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# Investigation of Genetic Variation in Some Wheat Cultivars Using RGA Molecular Marker

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## ABSTRACT

This paper investigates genetic variation of 30 foreign and domestic wheat cultivars by RGA molecular markers. DNA was extracted from leaf samples according to Dellaporta method (1983). Among the five pairs of RGA primers, three pairs of polymorphic primers were recognized. The highest and the lowest numbers of polymorphisms related to P14N and P54N markers. Percentage of polymorphic bands of P810, P54N and P14N markers were 42%, 33% and 39%, respectively. Cluster analysis was carried out in terms of presence or absence of the band by Jacard similarity coefficient based upon UPGAMA. Similarity coefficient ranged from 0.061 to 0.88. The highest similarity level was observed between Kuhdasht and Kerman cultivars, and the lowest similarity level was between TP981 and Lee.
In this paper, there is a significant correlation between genetic distance and geographical cultivation distance; such that foreign and Iranian cultivars were separated by similarity coefficient 0.44. However, the two cultivars Anza and Hyrmand did not follow this classification. Moreover, from genetic variation point of view, Iranian cultivars' place (with definite pedigree) in the cluster is subjected to their relativity.
**KEY WORDS:** Genetic variation; Wheat; Polymorphism; RGA markers.

## 1. INTRODUCTION

Regarding the worldwide cultivation of wheat, it has great economical and nutritional importance; such that 75% of world population uses wheat in their daily nutrition. Considering the world population growth, it is important to cultivate high yield cultivars (2). Knowing about genetic relatedness facilitates parent choice for crossing and using heterozygosis and obtaining high yield cultivars (5). A proper method in genetic variation investigation is using molecular markers demonstrating a high level of polymorphism. Specific RGA marker is considered as a marker in genetic variation investigation, designed on the basis of conserved motifs of resistance genes. These motifs exist in many plant genes responsible for resistance to fungal, viral and bacterial diseases, and nematode. Due to the high level of polymorphism, high repeatability, and easy interpretation, the result obtained from RGA markers can be an appropriate tool in genetic variation investigation.

## 2. MATERIALS AND METHODS

### 2.1. Plant materials

In this study, seeds of 30 wheat cultivars were collected from the collection of seed and plant breeding institute (Table 1). The sprouted seeds were planted in separate vases, and then were placed in 20-30° C.

Table 1. Name, pedigree and resistance status of wheat varieties

| Name | Iranian types | Resistance to yellow pitting | Foreign types |
|---|---|---|---|
| Attila (CM85836-4Y-0M-0Y-8M-0Y-OPZ | Shirodi | (R) | Chinese 166 |
| Attila (CM85836-50Y-0M-3M-0Y | Chamran | (S) | Lee |
| Byt/4/jar//cfn/sr70/3/jup "S | Hyrmand | (R) | Moro |
| STM/3/KAL/V534/JIL716) | Kavir | (R) | Compair |
| GV/D630//ALD "S"/3/AZD | Shiraz | (S) | Anza |
| ALVAND//ALDAN/IAS58) | Pishtaz | (R) | Yr1/6 Avocet S |
| KVZ/BUHO "S"//KAL/BB | Falat | (S) | Yr6/6 Avocet S |
| RSH/5/WT/4/NORLO/K54*2//FN/3/PTR/6/OMID//K Gods | Gods | (R) | Flanders AL/BB |
| 1-27-6275/CF1770 | Alvand | (S) | Jupateco S |
| TI/PCH/5/MT48/3/WT*//NAR59/TOTA63/4/MUS | Mahdavi | (S) | Heines Kolben |
| BOW "S"/NKT "S" (CM67428-GM-LR-5M-3R-LB-Y) | Tajan | (S) | Heines Peko |
| SPN/MCD//CAM/3/NZR | TOS | (S) | TP981 |
| KVZ/TIL71/MAYA"S"//BB/INIA/4/KARAJ2/5/A | Shahriar | (S) | Bolani NZA/3/PI/NAR//HYS |
| TR8010200-29R-1R-6R-0R | Kohdasht | (R) | MV17 |
| TAN/VEE//OPATA | Zagross | | |
| Kauz | Atrak | | |

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### 2.2. PCR DNA extraction

DNA extraction of plants leaves was carried out in two-leaf seedlings according to Dellaporta method (1983) (4). DNA quality and quantity was investigated by spectophotometry system in the wavelength of A260 and A280. The samples underwent PCR experiment using three pairs of primers (Table 2). PCR components were 2 µL of DNTP, 2.25 µL of buffer, 0.2 µL of Taq polymerase, 2 µL of primer, and 5 µL of sample DNA (1 ng/ µL ) (1). PCR thermal profile, including 94°C primary denaturation stage for 5 minute, 45 cycles containing (denaturation at 94°C for 1 minute, binding at 38°C for 1 minute), and final amplification temperature 72°C for 7 minutes, was utilized.

Table 2. RGA initiators list and their period.

| Initiator period | Direction | Initiator name |
|---|---|---|
| GGIAAIACIACICTIGCI | F | P1 |
| IAGIGCIAGIGGIAGICC | R | P4N |
| CTTITTGTIGTGAT | F | P5 |
| IAGIGCIAGIGGIAGICC | R | P4N |
| **ATCCTGGTGACIACICGI** | **F** | **P8** |
| **ATGICGCAAGTTGATIAG** | **R** | **P10** |

### 2.3. Electrophoresis and staining

PCR productions underwent electrophoresis in different systems. Initial electrophoresis was done in a horizontal in agarose 1%, in 80 v, for 45 minutes, using ethidium bromide staining. A vertical electrophoresis (Mod.Vss-1100) was utilized in polyachrilamid gel and TBE buffer (1X) for 1 hour and at 240v, in order to have better separation of bands. Moreover, to achieve a better band resolution, vertical electrophoresis (POWER PAC 3000), containing polyachrilamid 6% and TBE buffer (1X) was used. After 40 minutes of pre running in voltage 240v, the samples were running for 2 to 2.5 hours in voltage 1400v, and then were stained using $AgNo_3$ (Fig. 1, 2, 3).

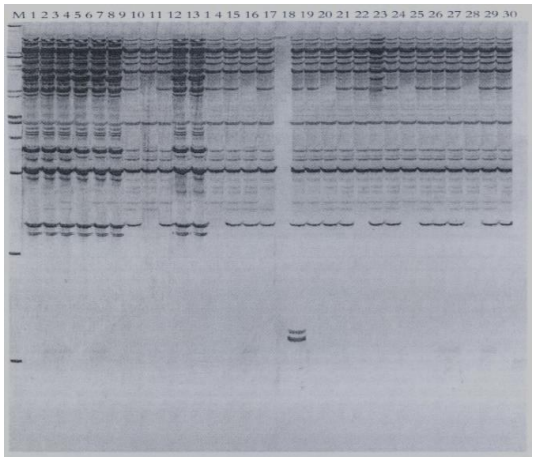

Fig. 1. Band pattern of amplified segments obtained by P54N primer.

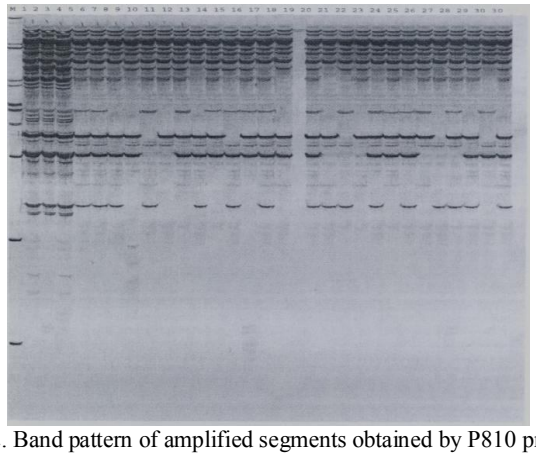

Fig. 2. Band pattern of amplified segments obtained by P810 primer.

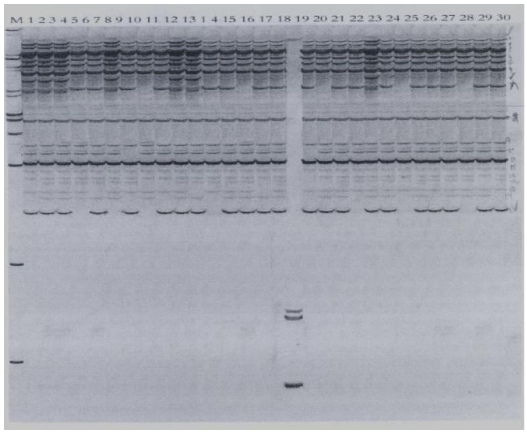


Fig. 3. Band pattern of amplified segments obtained by P14N primer.

### 2.4. Data analysis

Number of Bands and their rankings were recorded by 0 and 1based on bands presence or absence. Data analysis was carried out by SPSS software and UPGAMA method.

## 3. RESULTS AND DISCUSSION

Dendogram was drawn based upon Jacard similarity coefficient. Initial study of the dendogram shows that the cultivars bred in Iran and abroad (with some few exceptions), were grouped into two separate clusters (Fig. 4).

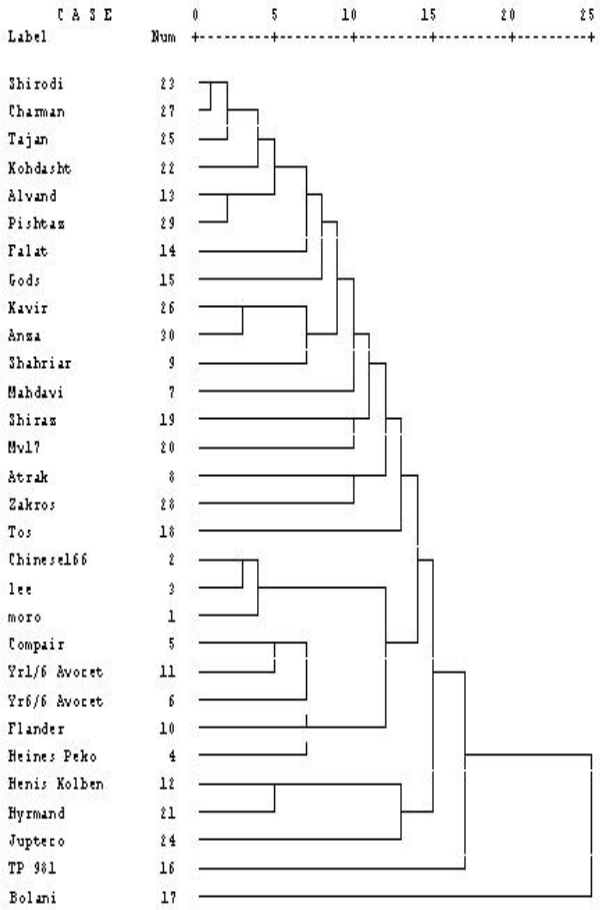


Fig. 4. dendogram determination of relativity of different genotypes using Jacard similarity coefficient.

Genetic separation of foreign and domestic cultivars can be explained by selection pressure on local resistance genes.

Within different breeding programs, the resistance genes which have been selected in each country are effective against physiologic pathogen of the region. So, placement of in the same cluster can be explained by their genetic relativity of their common resistance genes. Hence, RGA marker can indicate genetic variation of wheat cultivars.

In spite of separation of foreign and Iranian cultivars, the two Iranian cultivars, Hyrmand and Anza, do not comply with this classification; such that Hyrmand is placed in foreign cultivars cluster, next to Jupteco S. study of the pedigree of Hyrmand shows the presence of Jupteco S in its pedigree, and thus regarding its relatedness. Hyrmand is placed in the foreign cultivars cluster. Moreover, the foreign cultivar, Anza, is grouped in the Iranian cultivars cluster, next to Shahryar cultivar. This can also be explained similarly, since Anza belongs to Shahryar pedigree. In this paper, morphologic traits of Iranian genotypes such as growth type, sensitivity to chill, sensitivity to shattering as well as the cultivars' ancestors were comparewith band pattern. It was demonstrated that many genotypes with similar traits are grouped in the same subset. For example, Shirudi and Chamran cultivars which were in the same subset are similar in terms of the studied morphologic traits. Investigating their pedigree, it was specified that both have been bred from Attila. Gods and Falat cultivars, grouped in the same subcluster, have the common ancestors, BB and KAL, as well as having similarities in traits studied. The obtained results demonstrate proper compatibility between morphologic traits and genetic variation. This is also reported by Chen et al. (1998) on wheat RGA markers (3).

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