III.1 MOLECULAR MARKERS ASSOCIATED WITH RESISTANCE TO LEAF RUST IN BARLEY

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Abstract

Genetic variation and relationship among eight barley (Hordeum vulgare L.) genotypes (four cultivars; Giza 2000, Giza 126, Giza 123 and Giza 132 and four promising lines; line 1, line 2, line 3 and line 4 were evaluated to study the marker-assisted selection associated with resistance to leaf rust (Puccinia hordei) using Sequence-Related Amplified Polymorphism (SRAP) as a new marker and first time to be reported on Egyptian barley for barley rust resistance. Results showed that the eight barley genotypes were highly different in leaf rust reaction. Data generated by nine primer combinations were sufficient to discriminate among those studied barley genotypes. Primer combinations me2+em2 had specific bands related to leaf rust resistance with band size of 1250 bp, which was found only in the resistant genotypes, while other primer combinations were found in some resistant genotypes and not found in the other susceptible ones. The (Un-weighted Pair Group Method with Arithmetic Mean) UPGMA cluster analysis of the similarity data, grouped the eight barley genotypes into two main clusters according to their reaction to leaf rust. The first cluster included the resistant genotypes (Giza 123 and Giza 132) in sub cluster and other moderately resistant genotypes (line1 and line 2) in another sub cluster, while the rest of genotypes were found in the other cluster including susceptible genotypes. These results will be useful for barley germplasm management in terms of biodiversity protection and design of new crosses for disease resistance to leaf rust breeding program.

Keywords: *Hordeum vulgare* L., leaf rust (*Puccinia hordei*), sequence-related amplified polymorphism (SRAP), UPGMA cluster analysis

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a cereal crop that is grown throughout the world and is ranked fifth in world crop production. Barley can be grown in many different climatic regions due to its adaptability to diverse conditions. These climatic conditions include variable growing seasons, temperatures, and precipitation rates (Pomeranz, 1987). Barley, like all crops, is attacked by many disease-causing organisms. Some cause only minor damage while others can completely destroy the crops. One of the most devastating diseases is rust, which is caused by a fungus (*Puccini hordei*). This fungus can drastically reduce both the yield and quality of crops.

Deployment of resistant cultivars is one of the most effective and economical means of controlling barley leaf rust (Puccini hordei). Identification and incorporation of new and effective sources of resistance are crucial to the success of barley breeding programs. Barley leaf rust is of particular importance in climatic regions where the crop matures late in the growing season. This occurs to a great extent in both the winter and spring production regions of North Africa and other parts of the world (Mathre, 1982). Historically, rust diseases have had a major impact on barley crop yields. For this reason, the study of leaf rust resistance has become one of the major economic importance. The occurrence of leaf rust epidemics has increased in the past 15-20 years (Clifford, 1985). Yield losses as high as 32% in susceptible cultivars were reported by Griffey *et al.* (1994), based on estimates obtained from regression analysis of disease severity versus grain yield. It has been estimated that an average yield loss of 0.42% occurs for each 1% increment of leaf rust on the upper two leaves at the early dough stage of development (Griffey, *et. al.,* 1994 and Murray, *et. al.,* 1998).

Sequence related amplified polymorphism (SRAP) is a PCR based marker system as described by Li and Quiros (2001). The SRAPs is a simple and efficient marker system that can be adapted for a variety of purposes in different crops. It is simple, has reasonable throughput rate, discloses numerous co-dominant markers, targets open reading frames (ORFs), and allows easy isolation of bands for sequencing (Li and Quiros, 2001). With this unique primer design, SRAP markers are more reproducible, more stable, and less complex than RAPD and AFLPs. It could help to assess whether different management practices affect the genetic diversity of the individuals or populations. To date, SRAP markers have been used to determine genetic diversity in some crops such as; tomato (*Lycopersicon esculentum* Mill.) (Ruiz and Garcia-Martinez, 2005), persimmon (*Diospyros Kaki* L.F.) (Guo and Luo, 2006) okra (Gulsen *et. al.,* 2007), pea (*Pisum sativum* L.) (Esposito *et. al.,* 2007) sugarcane (*Saccharum sp.*) (Suman, *et. al.,* 2008) and citrus (Uzun *et. al.,* 2009). To date, there is no report on determining the genetic diversity and characterization of barley cultivars and/or genotypes by SRAP markers. Even other cereals like wheat just recently, some wheat genotypes were studied to find some marker associated with drought tolerance based on SRAP markers and found that these markers are evenly distributed throughout wheat genome and perfectly generate marker associated with drought (Khatab *et. al.,* unpublished data). This may apply to the other cereals and is likely to cause good genome sampling among barley genotypes resistant to leaf rust.

The objectives of the present study were to determine the relationships among some barley genotypes based on the SRAP markers and try to find markers associated with leaf rust resistance to use as a SCAR marker.

MATERIALS AND METHODS

PLANT MATERIALS

Eight barley genotypes were used in this study, the genotypes name, pedigree and reaction to leaf rust are shown in Table 1. The field investigation was carried out at Sakha Research Farm (North of Egypt), Barley Res. Dep., Field Crops Res. Inst., Agric. Res. Center during 2012/2013 growing seasons. Laboratory work was carried out at the Plant Genetics Laboratory, Institute of Genetic resources- Faculty of Agriculture, Kyushu University, Hakozaki 6-10-1, Fukuoka 812-8581, Japan.

Table 1. Name, pedigree and leaf rust reaction for the studied barley genotypes

No.	Genotype	Pedigree	Leaf rust reaction*
1	Giza 130	Comp. cross 229//Bco Mr/ DZ02391/3/Deir Alla 106	R
2	Giza 132	Rihane-05//AS 46/Aths*2Athe/ Lignee 686	R
3	Line 1	Alanda01/5/c101021/4/CM67/ U.Sask.1800//pro/CM67/3/dl70	MR
4	Line 2	Aths/Lignee686/5/Apm/RL/4/API/EB489-8-2-15-4//POR/U.SASK1766/3/ CEL/CL	MR
5	Line 3	Panniy/Salmas/5/Baca"s"/3/AC253// C108887/C105761/4/JLB70-01	MS
6	Giza 2000	Giza117/Bahteem52// Giza118/ FAO86 / 3/ Baladi16/ Gem	S
7	Giza 126	Baladi Bahteem/S D729-Por12762-BC	S
8	Line 4	M6476/Bon//JO/York/3/M5/Galt//As46/4/Hj34-80/Astrix/5/Nk1272	MS

** R= resistant and S= susceptible MR; moderate resistance and MS; moderate susceptible*

DNA ISOLATION, PCR AND GEL ELECTROPHORESIS

DNA was isolated using CTAB method from fresh leaves of the used eight genotypes of barley according to Doyle and Doyle (1990). The PCR reactions using eight SRAP combinations were used in this study as shown in Table 2. The reactions for SRAP were optimized and mixtures were prepared (in total volume of 25 µl). PCR cycling was carried out as the following program; initial denaturation at 94°C for

4 min, followed by five cycles comprising 1-min denaturation at 94°C, 1-min annealing at 35°C, and 30 s of elongation at 72°C. In the following 30 cycles, denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and elongation at 72°C for 30 s were carried out, ending with an elongation step for 10 min at 72°C. The amplified products were stored at4 °C. The PCR products were separated by electrophoresis using 2% agarose gel in 1 x TBE buffer against 100 bp DNA Ladder as a size marker. Bands were detected with ethidium bromide staining and visualized under UV light, photographed on Gel Documentation.

DATA ANALYSIS

Amplification profiles for the used eight barley genotypes as a result of SRAP application were compared with each other and DNA fragments were scored as a binary data, where (1) means present and (0) means absent. The data were used to estimate genetic similarity on the basis of number of shared amplification products (Nei and Li, 1979). The distance coefficients were calculated by the following statistical equation.

$$F= 2N_{xy}/ (N_x + N_y)$$

Where, F is the distance coefficient in which N_x and N_y are the numbers of fragments in genotypes x and y, respectively, and N_{xy} is the number of fragments differed by the two genotypes (Lynch, 1990). The electrophoresis patterns of the reproducible banding patterns of each primer which were produced by SRAP were chosen for analysis. Each band was scored as present (1) or absent (0), and pair wise comparisons between individuals were made to calculate the Jukes-Cantor coefficient using PAST program (Paleontological Statistics Version 1.94b) adapted by Hammer *et al.* (2001). Cluster analysis was performed to produce a dendrogram using un-weighted pair-group method with arithmetical average (UPGMA).

Table 2. Primers name and sequences

Name	Sequences SRAP 5'---- 3'	
me1+em1	TGAGTCCAAACCGGATA	GACTGCGTACGAATTAAT
me1+em2	TGAGTCCAAACCGGATA	GACTGCGTACGAATTTGC
me1+em3	TGAGTCCAAACCGGATA	GACTGCGTACGAATTGAC
me2+em1	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTAAT
me2+em2	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTTGC
me2+em3	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTGAC
me5+em1	GAGTCCAAACCGGAAG	GACTGCGTACGAATTAAT
me5+em2	GAGTCCAAACCGGAAG	GACTGCGTACGAATTTGC
me5+em3	GAGTCCAAACCGGAAG	GACTGCGTACGAATTGAC

RESULTS AND DISCUSSION

The SRAP marker system is becoming the marker of choice for characterization and genetic diversity studies in a wide range of plants. The study described in the present paper shows that SRAP analysis is a powerful tool also for the characterization of barley genotypes. In our study, the SRAP markers were used for the first time in barley and distinguished genotypes were efficiently shown with high level of polymorphism. On the other hand, some genotypes were distinguished by only one primer combination. Results showed that there were high levels of polymorphism in genotypes under study, especially when the genotypes were compared for the leaf rust reaction. Among the nine primers combinations used, me1+ em3 primer combination gave the highest polymorphism (84.6%) with 13; only two were monomorphic and the other 11 were polymorphic, followed by me2+em2 (71.4 %); two bands were polymorphic out of seven bands. However, me5+em3 has the lowest polymorphism (11.1%). The amplified fragments ranged in size from ~120 to 1500 (Fig.1 A and B). Some primers had specific bands found in some resistant genotypes such as primer me1+em3 had band with size ~300bp found in some resistant genotypes (Fig. 1 A). Other primers such as me2+em2 has some bands related to resistant genotypes with size ~1250 bp found only in all leaf rust resistant genotypes. Moreover, the same primer has other band linked with resistant genotypes with size ~550 bp and found in some resistant genotypes (Fig. 1 B).

A dendrogram (Fig. 2) based on the genetic similarity coefficient was constructed using the nine SRAP primers. In this dendrogram, the four barley genotypes Giza 130, Giza 132, Line 1 and Line 2 were classified into one group as resistant genotypes. The other genotypes; Giza 2000, Giza 126, Line 3 and line 4 were clustered together and formed another cluster as a susceptible genotypes. From similarity matrix the data showed high similarity between Giza 126 and Giza 2000 as susceptible genotypes as shown in Table 3.

High level of polymorphism in barley genotypes were previously reported based on their RAPD and ISSR data by Khatab *et al.* (2013) and based on SSR data Mariey *et al.* (2013). Genetic variation among barley genotypes were determined by using various marker systems and there were a gradation of polymorphism from RFLPs (low) to RAPDs (high) Mariey *et al.* (2012) to AFLPs (very high) (Adawy, 2008) in barley and a better resolution of relatedness among genotypes were done using SRAP marker. These results will be useful for barley germplasm management in terms of biodiversity protection and design of new crosses for disease resistance to leaf rust breeding programs.

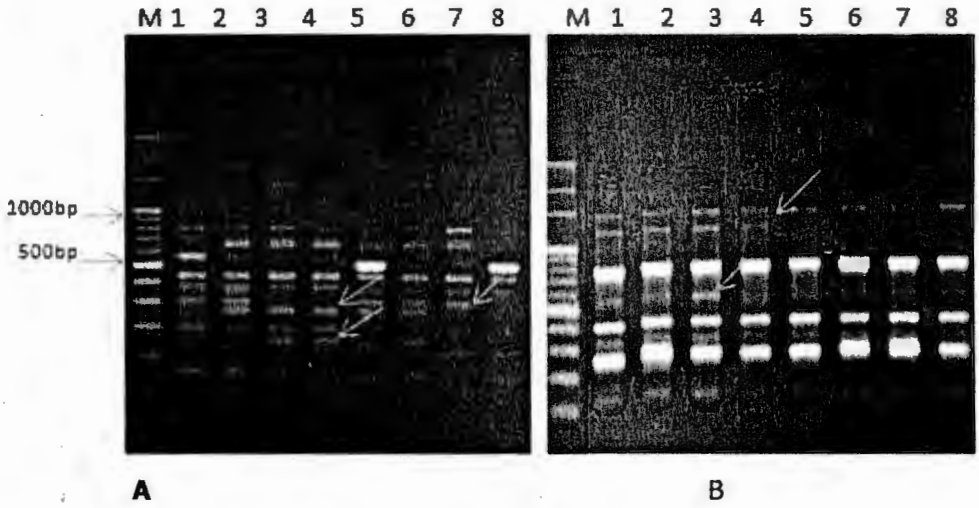

Fig. 1. Agarose gel electrophoresis using SRAP primer combinations me1+em3 (A) and me2+em2 (B) in eight barley genotypes. (M) marker, (1) Giza 123, (2) Giza 132, (3) line 1, (4) line 2, (5) line 3, (6) Giza 2000, (7) Giza 126 and (8) Line 4.

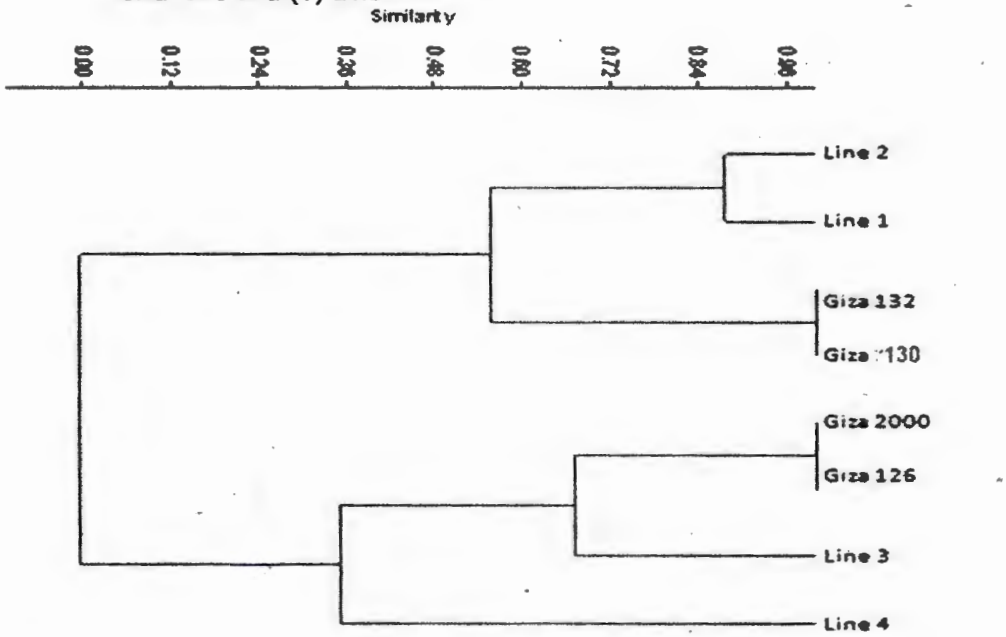

Fig. 2. SRAP based dendrogram of the eight barley genotypes constructed using Un-weighted Pair–Group Method with Arithmetic (UPGMA).

Table 3. Similarity matrix for SRAP primer combinations using eight barley genotypes.

	Line4	Giza126	Giza2000	Line3	Line2	Line1	Giza132
Line 4	1						
Giza126	0.37	1					
Giza2000	0.37	1	1				
Line3	0.30	0.67	0.67	1			
Line2	-0.08	0	0	-0.15	1		
Line1	-0.16	-0.12	-0.12	-0.30	0.87	1	
Giza132	-0.08	0.23	0.23	0.07	0.50	0.61	1

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٣-١ المعلمات الجزيئية المرتبطة بمقاومة صدأ الأوراق في الشعير

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يهدف هذا البحث إلى تقييم ودراسة التنوع الوراثي والقرابة الوراثية بين ثمانية تراكيب وراثية من الشعير و هي جيزة ٢٠٠٠، جيزة ١٢٦، جيزة ١٢٣، جيزة ١٣٢ وأربعة سلالات هي (سلالة ١، ٢، ٣، ٤) ومدى ارتباطها بصفة المقاومة لصدا الأوراق باستخدام الدليل الجزيئي القوى الSRAP كعلامة جديدة وأول مرة ينشر عن الشعير أو أصداء الشعير. وجد اختلاف بين الثمانية تراكيب وراثية من حيث قابليتها للإصابة أو المقاومة للأصداء. و كانت البيانات التي استخدم فيها تسعه تركيبات من البوداىء كافية لتمييز التراكيب الوراثية من حيث وجود بعض منها مقاوم وأخرى حساس. وباستخدام التحليل UPGMA لدراسة التشابه في التراكيب الوراثية الثمانية قسمت إلى مجموعتين رئيسيتين طبقا لرد فعلهم على المقاومة والحساسية لصدأ الأوراق. وتشمل المجموعة الأولى التراكيب الوراثية المقاومة أما بقية التراكيب الوراثية الحساسة توجد في المجموعة الأخرى. وسوف تكون هذه النتائج مفيدة لإدارة الأصول الوراثية للشعير من حيث دراسة التنوع البيولوجي وتصميم برامج التربية للمقاومة للصدأ.