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The comparison of ISSR and RAPD markers with different species of *Triticum*

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ABSTRACT

Considering genetic variety in Iran, we applied markers to determine genetic variety and phylogenic connections in different species. On the other hand, we made an attempt to compare RADP and ISSR molecular markers on different species of wheat considering the food consumption of Iran and easy sample to wheat. In this experimental study, markers were applied to consider different species of wheat and they were experimented by RADP and ISSR starters. The effect of marker was evaluated on different species of wheat by PCR test. A comparative effect of RADP and ISSR markers via PCR method signified that different species of wheat based on applied starters with a clear distance from DNA bands showed their similarities. In conclusion, the study results indicated to the effectiveness of different species of wheat on markers. Low cost and availability to wheat species and applying their DNA can be more effective in comparison to RADP and ISSR.

INTRODUCTION

Although from too many years ago human had considered drug crops of plants, the increasing production of these crops in fields and gardens turned to a new science since second half of the 20th century. Destroying and consuming growing plants of nature were placed by enjoying nature plants. Nowadays, the applying of genetic engineering methods and biotechnology allow forming DNA molecules with inheritance properties, and a significant progression has created in biological sciences. Modern biotechnology, a novel technology, is able to increase the function of plants through changing the genetic structure of crops and other plants.

Wheat (in science: *Triticum*) is a cereal grain. There are wild and domestic types of wheat. It is belonged to annual monocots magnoliophyta plants and Poaceae and a member of Gramineae family.

Wheat is among the oldest crop plants used by human, it is cultivated and harvested in a very great extent. Botany, it is a member of *Triticum* species divided in three different groups with determined chromosomes carrying all genetic properties of the family (3).Two diploids are more difficult than other crops, as some of them belong

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to the wheat species of genetics with four polyploidy chromosome chains, while other species got 6 chromosome chains. Diploids grow only in jungles and deserts and cropped Einkorn wheat belong to this group.Wild Tetraploids, and cultivated Emer and Durum belong to this group. Hexaploids involves bread wheat 90000 hectares of all 2.3 million hectares of water wheat are cultivated in cold regions.

PROPERTIES

Wheat contains all mineral salts. If you need to have different vitamins, take wheat. It is suggested to use wheat gruel with sugar and almond to stop breast bleeding. Wheat avoids gastric cancer.

RAPD MARKER

RADP marker starting a short oligonucleotid starter propagates in PCR reaction with low annealing temperature, a range of pieces of DNA pattern. One or more piece of polymorph productions are created as a result of changing in one open door in primer connection place ,and this polymorphism can be a genetic map (Kaiser and Polatski, 1995) RADP marker is distributed in the whole genome (Luine, Santanglo, 1994).

THE ADVANTAGES OF RAPD MARKER

It does not need the primary information on DNA orders to design and construct starters.

It allows a simultaneous consideration of numerous places in sample genome.

It does not need a prober, radioactive materials, etc.

THE DISADVANTAGES OF RAPD MARKER

The location of RAPD markers is not determined on the genetic maps.

The similarity and relationship between bands with similar movement on electrophoresis gel are not determined.The studies accomplished by Paul et al.(1996) show that RAPD markers show the genetic distances between people with little distance from each other in a less exact way in comparison to other markers. Therefore, applying markers to taxonomy studies in species should be considered.These markers have a dominant power and the disability of allele system in RAPD markers results in some limitations in the marker functions.

RANKING HARDSHIP

RAPD has a tendency to propagate repetitive parts of genomic DNA. As an example, too many repetitive orders of wheat genome are propagated through RAPD reaction.

ISSR TECHNIQUE

ISSR is a PCR-based technique contained a piece of DNA in a reproducible distance between two repetitive fields of unique micro-satellites with opposite directs. Usually, this technique used micro-satellites of 16-25 bp as a primer of a mono-primary targeting poly-genomic locus to propagate the successions between micro-satellites with different measures. Bi-nucleotides, Tri-nucleotides, quadra-nucleotides, or panta-nucleotides micro-satellite repetitions can be used as primers. Although applied markers are connected to 1-4 bases, and are enlarged based on these connections, they are able to connect to each point of DNA. This technique has integrated the advantages of AFLP and micro-satellites with RAPD comprehensiveness. The applying longer primers (16-25 mers in comparison to RAPD shorter primers (10 mers) allow to apply high temperature of connection (45-60`C) increasing the primer connection to determined points of DNA with more repetitive times results in high repetition of ISSR. The studies on repetition signify that only the weakest bands are not repetitive. Nearly 92-95% of scored pieces can be counted in DNA samples, and can be repeated in distinct periods of PCR when are identified by applying polyacrilamide. 25-50 nanograms of pattern DNA in each 20 micro-liter of PCR and 10 milligram of pattern DNA can produce the same propagated productions. The connection temperature of 45-60`C depends on the applied primer.

MATERIALS AND METHODS

HERBAL MATERIALS

Nine varieties of wheat species (existed in Minister of Agricultural Jihad) were applied to consider molecules. As a whole, nine leaf samples included fresh leaves of wheat species were collected.Starters applied 14 ISSR and 10 RAPD starters to consider genetic relationships.

THE EXTRACTION OF GENOMIC DNA

Changed CTAB method of leaf samples was applied to extract DNA before that samples were melted. To provide 100 milliliter buffer of 2% CTAB extraction, the components of table 4-3 were solved in 20 milliliter of distilled water, pH was 8 by applying chloric acid in one mullar. Then 2 grams of hexa decyl timthyl ammonium bromide was solved in hot distilled water, and it was added to the previous solvent. Finally, the solvent was 100 milliliter. The phases of DNA extraction are as following:

Table 4-3. The composed components of DNA extraction buffer

Buffer components	Amount	Final destination
Tris-HCL	1.211 g	100 millimolar
NaCl	8.19 g	4 molar
EDTA	0.744 g	0.2% (volume-volume)
CTAB	2 g	0.2% (weight-volume)
Merkapto etanol	200 microliter	0.5 molar
pvp	2 g	0.2% (weight-volume)

Before the experiment was started, CTAB buffer was heated in water bath of 65`C.

100 milligram of fresh and clean leaves was pounded in liquid nitrogen in a mortar. It is suggested to keep the dish and mallet (at least in connection with leaves) cold by adding some liquid nitrogen or putting in freezer to make Nucleases inactive.

Put the powder in a corner of the dish, and when buffer was melted, add 800 micro-liter of CTAB buffer to powder and mix thoroughly. The contents of mortar (dish) should be transferred to tube of 2 milliliter, and was kept in 60`C for 30 minutes in water bath (In this 30 minutes, the tubes were circulated for some times smoothly). The sample volume (800 micro-liters) of chloroform/ isoamyl alcohol (24:1) was added in room temperature. The tubes were turned some times in a smooth way to have a unified mixture. It was centrifuged for 10 minutes and 13000 turns. The above limpid part was removed (in 500-600 micro-liters), and was poured in another tube.

2/3 (almost 350 micro-liters) of cold isopropanol volume (-20`C) was added to each tube. It was centrifuged with 13000 turns for 10 minutes in 4`C. the tube contents were removed smoothly and DNA string was left at the bottom of the tube. Vacant tubes with DNA strings were inverted on a very clean place to have dry strings. 100 micro-liters of TE buffer was added to room temperature.800 micro-liters of Aminium acetate +cold ethanol was added (2.5 molar and 1.5 liter of aminium acetate+ 3.5 milliliter ethanol), the last step was accomplished on ice, it was inverted for some times, and then tubes were transferred to freezer. They were centrifuged with 13000 turns for 15 minutes in 4`C.

The above part was discharged. The tubes were inverted to become dry. 150 milliliters of TE buffer was added to each tube. The samples were kept in room temperature, then some other actions to evaluate quantity (spectro-photometry) and quality (electrophoresis on agarose gel of 1.5% DNA) (extracted DNA) were accomplished. The samples were diluted in 100 nanograms to subsequent application. Providing method of 0.8% and 1.5% agarose to determine quantity and quality and analysis of propagated pieces

X1EDTA was diluted by water in 9:1, and then X1EDTA was provided.

X1EDTA is put in the tube based on the electrophoresis tank volume by the help of cylinder, it is moved smoothly to have a good mixture. The solvent in tube is put in the microwave so that particles will be solved in a complete way and then a smooth solvent will be appeared. 0.2 micro-liter of DNA safe stain was added to gel to paint, then the gel was put in the gel dish. The solvent in the tube is poured on the electrophoresis tray. When 30 minutes was passed, agarose gel is stiff. The gel was put in the electrophoresis tank by X1EDTA.

9-3 sample preparation and electrophoresis of agarose gel

We add 5 milliliters of loaded buffer to each 5 milliliter of sample to have a good mixture, and then the sample is spilled into the sink. The first sink from the lift has been devoted to the measured marker. A 92-volt electric currency was connected to the tank. By passing 45 minutes, gel was removed from the tank, and then it was removed from the tray. Then a picture was taken from the gel in photography machine.

10-3 The components of polymerase chain reaction

The components presented in table 5-3 were applied to carry out PCR reaction in 12 micro-liter.

Table 5-3. The applied components to carry out PCR reaction

Component	Amount (micro liter)
PCR kate (Master mix)	5
Deionized water	5
Starter	1.1
Genomic DNA	1
Total	12.1

11-3 Time cycle and polymerase chain reaction steps

The polymerase chain reaction was accomplished in thermocycler (Bio Rad) in 4 minutes and initial compounding in 94`C via 10 initial touching down denaturation (so that the connection temperature of starter was

considered to be 5`C higher than the real connection temperature, and 0.5`C was decreased from the connection temperature to achieve the real connection temperature). This action allows to decrease similar bands of micro-satellites causing some problems in scoring the PCR typical cycles. It is suggested to apply 30 cycles containing 30 seconds of denaturation in 94`C to each starter in 45 seconds (to connect starters), and 2 minutes in 72`C to extend, and the last extension was carried out in 72`C for 7 minutes.

DATA ANALYSIS

The applied markers in this study are of dominated markers which are scored in interpreting gel as 1(indicating to the presence) or 0 (indicating to the lack).

CLUSTERING ANALYSIS

According to raw data obtained from ISSR and RADP molecular markers, the clustering analysis was accomplished by UPGMA method and applying the jaccard similarity coefficient to determine similarity between two individuals, Darwin 6 and Post 3 software.

THE ANALYSIS TO BASIC COMPONENTS

The analysis to basic components is another multi-variant method which is of high application in genetic variety analysis with clustering analysis. This method can be applied to present two-dimensional distribution of individuals in a field of plot signifying genetic similarity among them. PCA is a method to decrease data to construct relationships between two or more variants and to explain the changes of whole basic and primary data by some new independent variants called basic components.To decrease data is accomplished by linear changing of basic data to new independent variants called basic components, so that the first PC explains the maximum initial data, and the second PC explains remained changes after the first PC, etc. it should be noted that each PC explains those changes not been explained by other PCs.

As PCs are independent, each one presents different properties of basic data, and they should be interfered differently from each other. When PCA is applied to analyze molecular data, similarity matrix should be changed via the following formula to remove negative inert roots:

$$ij=Sij-Sio-Soj+SooóS$$

in which, Sij represents similarity coefficient between I,j individuals, Sio shows the average of similarity coefficient in n^{th} individuals, Soj shows the average of similarity coefficients of j^{th} individual, and Soo is the total average of similarity coefficients. This changing causes to move similarity matrix to zero root. These similarity properties calculated by any method will be kept.

RESULTS AND DISCUSSION

A study accomplished by Aslani (2013) on the genetic variety of molecular genotype of Mirabilis jalapa by applying ISSR marker showed that ISSR does not need radioactive materials and pattern DNA sequence. Therefore, ISSR was a good marker to consider genetic variety and relationship which was coincide with our results (Aslani,2013).

A study accomplished by Heidari Nejad on the genetic variety of African Violets varieties by applying RAPD marker signified that morphologic properties are influence by various factors with no effect on DNA. These factors which are similar in DNA based on the data, are similar to or different from each other in morphologic properties. Therefore, it is expected that RAPD data may have no similarity with individual grouping in planting and morphological properties by considering the effectiveness of environmental factors on these properties.

This result has been reported by Martinz-Gumz et al. (2003) on almond. Therefore, considering individuals based on planting and morphological properties can`t result in favorable results (Heidari Nejad, 2012).

Stephonova et al. (2014) accomplished a study on genetic variety of 16 species of Amaranthus of Caryophyllales family by applying molecular ISSR marker. Amaranth is a most important species existing in all around the world. In this study, ISSR method was applied to analyze variety in and among 16 species of amaranth. Eleven primaries were applied in this study. The dendrogram divided 16 varieties into 3 groups in which 2 groups belong to India and one group is belonged to Nepal. The similarity average was 0.154-1.000. This study indicated that ISSR is of high efficiency (Stephonova et al. 2014).

In a study accomplished by Ray et al. (2007) on the genetic relationships of Aramanthus which is belonged to Caryophyllales by applying molecular ISSR and RAPD markers, it was used from 18 starters and to ISSR marker and 15 starter to RAPD. The similarity coefficient of ISSR and RAPD markers were 0.45 and 0.47; respectively. Also, Cophenetic coefficient of both markers was 0.83. These coefficients refer to a good fitting between similar matrix and dendrogram, and both dendrograms showed a good similarity between species indicating that ISSR and RAPD markers are of high sufficiency to determine genetic relationships, and they are suitable tools to cluster species (Ray et al. 2007).

CONCLUSION

In general, the study results signified that there is a difference on the least and most distance of bands in different analyses. ISSR marker showed bands with less distance and more similarity in comparison to RAPD marker.

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