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Grouping Various durum wheat genotypes using molecular markers

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ABSTRACT

Information on genetic diversity of germplasm collections and determining genetic relations in breed material is prerequisite in plant breeding programs. The following research tries to study 129 genotypes from various locations (10 countries) using 20 pairs of EST-SSR markers. Considering the derived similarity matrix, two genotypes from Shush Region had the highest genetic similarity of 0.894 and two genotypes of 4 and 56 from Shush and Egypt had the lowest genetic similarity of 0.38. Cluster analysis results using Complete classification method grouping the genotypes in three main groups. The close relation between genotypes indicates the marker's nature, for these markers are designed based on completely preserved and expressible points of genome. Hence, the change and, as a result, variety between genotypes is unlikely.

Keywords: Non-irrigated Wheat, Yield, Genetic Diversity.

INTRODUCTION

Genetic diversity in plants and plant societies is of practical significance. Agriculture and producing food is highly related to using high-yielding plant genotypes use. Common agricultural breeding methods are based on collection of favorite genotypes among available genetic diversity and manipulation of some or all available and favorite traits in one genotype to produce a trading variety. Species diversity in an environment is related to production capacity and ecosystem sustainability [1]. Containing semolina, durum wheat or Triticum durum is among the main wheat verities which have a great significance in macaroni production and food supply in most countries, especially in European countries. It is currently proven that DNA-based molecular markers are of significant use for various goals. Among these markers, second generation markers such as SSR and AFLP are more efficient, comparing to first generation markers such as RFLP and RAPD. The use of EST markers has recently been recommended, for with their help in scoring alleles based on genome coding regions, only. Researches have suggested that approximately for each 9.2 k open EST sequence pairs, there is one SSR sequence [2].

Cluster analysis is referred to a set of multi-variable techniques which classification individuals so that similar individuals are Grouping based on studied traits. Accordingly, individuals in one cluster are more similar to the individuals in the same cluster, comparing to individuals in other clusters.

UPGMA[1] and Ward's minimum variance method have the highest application in cluster analysis among all hierarchical cluster analysis. Other methods such as single linkage and complete linkage are applied by some researchers in analyzing genetic diversity. After forming the primary core for each cluster which is formed based on similarities between two individuals, in UPGMA similarities or distances between each individual and other

[1] *Unweighted Pairs Group Method Using Arithmetic Average*

individuals in the same cluster are considered as the average distance or similarity between that individual and other individuals of the same cluster [3].

One of the methods used for comparison between various classification algorithms efficiency is to determine the cophenetic correlation coefficient, in which, the correlation between similarity matrix or distance matrix as a the cluster analysis input and cophenetic matrix which is based on dendrogram as the analysis output are calculated.

Fit degree could vary in the range of $0 \leq r < 10.9 \leq r$ is excellent fit, $0.8 \leq r < 0.9$ is good fit, $0.7 \leq r < 0.8$ is weak fit and $r \leq 0.7$ is very weak fit. However, low cophenetic coefficient molecular data does not imply the inefficiency of the algorithm, but the fluctuation between data due to the lost data [3].

The following research tries to study the durum wheat genetic closeness using molecular markers.

MATERIALS AND METHODS

The plant materials used in this research included 129 Iranian (101 genotypes) and international (28 genotypes) durum wheat genotypes. Iranian genotypes were provided from Tehran University Pardis seeds collection and international genotypes were provided from Seed and Plant Improvement Institute in Iran. Seeds were used after 2 years of purification (Table 1).

All stages for this research were conducted at Genomics Research Center in Agricultural Biotechnology Research Institute of Iran located in Karaj. Young leaves were used for sampling for each genotype and DNA extraction was conducted by modified Dellaporta et al (1993) method [4]. Polymerase chain reaction was conducted by Perkin Elmer 9600 Thermal Cycler at the volume of 15 ML and based on Röder et al (1998) method [5]. 6 markers based on Gupta et al (2003) and 14 markers based on Saha et al (2004) were used [6,7]. Polyacrylamide gel electrophoresis was used for differentiation and silver nitrate staining was used for processing of reproduced fragments. Using Bio Rad scanner and Magic Scan software, gels were photographed and bands were scored zero (presence of band) and one (lack of band). Similarity matrix was calculated, using Dice's coefficient. Cophenetic correlation coefficient was used in cluster analysis which is shown as "r". To calculate "r", dendrogram was transformed into its equivalent. This equivalent is called "Cophenetic correlation matrix". Subsequent to calculating this matrix, it was compared with similarity matrix [3]. NTSYSpc ver, 2.02 software was used to determine the genetic differences and drawing dendrogram.

Table 1. Overall Distribution of samples

Genotypes type	Native genotypes of Iran	Iranian line	Foreign varieties
Number	67	34	28
Location	North - North West - West - South West	North - North West - West	Hungary - Russia - Syria - Egypt - Turkey - Libya - Algeria - Morocco - Mexico - India

RESULTS AND DISCUSSION

Cophenetic Correlation Coefficient

Dice's coefficient is used for drawing cluster diagrams and these diagrams had the cophenetic coefficient of r=0.40765 (Table 2). On cluster analysis using molecular data, researchers have shown that low cophenetic coefficient molecular data does not imply the inefficiency of the diagram, but the abnormal conditions in data, especially in molecular data [1].

Grouping Genotypes

The best cluster in this research for total genotypes was cluster related to COMPLETE algorithm; that is, this method simulated the data better, comparing to other methods. Also, Dice' genetic similarity calculation method had better results among other methods (Figure 1). Although the cophenetic correlation in dendrogram was around 0.4 which is not high, considering its significancy at 1% and acceptable separation of genotypes, this dendrogram was chosen.

Considering the derived similarity matrix, two genotypes from Shush Region had the highest genetic similarity of 0.894 and two genotypes of 4 and 56 from Shush and Egypt had the lowest genetic similarity of 0.38 which seemed normal due to their distances and their location in two different latitudes.

Considering the similarity matrix derived from SSR markers, the highest similarity was related to two samples from Ahvaz region and the lowest genetic similarity was related to two samples from Kermanshah and Khorramabad [3].

Groups Derived from Cluster Analysis

Genotypes were grouping in three groups of A, B and C:

Group A, which included the highest number of both Iranian and international genotypes, contained several sub-groups. Most samples from west of Iran such as Kermanshah and Ilam formed one group together and most groups from south of Iran such as Khorramshahr, Shush and Ahvaz formed one group. Since most international genotypes would be in the same group with Iranian genotypes, it could be concluded that these genotypes most likely had similar genetic characteristics and they have originated from geographical environment similar to each other. Also, it could be said that there cultivars are from the same ancestors. For instance, having a sample from Turkey and one from west of Iran could prove this fact.

In this group, most international genotypes formed one group with genotypes from south of Iran (for instance, samples from Mexico, Hungary, Morocco, Syria and Egypt formed one group with samples from Khorramshahr, Shush and Ahvaz). There could be two explanations for this fact; it is either due to the technical weakness (deficiencies, the low number of markers and low polymorphism), or due to the genetic similarities between genotypes.

The high geographical distribution of samples in this group could be due to their common ancestors. However, these cultivars are planted during the time, by transporting them into new locations and forming compatibility [3].
Generally, it could be said that Group A, as the largest group, had the highest diversity and grouping this group into subgroups resulted into homogeneity among subgroups. For instance, several subgroups including samples from west of Iran, two subgroups including international samples and one subgroup including samples from south of Iran were completely separated.

One remarkable fact about this group is that 30 local samples were in this group and all these 30 samples were consecutive. Accordingly, this marker along with separating genotypes according to their geographical distances could separate genotypes in terms of local and crop. Similar to Thiel et al (2003) who could separate winter cultivars from spring cultivars using EST-SSR markers, but they could not spate the genotypes, geographically [9]. Separating local genotypes from lines could only be conducted using this marker and the two previous markers (SSR and AFLP) could not perform the same.

Unlike Group A, Group B included one genotype from Egypt region, only, which due to its huge differences with other genotypes formed a separate group. Studies on markers used in this study suggested a huge difference between this genotype and other genotypes. This difference could be explained by the changes in coding regions in some genes.

Group C includes most samples from west and northwest of Iran along with four samples from Hungary. These results are completely in accordance with results derived from SSR markers. Using SSR markers, Omidbakhsh (2005) could separate samples from west and northwest of Iran from other genotypes. He claimed that if cultivars in on genetic cluster are geographically in a more limited region the prediction of sharing the same ancestor is more likely and no cultivars have entered the region from other regions. To have a more diversity in the region, using cultivars from other regions with lesser similarities are required. Also, to preserved germplasm, a sample from above community could be used [3].

This group separates local genotypes from lines, so that if the group is divided into two subgroups, the second group includes local genotypes. The first subgroup is divided into two subgroups and the first subgroup includes local genotypes and the second subgroup includes cultivars and lines.

Considering the nature of ESR-SSR, the research results are close to what is expected. Since these markers are designed based on completely preserved points of genome, the difference between genotypes in comparison with other markers (SSR, AFLP) is so little. Hence, genotypes are groups closely.

As it could be observed, results from this cluster analysis are in accordance with SSR marker results, for the nature of these two markers is similar to each other. Relative separation of local genotypes from lines is a point which the two previous systems (SSR, AFLP) missed and this shows the efficiency of EST-SSR system.

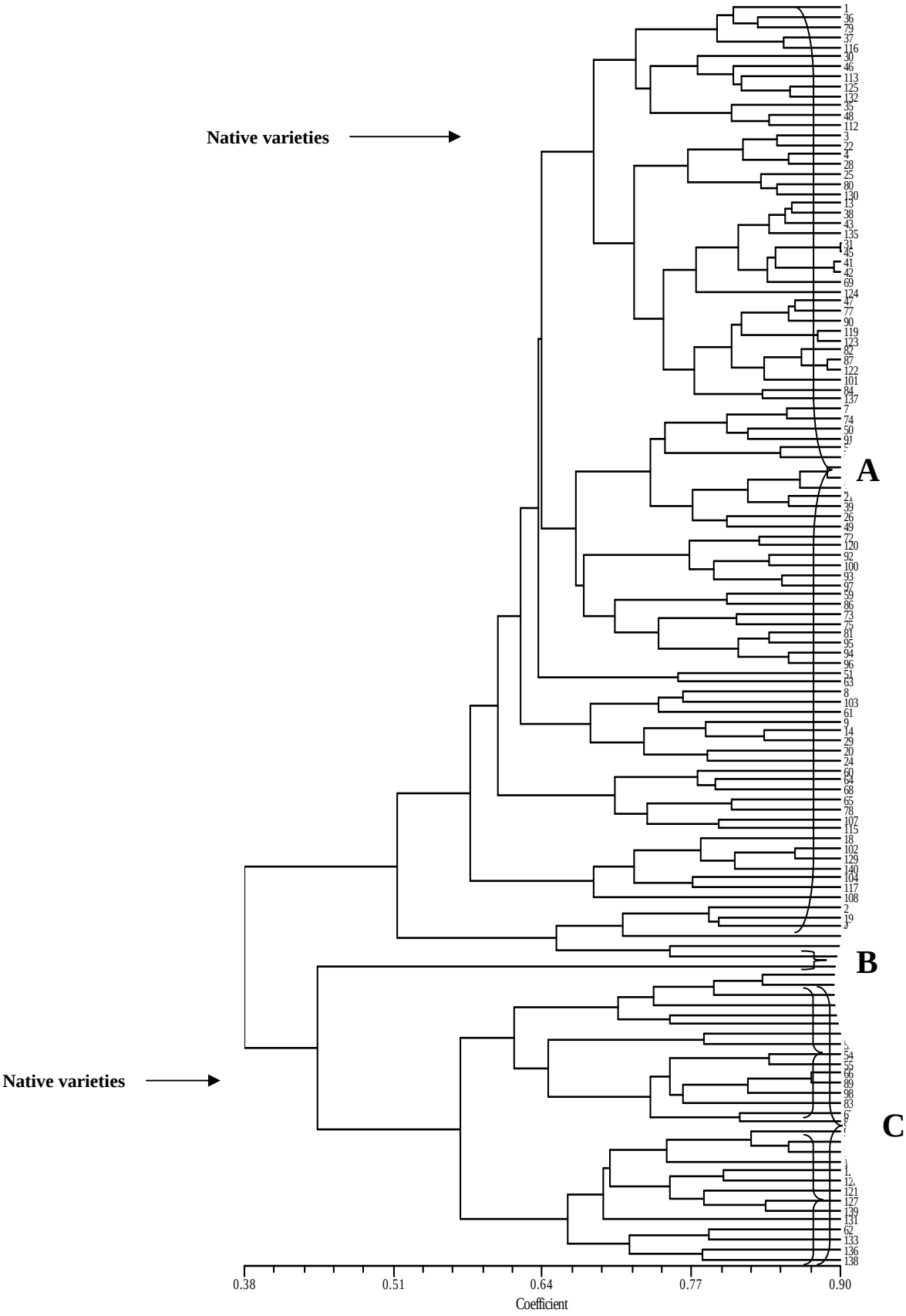

Figure 1. cluster analysis Durum wheat samples

Wong et al studied 60 durum wheat genotypes from seven countries using EST-SSR markers. Cluster analysis of these 60 genotypes suggested that similar samples which have common geographical ancestor are completely separated. To put it another way, genotypes from each country are not grouping separately [10].
In a study on 64 durum lines using EST-SSR markers, Eujayl et al. indicated that samples from one geographical region are completely separated. For instance, 5samples from Syria had a high distance among which samples from the U.S., Iran, France and Italy could be observed [11].

Using SSR, AFLP markers, Omidbakhsh and Ahkami (2005) indicated that these two systems are completely efficient in separating various samples, while EST-SSR marker separated similar samples which originated from one geographical region [3,8]. For instance, having 4samples from Hungary in Group C and having 4 other samples from the same region in Group A could approve that these results are completely in accordance with Wong (2007) and Eujayl (2001), [10,11]. Hence, EST-SSR markers could separate samples which could not be separated by previous markers (SSR, AFLP).

Table 2. Cophenetic Correlation Coefficient Related to Dendrograms Derived from Various Methods

Cophenetic correlation	Dice	Jaccard	Simple matching
Complete	0.40765**	0.43913	0.35422
UPGMA	0.77197**	0.759**	0.61803
WPGMA	0.67484**	0.6314	0.52839

*** Significant at the 1% level*

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