Using the Anchored Microsatellite Primed Polymerase Chain Reaction (AMP-PCR) Technique to Study Genetic Diversity in Four Local Tomato ("Lycopersicum esculentum") Cultivars in Palestine

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Using the Anchored Microsatellite Primed Polymerase Chain Reaction (AMP-PCR) Technique to Study Genetic Diversity in Four Local Tomato (*Lycopersicum esculentum*) Cultivars in Palestine

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Summary

Although there are only four local cultivars of tomato in Palestine, the genetic variation among these cultivars has not been studied yet. The genetic variation among the four local cultivars and their regenerant plants was determined using anchored Inter Simple Sequence Repeat (ISSR) regions as a preliminary step towards developing a tomato cultivar resistant to abiotic and biotic stresses through gene transfer and molecular marker-assisted breeding,. Ten 3'-anchored semi-specific primers were employed. The results showed that the least similarity exists between cultivars H3 and P1 (74%) while the highest is between cultivars B2 and R4 (93.3%). These results demonstrate the existence of genetic variation among the local cultivars, which is an important step for the ongoing breeding and selection programs.

Introduction

Simple Sequence Repeats (SSR), also called Microsatellites, are highly polymorphic regions of many animal and plant genomes. Such variable regions may assist in genotyping closely related cultivars which have low genetic diversity such as in the case of tomato (Bredeneijer et al., 1998). Moreover, the variation in microsatellite sequences and length could be used as genetic markers in breeding programs when found attached to a gene.

Inter Simple Sequence Repeats (ISSR) are multiloci fragments that are produced by microsatellite primed (MP) PCR.

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In order to increase the specificity of the microsatellite primer, one or more none repeat bases are added to the sequence of the microsatellite primer.Such primers are called anchored primers. In contrast to the non anchored primers, anchored primers with dinucletide and AT-rich trinucletide repeats produce resolvable banding profiles. Products of PCR amplification of such a primer are called Anchored Microsatelltie Primed AMP markers. These markers have high reproducibility and polymorphism.

After the first description of this method (Zietkiewicz et al., 1994), it has been reported in various fields of molecular genetics such as linkage analysis, fingerprinting and genotyping. For example, ISSR markers were engaged to identify closely related citrus cultivars (Fang and Roose, 1997).

Also, it was compared with RAPD markers in a study of the genetic variation within and among populations of a wild rice *Oryza granulata* in China (Qian et al., 2001). These researchers concluded that ISSR are more reliable than RAPD markers because of their reproducibility and highly polymorphic bands. A similar conclusion was raised by Yong-Cui Hou et al., (2005) when they compared the two markers to study genetic diversity in barely. ISSR markers have also been employed to determine genetic instability cause by somaclonal variation in the early stages of in *In vitro* cultures of cauliflower (Leroy et al., 2000). Moreover, ISSR markers has been employed to study the genetic diversity in many plants and animals such as in mulberry (Awasthi et al., 2004), common bean (Gonzalez et al., 2005), barley (Yong-Cui Hou et al., 2005), the insect Noctuids (Luque et al., 2002), and many other organisms.

Tomato, *Lycopersicon esculentum,* is one of the most important vegetables in Palestine occupying about 29.8% of the total area of vegetable cultivation (Palestinian Central Bureau of Statistics, 2010). Historically, Palestinian farmers have been cultivating local tomato cultivars for centuries. However, in recent years, these cultivars have been replaced by superior commercial cultivars developed in Israel. The Palestinian Agricultural Relief Committees has initiated a program to characterize the local cultivars of vegetables and preserving their seeds. So far, some phenotypic traits of four Palestinian cultivars of tomato have been characterized. Cultivars Ramallah-Jiljilaweh1920 (R4), Hebron K/21(H3), Jenin 2/2556 (P1) and Jenin 2/2520 (Jenin2) were collected from Ramallah, Hebron and Jenin areas respectively, and self-crossed for several generations. These cultivars of tomato, however, have not been genotypically characterized. Such studies are a prerequisite step towards improving their phenotypic characteristics

including crop quality and capacities for disease resistance through conventional breeding or genetic engineering techniques. In this work, we used the AMP-PCR to study the genetic variations among four Palestinian tomato cultivars.

Materials and methods

Tomato cultivars

The seeds of four Palestinian tomato landraces were obtained from the Jericho Botanical Garden, Palestinian Authority: Jiljilaweh: 5/1920 line, from Ramallah, Hebron's line K/21 (rain-fed tomato) from Dura, Pure line 2/2520 from Jenin, and Pure line 2/2556 from Jenin. The names of the four cultivars were abbreviated as R4, H3, B2, and P1 respectively.

DNA extraction

Total genomic DNA was extracted from the tomato cultivars and their regenerants using the DNeasy™ System (QIAGEN) according to the manufacturer's instructions. The quality and the DNA concentration of the extracted DNA were determined by the electrophoresis method (Sambrook et al., 1989) and also by the spectrophotometer method.

PCR conditions

Add 20-30ng of the genomic DNA, 60pg Oligonucletide, 200μM dNTPs, 1X of the Polymerase buffer (Sigma), 1mM of $MgCl_2$, and 1.5 units of the Taq DNA Polymerase enzyme (Sigma) to a final volume of 25μl.

PCR cycles

The reactions were incubated in a thermocycler (MJ research) at 94°C for 5 min, then 35 cycles of 94°C for 30 seconds, (Tm, see table 1) for 90 seconds, and 72°C for 90 seconds followed with a final cycle of 72°C for 5 minutes.

Gel electrophoresis

PCR products were separated by electrophoresis in 1.5% agarose (w/v) gels in 1X TBE buffer at 120V for 1.5 hours. PCR bands were visualized by staining with ethidium bromide.

Primer sequences

Ten primers were used in this study. The names, sequences and annealing temperatures of the primers are shown in Table 1.

Table 1. The primers used in this study.

Primer	Sequence 5`→3`	T (°C)
MT5	$(CA)_{10}G$	60
MT6	$(CA)_{10}T$	57
MT7	$(TAA)_6G$	40
MT9	$(ACC)_6G$	60
MT1	$(CTAT)_5G$	50
MT2	$(CTAT)_5A$	48
MT3	$(CT)_{10}G$	60
MT4	$(CT)_{10}T$	57
MT8	$(TAA)_6C$	40
MT10	$(ACC)_6T$	57

Results and discussion

In this study, ten 3'-anchored semi-specific primers were employed of which eight produced polymorphism. The two primers that failed to generate amplification products in all of the tomato cultivars are MT1 and MT2. Although, primers MT3 and MT7 resulted in several ISSR bands, these products were observed only in part of the tested cultivars. This might not necessarily be due to the absence of the microsatellite sequences but could also be a result of a mismatch of the anchoring nucleotide. While only one primer (MT4) generated similar binding profiles, 60% of the primers produced polymorphic bands among the cultivars. Three of these primers: MT5, MT6, and MT10 generated polymorphic bands in all of the cultivars, whereas primers MT9, MT3, and MT8 produced polymorphism only in either 1 or 2 of the cultivars. A monomorphic band was generated in two of the cultivars by primer MT7. Figure 1 shows some banding profiles.

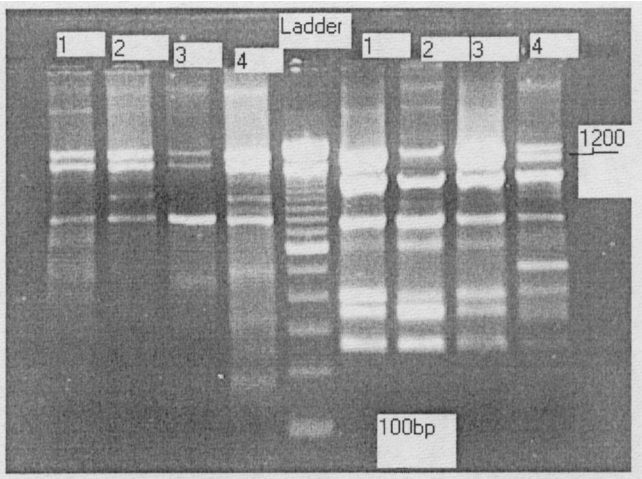

Figure 1. Anchored Microsatellite Primed Polymerase Chain Reaction (AMP-PCR) products for four Palestinian cultivars using the primers: left of ladder $(CA)_{10}G$ and to the right of ladder $(ACC)_6G$. 1: B2, 2: R4, 3: H3, 4: P1

The genetic similarities among the cultivars (P1, B2, R4, and H3) were calculated as the average of the calculated coefficients of similarity (F) for the DNA patterns generated by each primer. The F values were determined by a pair-wise comparison. The results showed that the least similarity exists between cultivars H3 and P1 (74%) while the highest is between cultivars B2 and R4 (93.3%). Moreover, each of the cultivars B2 and R4 exhibited a higher level of genetic relatedness to cultivar P1 (86.3% and 86.8% respectively) than to cultivar H3 (78.3% and 81.7% respectively). Although the chances for changes in these sequences due to regeneration are low, we amplified the DNA of regenerants from the four cultivars using the same primers. As expected, the banding profiles of the regenerants were identical to those obtained from the original plants. The present results document for the first time the existence of genetic variation among the local cultivars, which is an important step for the ongoing breeding and selection programs.

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