective method to determine genetic diversity. For this purpose, DNA markers can be used safely, especially in plant breeding and gene mapping studies (Reddy *et al*., 2002).Identification of the DNA markers belonging to plant species and creating genomic maps of these plants will facilitate to develop new varieties in short time, will save time by shorten the breeding period and will provide reliability and convenience in the selection process (Michelmore *et al*., 1991). In recent years, specific sequence differences between two or more individuals could be identified with molecular marker techniques, Besides, developing a detailed genetic map of marker linked to a trait under study is an important tool for increasing the breeding efficiency. Therefore, genome maps of many crops which have agricultural importance have been established (O'Brien, 1993).In this study, genetic differences among spring triticale varieties registered in Turkey were determined by ISSR-PCR method. Important cultivar based on genetic variation were identified for further breeding programme.

Material and Methods

DNA Isolation

In this study, five spring and/or facultative triticale (X. *Triticosecale* Wittmack) cultivars (Alperbey, Aysehanim, EgeYildizi, Mehmetbey, Tacettinbey) were used to determine the genetic distance/ similarity. Twenty-five seeds of each of the Triticale cultivars were germinated by placing seeds in 9 mm petri dishes lined with filter papers and kept in growth cabin (Binder KBW 400). The germinated triticale seeds were planted in pots which were maintained in controlled greenhouses. Then, plants were harvested after having 3-4 tillers and DNA isolation were done according to modified Doyle and Doyle (1987) method by using the samples obtained from young leaves.

Firstly, the amount of isolated DNA was determined by spectrophotometer (Thermo Scientific Nanodrop 2000c) since equal amounts of DNA from each sample would be used in the PCR reaction. Then, the DNA samples were diluted with TE (Tris-EDTA) buffer according to amount of DNA calculated from the OD results for PCR analysis of ISSR primers.

ISSR Amplification

ISSR protocol developed by Zietkiewicz *et al.,* (1994) was used for the amplification of DNA obtained from Triticale cultivars. The volume was kept similar in each probe as 25 microliter amplification reaction solution; 75 mM Tris-HCl, pH=8.8, 20 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 100 mM dATP, 100 mM dTTP, 100 mM dGTP, 100 mM dCTP, 0.2 mM primer, 1.0 unit Taq DNA polymerase and 10 ng DNA. The binding temperature was adjusted (50-55 °C) according to the Tm temperatures of the primers. Generally, temperatures above 2-3°C of the Tm of the primers gave favourable results in terms of amplification. Then, the primers were incubated at 70-75°C for amplification with Taq polymerase (Palumbi *et al*., 1991). Temperature and cycles adjusted in that: Preliminary denaturation during 2 min at 94 ºC, denaturation for 36 cycles during 1 min at 94 ºC, adhesion of primers to DNA during 1 min at varying Tm according to primers, primer elongation during 2 min at 72 ºC and last primer elongation during 10 min at 72 ºC. The reactions were performed eppendorf tubes using Eppendorf Mastercycler Pro S PCR System.

PCR products were analyzed on a 2% agarose gel prepared in 0.5 X TBE (Tris / Borate / EDTA), in an electrophoresis (ThermoMidicell Promo) tank containing 0.5 X TBE buffer, at 100 V during 2-3 hours. After electrophoresis, the DNA was stained with ethidium bromide during 20 minutes in order to visualize and evaluate the DNA. Emerging DNA bands examined under UV light (VilberLourmat) and then they were photographed on a Kodak Gel Logic 200 Imaging System.

Evaluation of the Bands

PCR results were evaluated taking into account the gel electrophoresis views of the ISSR-DNA bands formed. ISSR amplification products were evaluated as having (1) or absent (0), and the obtained data were analyzed in IBM SPSS 20 (SPSS) statistical package program. The genetic similarity index was calculated according to Jaccard. (Jaccard , 1912)

Based on the genetic distance matrix, a dendrogram was constructed using the Unweighted Pair Group Method (UPGMA).

Results and Discussion

Twenty different primers which have 17-18 base pair lengths were used in the study. Sixteen from them exhibited polymorphic bands and the other four primers were showed monomorphic band formation. Out of total ISSR-PCR 145 bands , 103 were polymorphic resulting from 16 primers (Table 1). When the bands were examined assigned to individual primers; it was seen clearly that Primer UBC 821 gave the most polymorphic band, Primer UBC 813 gave the least polymorphic band, primers UBC 813, UBC 826, UBC 827 gave the most monomorphic bands, and the primer UBC 821 gave the least monomorphic band (Table 1).

Triticale cultivars were mainly clustered in three groups. Alperbey and Tacettinbey took place in the first group, Mehmetbey and Aysehanım in the second group and EgeYıldızı in the third group respectively (Figure 1).

The genetic distance values between triticale cultivars ranged from 0,509 to 0,712. The highest genetic similarity was observed between Alperbey and Tacettinbey and followed them Mehmetbey and Ayşehanım with 0.627 (Table 2). The lowest genetic similarity was observed between Mehmetbey and EgeYıldızı while Ayşehanım in the same group as Mehmetbey were found more similar with EgeYıldızı (Table 2)

Conclusion

This study was concerned with assessing genetic distances/similarities among five Triticale cultivars using ISSR markers and genetic information related to triticale cultivars based on genetic similarities gained from Cluster analyzes. Totally 145 bands gained from 16 primers formed 103 polymorphic and 42 monomorphic bands. Maximum of polymorphic bands were formed by primer UBC 808 while most of monomorphic bands were formed by primers UBC 813, UBC826, UBC 827. From the three groups obtained, the first group contained Alperbey and Tacettinbey, the second group contained Aysehanım and Mehmetbey and the last group contained only EgeYıldızı. Alperbey and Tacettinbey were the most similar cultivars to each other genetically.

This study is important in terms of revealing DNA isolation method and PCR conditions that can be used in triticale. The information gained from this study may be used in further similar research. The ISSR technique can be used effectively in the determination of DNA polymorphism in the triticale and genetic characterization.

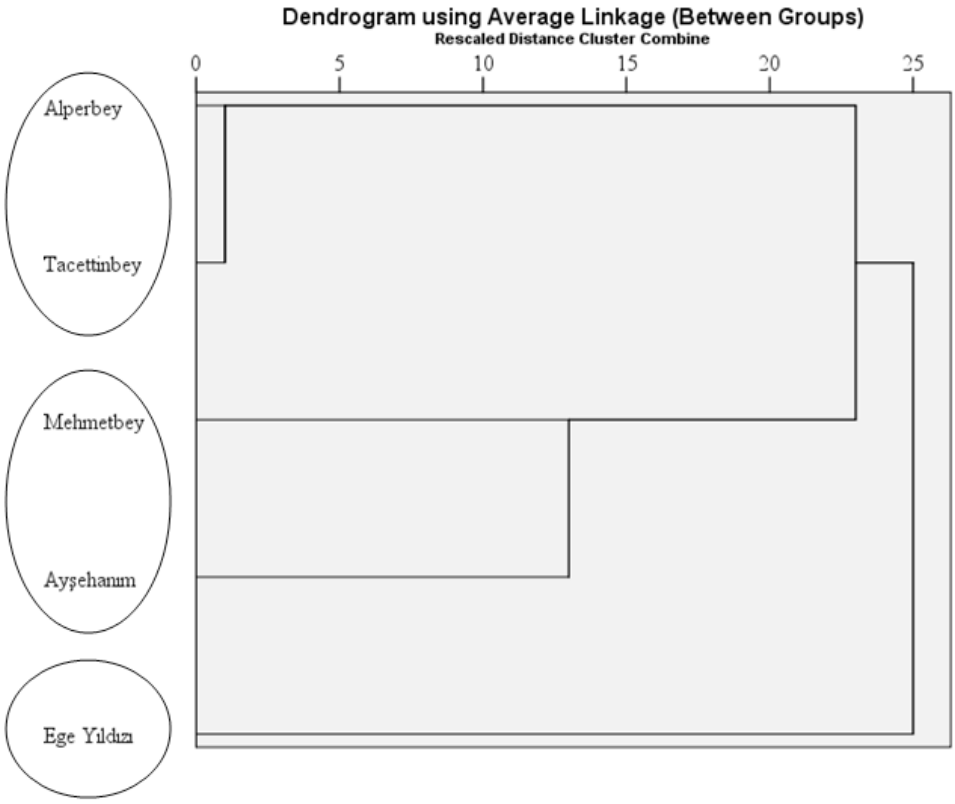
Table 1. Amount of total bands received from amplification of ISSR primers, number of polymorphic bands and polymorphism rate (%)

Primer Name	Number of Polymorphic Bands	Total Number of Bands	Polymorphism Rate (%)
UBC 808	14	16	87,5
UBC 809	-	-	-
UBC 810	4	8	50,0
UBC 812	6	9	66,7
UBC 813	4	9	44,5
UBC 818	4	6	66,7
UBC 820	4	**6**	66,7
UBC 821	8	8	100,0
UBC 822	-	-	-
UBC 824	3	5	60,0
UBC 826	3	8	37,5
UBC 827	5	10	50,0
UBC 829	-	-	-
UBC 830	10	13	77,0
UBC 834	11	13	84,7
UBC 835	5	7	71,5
UBC 840	11	12	91,7
UBC 843	-	-	-
UBC 844	5	6	83,4
UBC 847	6	9	66,7
TOTAL	103	145	71,1

Table 2. Similarity coefficient obtained from cluster analyses according to Jaccard.

	2:Tacettinbey	3:Mehmetbey	4:Ayşehanım	5:Ege Yıldızı
1:Alperbey	,712	,540	,523	,516
2:Tacettinbey		,610	,592	,549
3:Mehmetbey			,627	,509
4:Ayşehanım				,619

Figure 1. Dendograms of some spring and facultative triticale cultivars using ISSR Markers



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