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Evaluation of Watermelon Retrotransposon Elements in Melon

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Abstract: Melon (*Cucumis melo* L.) is an important vegetable crop in Iran and it is regarded as the most morphologically diverse species in the genus *Cucumis*. Study of genetic variability is very important to understand ecological adaptation of natural plant populations and to estimate of it potential for breeding. The aim of this research was to reveal most effective watermelons retrotransposon-based primers for analysis of Iranian melon population. The 25 primers were tested to find out those showing highest level of polymorphism in samples involved in the investigation. Selected primers can be applied to Iranian melon genetic resources consisting from breeding varieties and populations.

Key words: *Cucumis melo* L., genetic variability, retrotransposon, primers, Iran

INTRODUCTION

Cucurbitaceae or Cucurbit family are important crops widely distributed in the warmest areas of the world supplying humans with edible products and useful fibres. Melon (*Cucumis melo* L.) was first described by Linne 1753 in species *Planetarum*. It is a member of the family Cucurbitaceae represented by some 118 genera and 825 species (Jeffery, 1990). Melon is found in very dry areas. The geographical distribution of wild melon is: Africa, Asia (Iran, China, India, Japan, Korea and also) and Australia (Kirkbride, 1993). *C. melo* includes a wide range of cultivars and its polymorphism in leaf, flower, fruit shape and color creates difficulties on the systematic classification so that the one proposed by Naudin who grouped the species into the 5 botanical varieties of Cantalupensis, Reticulatus, Saccharinus, Inodorus and Flexuosus was and is the most used.

High genetic diversity in melon has been studied using either biochemical isozyme markers (McCreight *et al*., 2004) or different molecular markers (Garcia-Mas, 2000) such as RFLP (Restriction Fragment Length Polymorphism) (Silberstein *et al*., 1999), RAPD (Randomly Amplified Polimorphic DNA) (Luan *et al*., 2008) and SSR (Simple Sequence Repeats or micro satelites) (Kacar *et al*., 2012). Due to the abundance of Retrotransposons (RTNs) in plant genomes and their ability to create new copies they been used as molecular markers (Kalendar *et al*., 1999). In 1956, McClintock (1984) found some mutagenesis factors, called transposable elements that can be divided into 2 main classes: DNA transposons that move through their intermediate DNA using a cut and paste mechanism and retrotransposons that increase their copy number through a cycle of transcription and integration back to the genome on condition that the older copy still persists and causes increase of genome size. In many crop plants, between 40-70% of the total DNA comprise retrotransposons (Bennetzen, 2000). Due to the features such as integration activity, persistence, dispersion, conserved structure, sequence motifs and high copy number; retroelements can widely be used as molecular markers today. There are several marker systems based on the analysis of distribution of different retrotransposons in genome. Sequences of LTR retrotransposons are suitable to identify polymorphism of analyzed forms belonging to a single species by differnt PCR-fingerprinting techniques: IRAP (Inter-Retrotransposon Amplified Polymorphism), REMAP (Retrotransposon-Microsatellite Amplified Polymorphism) and SSAP (Sequence-Specific Amplyfication Polymorphism) Methods (Kalendar *et al*., 1999). A major disadvantage of RTN-based molecular markers is a necessity of development the own molecular marker systems for each plant species depending on specific retrotransposon sequences. Till now, they are not available markers for all crops of the interest. However, related species have similar RTN sequences, meaning that primers designed based on LTR sequences of an RTN can be readily used across species lines, among closely related genera and even sometimes between plant families (Lou and Chen, 2007). In this study, several IRAP and REMAP primers from watermelon were tested to select those which recover most of polymorphism of melon samples involved in the investigation.

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Table 1: Studied melon populations, hybrids and inbred lines

Names	Abbreviation	Origin	Botanical varieties
Khatooni Farimani	KhF	Iran-Fariman	Inodorus
Dargazi Tashkandi	DTa	Iran-Dargaz	Inodorus
Zivari Shahroud	ZSh	Iran-Shahroud	Inodorus
Chahpaliz	Cha	Iran-Mashhad	Inodorus
Minoo	Min	Iran-Gonabad	Inodorus
Shadegani E	ShE	Iran-Ahvaz	Inodorus

Table 2: Sequences and source of the primers used in this study

Primers	Sequence (5'-3')	Primer source
LTR2452	TCCTGGTAACACTATGGATACGAC	Watermelon retrotransposon
LTR2453	CTTATACGTCTGAAGGACAGGGTTTC	Watermelon retrotransposon
LTR2467	ACGGTTACGGGCGTGTTCCTCTTCCA	Watermelon retrotransposon
LTR2476	GACTTCAAGCTACTTCGAATGGGTTGTC	Watermelon retrotransposon
A7	AGAGAGAGAGAGAGAGAGAGT	ISSR
UBC808	AGAGAGAGAGAGAGAGC	ISSR
UBC811	GAGAGAGAGAGAGAGAAC	ISSR
UBC816	CACACACACACACACAT	ISSR
UBC825	ACACACACACACACACT	ISSR
UBC826	ACACACACACACACACC	ISSR
UBC834	AGAGAGAGAGAGAGAGY*T	ISSR
UBC840	GAGAGAGAGAGAGAGAY*T	ISSR
UBC855	ACACACACACACACACYT	ISSR
UBC880	GGAGAGGAGAGGAGA	ISSR

MATERIALS AND METHODS

The 30 genotype from 6 population (5 plants from each population) were used to detect best suited (polymorphic) primers. The populations were provided from Iran; Khatooni Farimani (KhF), Dargazi Tashkandi (DTa), Zivari Shahroud (ZSh), Chahpaliz (Cha), Minoo (Min) and Shadegani E (ShE) (Table 1).

Fresh leaves were used for DNA extraction as described previously (Ausubel *et al.*, 1995) with slight modifications. The quality and concentration of the DNA were measured using a spectrophotometer (Eppendorf Biofhotometer, Germany) and electrophoresis in a 0.8% (w/v) agarose gel.

The amplification profile (according to Abdollahi Mandoulakani's Method) (Mandoulakani *et al.*, 2012) composed of an initial denaturation at 94°C for 4 min, followed by 35 cycles at 94°C for 45 sec, 53-55°C for 40 sec, 72°C for 2 min and a final extension of 5 min at 72°C. PCR products were separated by electrophoresis using 1.8% agarose gel in 0.5×TBE buffer with constant voltage of 65 V for 3-4 h.

Gels were stained by ethidium bromide then visualized under UV light and photographed using a gel documentation system.

In this research, researchers use 4 watermelon's RTNs primer and 10 ISSR primers (Table 2). Four primers isolated from watermelon, produce 10 IRAP (4 single and 6 IRAP primer combinations) and 14 REMAP primers including 4 IRAP primers combined with 10 3'-anchored ISSR primers were used to study RTN activity and analyze genetic diversity in 30 melon genotypes.

Table 3: List and Tm of the used IRAP and REMAP primer combinations in the current study

Primers	Tm
IRAP	
LTR2467	53
LTR2476	54
LTR2453	53
LTR2467+LTR2453	55
REMAP	
LTR2467+855	53
LTR2476+825	50
LTR2467+826	52
LTR2452+855	52
LTR2452+825	50
LTR2452+808	50
LTR2452+840	50
LTR2453+A7	50

Tm: Annealing temperature

RESULTS AND DISCUSSION

At last, from 50 tested primers, 12 primers (4 IRAP and 8 REMAP) show high PCR efficiency in melon genome (Table 3). This primers indicate 94% polymorphism between melon genotypes (Fig. 1). The 38 primers gave monomorphic products.

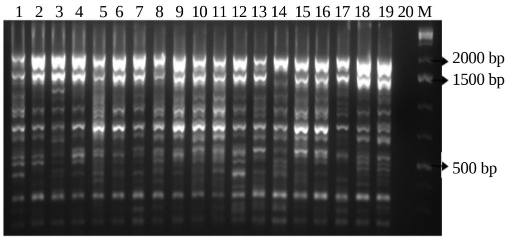

Fig. 1: Polymorphism detected by IRAP primer LTR2467. Lanes from left to right; 1-4: individuals from population Khatooni Farimani; 5-8: individuals from population Dargazi Tashkandi; 9-12: individuals from population Chahpaliz; 13-16: individuals from population Zivari Shahroud; 17-19: individuals from population Shadegani E; M: 1 kb O'GeneRuler™ DNA ladder (Fermentas) in base pairs

CONCLUSION

Watermelon LTR-based primers produced polymorphic banding patterns, suggesting that these elements may have relatives in *C. melo* and probably come from the common ancestors before the divergence of melon and watermelon in evolutionary processes. Mandoulakani *et al.* (2012) stated that RTNs Lore1 and 2 characterized in the model legume *Lotus japonicus* are transpositionally active in *Medicago sativa*. LTR2452 from watermelon may not be as a form of local clusters in melon genome, since no bands were amplified when this RTN primer used alone in IRAP reactions. Primer LTR2453 generated much polymorphism in combination with LTR2467 indicating the insertion of this RTN near or into the other RTNs in melon genome.

The insertion of the RTNs near or into each other has been reported in plant genomes (Carvalho *et al.*, 2010). The generated REMAP patterns resulted from the proximity between SSRs and the LTR region (Kalendar *et al.*, 1999).

Moreover, the multiplicity of REMAP loci per each melon genotype reflected the insertion events between LTR sequences of the used RTNs and the SSR regions. The insertion of RTNs near different SSR motifs has been already stated in wheat (Carvalho *et al.*, 2012). These results might be useful in genetic diversity investigation and designing melon breeding programs and defining strategies for germplasm conservation.

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