MOLECULAR CHARACTERIZATION OF EGYPTIAN WATERMELON PROMISING LINES FOR RESISTANCE TO *FUSARIUM* WILT

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Abstract

Sequence characterized amplified region (SCAR) and random amplified polymorphic DNA (RAPD) were used to identify new watermelon promising lines for *Fusarium* wilt resistance. Six promising lines namely, 74, 6311, 95, 85, 84, 84g compared with Sugar Baby cultivars as a susceptible genotype were used in this study. SCAR, related to resistance watermelon with 898 bp distinguished the resistant lines which was consistent with the field reaction. RAPD markers were used for characterization of the promising lines, three additional bands were provided related to primers 2, 5 and 6. Based on phylogenetic tree, there were two clusters, the first one consisted of two genotypes Sugar Baby and line 6311 as susceptible genotypes. While, the second consisted of two subclusters at the level of 80% genetic similarity, one of them has the three resistant genotypes, lines 74, 84 and 84g. The second subcluster consisted of two lines 85 and 95 which were pre-identified as resistant lines. Thus, these markers could be useful for identifying *Fusarium* wilt-resistant genotypes in breeding programs using segregating progenies of watermelon.

Introduction

Watermelon (*Citrullus lanatus* L.) is one of the most important summer cucurbit. *Fusarium* wilt (FW) is one of the economically most important diseases that infect watermelon in Egypt. It is caused by the soil born fungus *Fusarium oxysporum* which considered as one of the most serious diseases of watermelon. Yet, there are no fungicides currently labeled for treating FW. Thus, one of the best ways to manage FW disease at present is based on breeding for resistant lines (Diener and Ausubel, 2005). Most of the previous studies on watermelon breeding were done to study combining ability and heterosis analysis (Armstrong and Armstrong 1975; Rajan *et al.*, 2002). Losses due to FW have been increasing recently, because, firstly there is no resistance in most commercial watermelon cultivars. Secondly, there has been an increase in the incidence of seedborne pathogens transmission (Egel and Hoke, 2007), and thirdly, the increased levels of soil pathogen inoculum (Beckman, 1990). On the

other hand, resistance in commercial cultivars is often no longer effective due to the presence of the highly aggressive races of the pathogen (Martyn, 1987). The resistance to FW in watermelon is controlled by a single dominant gene (Henderson *et al.*, 1970; Netzer and Weintall, 1980).

A major limitation of using Isozyme/protein markers is the limited polymorphic loci which can be detected in watermelon (Biles *et al.* 1989). A new marker system, which offers a large number of polymorphic loci, is needed to develop a genetic map with high density or map the genomic regions around the genes of interest. DNA markers have introduced a new dimension to the development of genetic maps and the mapping of agronomically and physiologically important characters since they have the potential to reveal an almost unlimited number of polymorphisms. The commonly used DNA markers are sequence characterized amplified region (SCAR) and random amplified polymorphic DNA (RAPD). The RAPD markers have been used for many genetic studies of plant crops and are suitable for marker assisted selection in applied breeding. (Wechter *et al.* 1995; Lee *et al.* 1996; Morales *et al.* 2002; Park *et al.* 2004a, b). On RAPD-based study, Levi *et al.* (2001) found low genetic diversity among watermelon cultivars. Unlike RAPD, SCAR is effective and sensitive (Clercq *et al.* 2007; Dias *et al.* 2007), its high specificity allows direct tagging of a single locus of a genome and commonly used as genetic markers by plant breeders to accelerate breeding programs (Huaracha *et al.* 2004). RAPD and SCAR were used to construct a linkage map of resistance and described as a powerful molecular tool for marker disease screening (Xu *et al.* 2000; Hawkins *et al.* 2001; Juchum *et al.* 2007; Ngezahayo *et al.* 2007). This approach is a highly efficient identification tool to screen molecular markers, especially when dealing with a single dominant gene (Liebenberg and Pretorius 2004).

The present study amid to investigate SCAR and RAPD molecular polymorphisms, and identify polymorphic markers for developing a molecular marker(s) associated with *Fusarium* wilt resistance gene(s) in Egyptian watermelon, which will be necessary to reliable screening methods for future breeding programs.

Materials and Methods

Plant genotypes

Six watermelon promising inbred lines namely, (74, 6311, 95, 85, 84, 84g) and Sugar Baby cv., as susceptible for FW were used in this study. Watermelon seeds were kindly provided from Vegetables Department, Institute of Horticulture Research, Agricultural Research Center, (ARC) Egypt. Field traits such as, average fruit weight, No. of

fruits/plant, total fruits No., earliness, yield/plant and reactions of FW were recorded.

Molecular analysis

DNA was isolated by CTAB method (Doyle and Doyle, 1990). Seven 10-mer arbitrary primers in RAPD and one pair specific SCAR primer for wilt resistance based on Lin *et al.*, (2009) were used. Sequences of all primers are shown in Table (1). For RAPD and SCAR analysis, PCR amplifications were carried out in a total volume 25 µl containing 2.5 µl 10 x buffer, 2 µl 25 mM MgCl2, 2 µl 2.5 mM dNTPs, 2 µl 10 pmol primer, 1 µl 50 ng of genomic DNA and 0.25 µl Taq DNA polymerase (5 units/µl). PCR cycling was carried out as the following program, one cycle at 95 °C for 5 min., then 40 cycles were performed as follows: 30 s at 95 °C for denaturation, 1 min at (based on primer) for annealing and 2 min at 72 °C for extension. Reaction was incubated at 72°C for 10 min then at 4°C. The PCR ampilified products were separated by electrophoresis using 1.2% agarose gel in 1 x TAE buffer against λ HindIII DNA Ladder as a size marker. Bands were detected with ethidium bromide staining and visualized under UV light, then photographed on Gel Documentation. RAPD bands were scored as (0) for absent and (1) for present. Phylogenetic tree were done by NTSYSpc version 2.1 software (Rohlf , 2000).

Table (1): Primer sequences of RAPD and SCAR used in PCR analysis

Primer No	Primer sequence 5' 3' →
1	AGGTCACTGA
2	CACATGCTTC
3	GGGTAACGCC
4	GTGACGGTAG
5	TACCTGGAGC
6	GTGATCGCAG
7	GAAACGGGTG
Ga5-SCAR GsF5/GsR5	F- CTGAGACGGAGCAAA R- CTGAGACGGAGTGTT

Results and Discussion

Yield and Morphological Characteristics

Agronomic characters are very crucial in evaluation of crops in field conditions. Highly remarkable differences were observed for average fruit weight, no. of fruits /plant, total fruits, earliness and yield /plant. Reactions of FW under field conditions were recorded. Data presented in Table (2) proved that the tested watermelon genotypes were different in most studied field traits. Some lines such as 84 and

84g were the best for the average of fruit weight and total fruit / plant and exhibited resistance for FW. Similarly, line 85 was the best for no. of fruit / plant and have economically important trait which was very early. Moreover, the aforementioned lines (84, 84g and 855) and line 74 showed highly resistant throughout the natural infestation study. Watermelon genotypes as shown in Table (3), showed high resistance under natural infestation such as lines 74, 84 and 84g where they showed 2, 4 and 6 % of wilted plants, respectively. Other genotypes such as lines 95 and 85 revealed moderately resistance against FW under field conditions by the rate of 18 and 12% wilt disease incidence, respectively. Contrary, line 63 showed 49% wilted plants and was more related to Sugar Baby cultivar as sensitive genotypes.

Table (2): Means of the yield related traits for watermelon genotypes under field natural conditions

No.	Genotypes	Average fruit weight (kg)	No. of fruits /plant	Total fruits Yield /plant (kg)	Earlines s	*Fusarium* Resistance
1	74	5.0	4	20.0	middle	Resistant
2	6311	6.0	4	24.0	Early	Sensitive
3	Sugar Baby	3.5	5	17.5	Early	Sensitive
4	95	6.0	4	24.0	middle	Normal
5	85	2.5	6	15.0	Very early	Normal
6	84g	7.0	4	28.0	middle	Resistant
7	84	7.0	4	28.0	middle	Resistant

Table (3): Percentage of reactions of watermelon genotypes to *Fusarium* wilt under field natural conditions

Genotypes	Survival plant (%)	Wilted plants (%)
74	98%	2%
63	51%	49%
Sugar Baby	48%	52%
95	82%	18%
85	88%	12%
84	94%	6%
84g	96%	4%

Molecular genetic studies

The analysis of PCR amplified products using the specific Ga5-SCAR SCAR primer set (GsF5/GsR5) against DNA of the seven watermelon genotypes are shown in Fig (1). The amplification of specific band with 898 bp size was occurred only with the FW resistant or moderate (normal) lines. On contrary, no amplification

products could be detected when the susceptible genotypes DNA, i.e., 6311 and Sugar Baby were used. These findings confirmed the previous report of Lin *et al.*, (2009) about discrimination of watermelon resistance or susceptibility to FW disease. Moreover, this specific molecular marker exhibited highly harmony with field data related to FW, since it occurred only with resistant or normal lines.

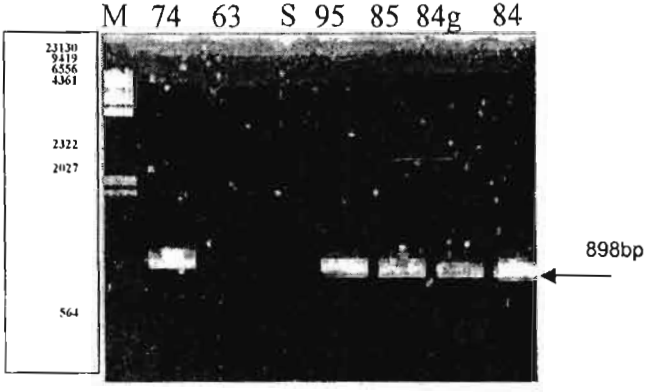

Fig. (1): SCAR products using primer GsF5/GsR5 specific to *Fusarium* wilt in watermelon

Out of the seven used random primers, only three No., (2, 5 and 6) produced polymorphic bands. Meanwhile, the other four primers showed either monomorphic banding patterns or no amplification products at all. The RAPD primers produced 25 scorable RAPD markers of which 23 (92%) were polymorphic. To generate a marker by RAPD markers for new Egyptian promising lines, three additional bands related to FW were provided. Figure 2a showed a polymorphic band with about 750 bp size when primer No. 2 was applied with the DNA of some resistant genotypes. Moreover, this band was not detected with either normal or susceptible genotypes. Primer no. 5 showed a band with size of 500 bp in most resistant and normal lines except line 74 Fig. (2b). Moreover, no amplified bands were produced against any of the susceptible genotypes. Another alternative polymorphic band with 1500 bp was detected when both of genomic DNA of resistant and normal watermelon lines of FW was applied against primer no. 6 (Fig 2c). It is clearly noticed that no amplification of this band could be occurred when the two susceptible genotyoes were used. *Fusarium* wilt is one of the most destructive diseases which affect the production of cucurbits crops which includes watermelon. Paulus *et al.*, (1976) reported that some watermelon cultivars tested under field conditions were very susceptible. Therefore, a more efficient breeding program for resistant watermelons is necessary. In addition, DNA markers associated with resistance or sensitivity to FW could be used to rapidly assay large

numbers of individual melon plants to ascertain the introgression of resistance (Joobeur *et al.*, 2004).

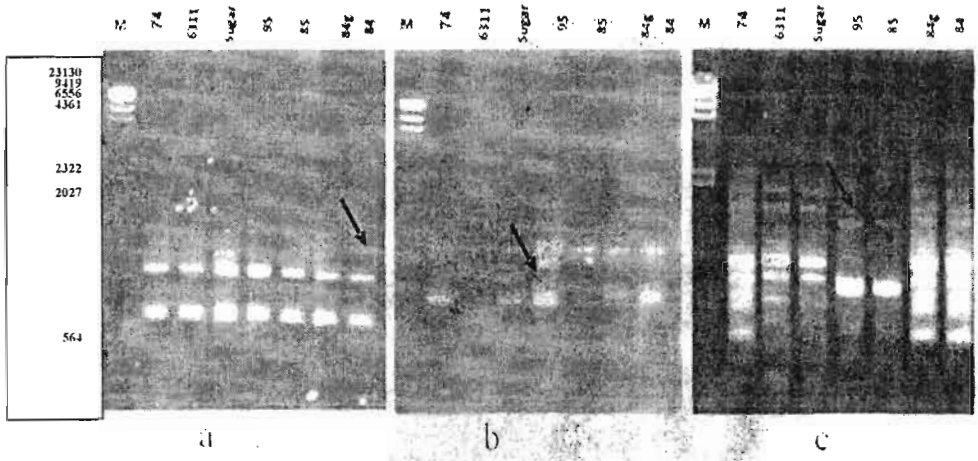

Fig. (2): RAPD pattern profile of Egyptian new watermelon promising lines and sensitive cultivar Sugar Baby using primer 2 (a) , primer 5 (b) and primer 6 (c).

These results showed the importance of RAPD and SCAR to detect molecular markers based analysis for disease-resistant in Egyptian watermelon which were agreed with these of Wenzel, (1992), who emphasized the potential of DNA markers-based diagnosis for environmental stress tolerance in plants. In Taiwan watermelon, Lin *et al.*, (2009) found a RAPD positive marker that could be used to identify watermelon lines resistant or tolerant to FW.

Based on phylogenetic tree as shown in Fig. 3, there were two clusters, the first one consisted of the two genotypes Sugar Baby and line 63 as susceptible genotypes. While, the second cluster consisted of two subclusters at the level of 80% genetic similarity, one of them has the three resistant genotypes (lines 74, 84 and 84g). The second subcluster consisted of two lines 85 and 95 which were known as normal lines.

Differences in genotypes susceptibility to FW were reported by several investigators (El-Kazzaz and Ashour, 2004; Mohammed *et al.*, 1981). However, identification of resistant genotypes is often difficult, especially when the genetic bases for the host-pathogen relationship is unknown. Thus, using genetic molecular markers cloud be useful to detect genotypes and their resistance reaction. We highly recommended to use lines 74, 84 and 84g in the next watermelon breeding programs.

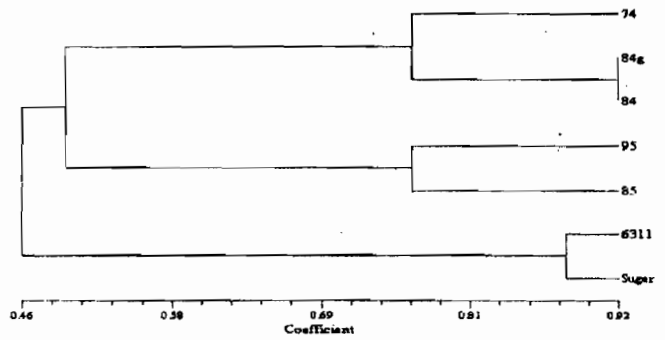

Fig. (3): RAPD based dendrogram of Egyptian new watermelon promising lines and sensitive cultivar Sugar Baby

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الملخص العربى

التوصيف الجزينى لسلالات بطيخ مصرية مبشرة مقاومة للذبول الفيوزارمى

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لتحديد المقاومة للذبول الفيوزارمى فى سلالات مبشرة من البطيخ وهى ٧٤و٦٣و٩٥و٨٥و٨٤و٨٤g مقارنة بالصنف الحساس Sugar Baby. تم استخدام اثنين من من الادلة الجزينية المعتمدة على الـ PCR (تفاعل البلمرة المتسلسل) وهى SCAR and RAPD ووجد ان الـ SCAR بحزمة وزنها ٨٩٨ قاعدة كان مرتبط ومميز للسلالات المقاومة واعطى نتائج مرتبطة ومعضضة للبيانات الحقلية لمقاومة المرض.
ولعمل دليل جزينى جديد خاص بهذة السلالات المصرية تم استخدام الـ RAPD وتم التحصل على ثلاثة حزم اضافية من استخدام البوادىء ارقام ٢و ٥ و٦ وكان لها علاقة بالسلالات المقاومة. بناء على شجرة النسب الوراثية كان هناك مجموعتان رئيسيتان الاولى تضم الصنف Sugar Baby والسلالة ٦٣ كتراكيب وراثية حساسة. المجموعة الثانية تحتوى على تحت مجموعتين الاولى تضم ثلاثة تراكيب وراثية مقاومة (سلالة ٧٤و ٨٤و ٨٤g) والاخرى تضم السلالات ٨٥ و٩٥ وهى مقاومة ايضا. وتوضح هذه الدراسة أن هذه الادلة الجزيئية يمكن أن تكون مفيدة في تقييم التنوع الوراثي وعمل مجموعات قبل تقييمهم لمقاومة مرض الذبول الفيوزارمى. وهكذا، يمكن لهذه الادلة أن تكون مفيدة لتحديد التراكيب الوراثية المقاومة لمرض الذبول الفيوزارمى في برامج التربية باستخدام سلالات من البطيخ من اجيال الانعزالية. ولذلك، يمكن استخدام هذه السلالات المبشرة من البطيخ فى برامج تربية أصناف جديدة ذات سمات اقتصادية جيدة، بالإضافة إلى مقاومة مرض الذبول الفيوزارمى.