J. Basic. Appl. Sci. Res., 2(1)233-236, 2012
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ISSN 2090-4304
Journal of Basic and Applied Scientific Research
www.textroad.com

Study of Variation of Disease Resistance Genes in Wheat using RGA Molecular Markers

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ABSTRACT

Comparison of nucleotide sequences of cloned resistance genes of plants demonstrate some conservative regions called Resistance Gene Analogues (RGA). Based on RGA sequences, some degenerated primers were designed and employed in the study of resistance mechanisms and cloning of resistance genes. In this study, we have used five pairs of RGA primers and three electrophoresis systems to study the variation of disease resistance genes and genetic variation in 30 local and foreign wheat cultivars resistant and sensitive to yellow rust. The results demonstrated sharp bands of PCR products in vertical electrophoresis (dimensions: 400*400*0.4 mm, 1600 volt, and 2-2.5 hours) with polyacrylamid denaturing gel. Data analysis using SPSS software and UPGMA method showed the three pairs of selected primers with total 1886 bp resulted in 33-42% band polymorphism. Similarity Jacard dendogram study demonstrated that although cultivars were not categorized in separate groups, all yellow rust sensitive cultivars were all placed in the lower part of the dendogram. The results were indicative of the efficacy of RGA technique for evaluation of genetic variation and variation of resistance analogues. Nevertheless, considering the contribution of two isolated characteristics in separation and clustering of cultivars, interpretation of the results was complex.
KEY WORDS: Genetic variation; Wheat; Polymorphism; RGA markers.

1. INTRODUCTION

Many plant genes responsible for resistance to different pathogens such as viruses, fungi, bacteria and nematodes have been isolated and cloned (Jahal and Brigg 1992, Baker et al. 1997). Study of nucleotide sequences of resistance genes indicate that although they do not have a high degree of homology, they have specific conserved domains, called resistance gene analogues. Sequences of conserved domains are used as molecular RGA markers in PCR amplification for cloning of resistance genes. For instance, using degenerated RGA primers on the basis of LRR sequence conserved in RPS2 resistance genes of Arabidopsis thaliana and N tobacco, a cloned sequence in potato was isolated which was homologous to known resistance genes and was completely linked with locus Grol that relates to blight resistance (Leister et al. 1996). Fuillet et al. (1997) cloned gene LR10 which is responsible for leaf color in wheat using RGA markers. Designing some RGA markers and using them in segregating bulk genome lines of wheat, Keshavarzi et al. (2005) labeled the genes contributing in resistance to yellow rust (1). Moreover, RGA markers have been used in construction of the genetic map of some genes (Kanazin et al. 1996) and coincident study of variation of resistance genes and germplasm genomes of rice, wheat and barley (Chen et al. 1998). The aim of this study is to evaluate the variation of pathogen resistance genes in 30 local and foreign wheat cultivars modified for yellow rust resistance using degenerated RGA. Furthermore, the efficiency of these markers for evaluation of the genetic variation of cultivars, considering their pedigree and the role of coincident contribution of two different characteristics (variation of resistance genes and genetic variation) in clustering of cultivars is studied.

2. MATERIALS AND METHODS

Plant material: 30 local and foreign wheat cultivars modified for resistance against yellow rust and some yellow rust sensitive cultivars were used.

DNA extraction and PCR:

DNA was extracted from the leaves in two-leaf seedlings according to Dellaporta (1983) method (4). The quality and quantity was DNA was evaluated by spectrophotometer. PCR reactions (10×) were carried out using 10 pmol of each RGA primer (Keshavarzi et al. 2005, Chen et al. 1998). PCR cycles consisted of the initial denaturing step for 5min at 94°C, then 45 cycles of 1min at 94°C, 1min at 45°C, and 12min at 72°C, and the final extension

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step which lasted 7min at 72°C (Chen et al. 1998). The name and sequence of primers used in this study are presented in Table 2.

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		

PCR products of different systems underwent electrophoresis as follows. The initial electrophoresis was performed horizontally in agarose 1-1.4%, with 80 volt, for 45min, and using ethidium bromide dye. Denaturing polyacrylamide gel 6% in small scale vertical system (200*200mm), 240 volt, and for one hour was used to enhance the banding resolution. Finally, vertical DNA sequence finder electrophoresis (dimensions: 400*0.4mm) by denaturing polyacrylamide gel, 1600 volt, and for 2-2.5 hours was carried out. Dyeing was done by silver nitrate and images were taken by a scanner.

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**

Data analysis:

In each gel, a 0,1 matrix was constructed for each cultivar, which was transformed into similarity matrix using similarity Jacard coefficient. Cluster analysis was done using SPSS software and UPGMA method, and the similarity dendogram was drawn.

3. RESULTS AND DISCUSSION

In agarose gel, an 8-100 bp smear or limited banding is usually observed. Although it is not suitable for scoring, it is an indicator of successful genome propagation (Chen et al. 1998). Polyacrylamide electrophoresis in small scale vertical systems leads to relative improvement of banding. However, the optimized condition and various polymorphous bands of each primer were obtained in large scale electrophoresis system (DNA sequence finder) (Fig.1). Considering these findings, it was revealed that many bands in small scale vertical system were overlapping and were not separated adequately. DNA sequence finder electrophoresis system is able to discriminate the differences at the level of one nucleotide. Until 1998, only agarose gel and RFLP were used for PCR products based on RGA primers. Thus, heterogeneity of a unique band obtained by agarose gel was frequently reported (Leister 1996, Kanzan 1996, Yu 1996). By using polyacrylamide gel and large scale electrophoresis system, Chen et al. overcome the problem of band overlap in 1998.

Analysis of obtained bands demonstrated that the three RGA primers produced high degrees of polymorphism (Table 3). Totally, the three primers propagated 1486 main bands, out of which 32-42% were polymorphous. The highest number of bands and the highest percentage of band polymorphism were produced by primer P810.

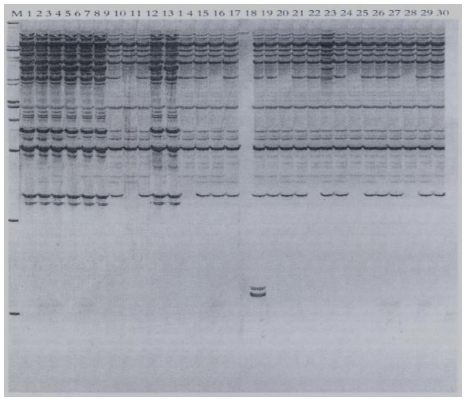

Fig. 1. Banding pattern of propagated fragments by primer P54N.

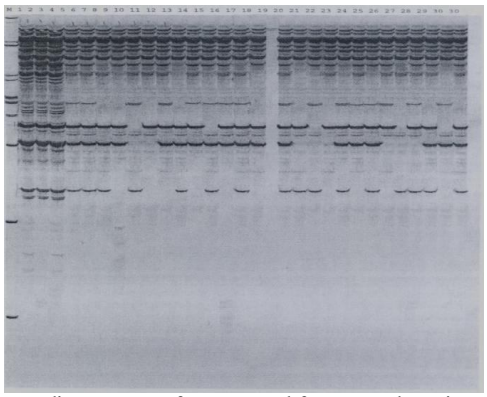

Fig. 2. Banding pattern of propagated fragments by primer P810.

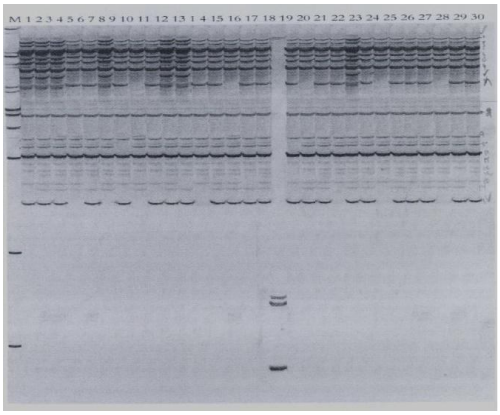

Fig. 3. Banding pattern of propagated fragments by primer P14N.

In evaluation of resistance gene variation in wheat cultivars modified for resistance to yellow rust using RGA primers, Chen et al. (1998) reported 27% of polymorphism. They considered the relatively low percentage of polymorphism to a result of close relativity of studied cultivars; since all studied cultivars were of the same line modified for resistance to yellow rust. The percentage of polymorphic bands obtained in this study was higher compared with the results obtained by other primers (3). For instance, the wheat polymorphism percentage using AFLP and ISSR primers reported to be 17 and 24%, respectively (Ma 1998, Nagoaka and Ogihara 1997). The dendogram constructed on the basis of similarity Jacard coefficient is illustrated in Fig. 4. Primary study of the dendogram showed that modified Iranian and foreign cultivars (except few cultivars which be mentioned later) were segregated into two isolated clusters. The genetic segregation of local and foreign cultivars can be explained by selection pressure on local resistance genes.

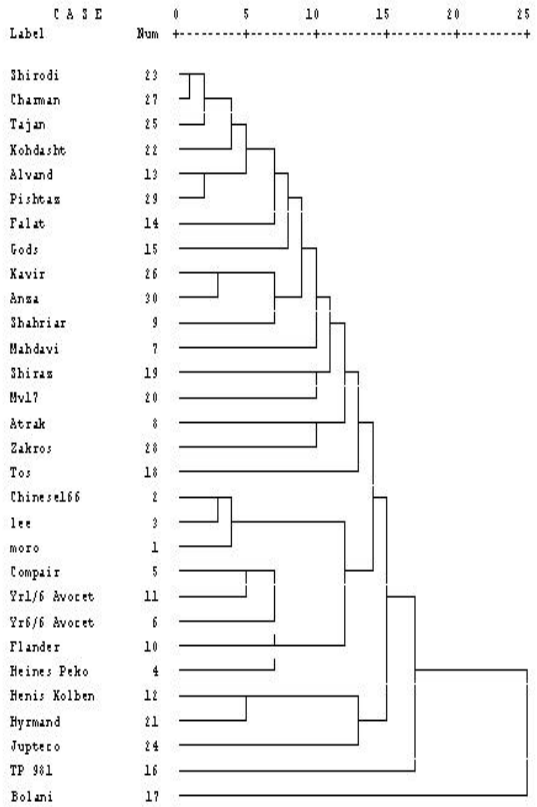

Fig. 4. Dendogram of relativity determination between genotypes using similarity Jacard coefficient.

In this regard, through breeding programs in each country, some resistance genes are selected and amplified which are effective against physiological pathogen species of that region. Thus, the reason for Iranian cultivars being placed in one cluster is their genetic relativity, because of their common set of resistance genes. Therefore, RGA primers can be indicative of the level of similarity and variation of resistance genes of cultivars.

Cluster analysis of cultivars based on variation of disease resistance genes also demonstrated more or less similar results. For instance, all cultivars sensitive to yellow rust, including Bolani, Jupateco, Heines, Peko, Heines Kolben, Yr6/6 Avocet, in the lower part of the dendogram next to each other; and other resistance or semi-resistance cultivars were quietly segregated from them. The presence of the resistance cultivar, Hyrmand, among sensitive cultivars can be due to existence of Jupateco S in its pedigree. Moreover, the presence of the sensitive cultivar, Lee, can be explained by the similarity of resistance genes, since Lee and Chinese 166 which were assigned to the same subcluster, possess the same yellow rust resistance genes, i.e., Yr6/17, 24, 35, 43, 45 (5).

Acknowledgement

We thanks very much from research assistant of Young Researchers Club of Islamic Azad University (Ahvaz Branch) that provide finance of this research project.

REFERENCES

1- Hlajyan, T., 1381, Nshanmnd a gene or genes for resistance to yellow rust in wheat using molecular markers RGAP and RAPD. Biotechnology M.Sc. Thesis, Faculty of Agriculture, Ferdowsi, Mashhad. Page 76

2- Khodabandeh, N. 1372. Grains. Tehran University Press, 506 pages

3- Chen,X.M., Line,R.F. and Leung,H.(1998). Genome Scanning for resistance gene analoge in rice, barlay and wheat by high solution electrophoresis. Theor. Appl. Genet.97:345-355

4- Dellaorta. S.C, Wood, F. and Hicks J.B.(1983). A Plant DNA Mini Preparation: version II. Plan. Mol.Bio Reporter 1:19:21

5- Lefebvre, V. and Chevre, A.M.(1995). Tools for marking plant disease and Pest resistance gene review. Agronomic., 15;3-19.