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**Research Article**

# Retrotransposon analyses in Cucurbitaceae family

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## Abstract

Retrotransposons are class I mobile elements, moving via an RNA intermediate. There are many retrotransposon-based molecular markers. Here, Nikita and Sukkula retrotransposons were investigated in Cucumis sativus L., Citrullus lanatus and Cucumis melo L. by using IRAP (Inter-Retrotransposon Amplified Polymorphism) molecular marker method. Nikita and Sukkula band profiles were similar in the plants, indicating only homomorphic band profiles. These barley-specific retrotransposons were identified in these three plant species for the first time. Findings could provide valuable information for understanding genomes of these plants and evolutionary relationships among them..

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## 1. Introduction

Cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus*) and melon (*Cucumis melo* L.) belonging to Cucurbitaceae family are economically important cultivated plants. According to Turkish Ministry of Agriculture and Forestry the production of cucumber, melon and watermelon, 1.828.273, 1.753.942 and 4.031.174 tonnes in 2018, respectively (TÜİK, 2018). Cucumber, eudicot diploid plant species (2n =2x = 14), is originated from Asia and cultivated around 1.500 BC (Jeffrey, 1980; Renner et al., 2007; Sebastian et al., 2010). *C. sativus* var. *sativus* L. and wild type *C. sativus* var. *hardwickii* (Royle) Alef. are two important cucumber varieties and provide valuable information as primary gene pool.

The watermelon consists of several subspecies with genetic diversity and germplasm resources (Zhang et al., 2018). Modern cultivated watermelon (*C. lanatus* subsp. *vulgaris*) is one of the subspecies, representing the sweet (dessert) watermelon group (Jeffrey, 2001). The

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genome size is 425 Mb with 22 chromosomes (2n =2x= 22) (Arumuganathan and Earle, 1991). Melon is another important vegetable crop species in this family, containing 12 chromosomes and 450-500 Mb of DNA (3-4 times bigger that than *Arabidopsis*) (Huala et al., 2001). Similar to cucumber, recent data suggest that the origin of melon is Asia (Sebastian et al., 2010). Melon is an attractive model for analysis of fruit traits (Galpaz et al., 2018; Huang et al., 2019) and sex determination (Pawełkowicz et al., 2019). At the same time, several genetic and molecular tools have also been improved over decades, consist of genetic maps (Liu et al., 2019), microarrays (Gómez-Aix et al., 2016) etc.

Transposons are major components of eukaryotic genomes. Different studies showed that ratios of transposons are variable in Cucurbitaceae family. Huang et al. (2009) published draft genome sequence of *Cucumis sativus* L. They reported that *Gypsy* and *Copia* LTR retrotransposons are major transposable element classes and comprise 10.4% of the genome. In melon, the retrotransposon elements comprise 14.7% while DNA transposons are 5.0% of the genome (Garcia-Mas et al., 2012). In watermelon, a total of 45.2% (159.8 Mb) of assembled genome were identified as transposable element repeats and *Gypsy*-type and *Copia*-type LTRs are predominant in this percentage (Guo et al., 2013). Transposons are one of the important drivers of evolution in terms of structural variation and copy number variation (Morgante et al., 2007). Here, the aim was to analyse the *Nikita* and *Sukkula* retrotransposon movements by using retrotransposon based molecular marker technique, IRAP, in closely related species, cucumber, melon and watermelon genomes, to understand the role of retrotransposons in species identification.

## 2. Materials and Methods

Genomic DNAs (gDNAs) were extracted from 17-day old seedlings of cucumber, melon and watermelon grown under greenhouse conditions via using isolation method reported by Kidwell and Osborn (1992). After isolation, concentration of gDNAs was measured via spectrophotometer (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific). The movements of barley-specific retrotransposons (*Nikita* and *Sukkula*) among three different plant species were analysed with IRAP-PCR. Primers used in IRAP-PCR were indicated in Table 1.

**Table 1**. Primer sequences

| **Primer name** | **Sequence (5'-3')** | **Reference** |
|---|---|---|
| *Nikita* | ACCCCTCTAGGCGACATCC | Leigh et al., 2003 |
| *Sukkula* | GGAACGTCGGCATCGGGCTG | |

IRAP-PCR reactions were performed in 20 µl reaction mixtures consisting of 9.4 µl of nuclease-free $dH_2O$, 2 µl of reaction buffer, 2 µl of $MgCl_2$ (2.5 mM), 1 µl dNTP mixture (0.3 mM), 2 µl of primer (1 µM/µL), 3 µl of 20 ng/µl template genomic DNA (3 ng/µl) and 0.6 µl of Taq polymerase (0.15 U/µl) (EP0703, Thermo Scientific). Final concentrations of components were indicated in parenthesis. PCR conditions were adjusted as follows an initial denaturation step at 94ºC for 3 min followed by 30 cycles of 94ºC for 30 s, annealing for 30 s and 72ºC for 3 min. The reaction was completed by a final extension step at 72ºC for 10 min. Annealing temperatures of *Nikita* and *Sukkula* were 52ºC and 56ºC, respectively. All analyses were performed with five independent biological replicates.

After IRAP-PCR, products and molecular weight marker (Gene Ruler™ 1 kb DNA Ladder, SM0313, Thermo Scientific) were resolved on 1% agarose gel in 1X Tris–Borate–EDTA buffer at 100 V for 120 min and photographed on a transilluminator. After agarose gel electrophoresis, gels were photographed on a UV transilluminator. *Nikita* and *Sukkula* band profiles of cucumber, melon and watermelon were investigated.

## 3. Results and Discussion

In cucumber, IRAP-PCR of *Nikita* retrotransposon showed homomorphic band profiles with the length between 250 and 10.000 bp. Similar band lengths were also observed in *Sukkula* band profiles (Figure 1).

Barley-specific retrotransposons were determined in other plants (Karlik et al., 2019; Marakli et al., 2019). In these studies, results showed that transferability of retrotransposons provide valuable information about species. Therefore, *Nikita* and *Sukkula* retrotransposons were identified for the first time in cucumber to figure out the relationships between species. In cucumber, there are dominant and codominant markers have been used for germplasm analyses, mapping, disease resistance etc. These markers can be summarised as microsatellites (Jat et al., 2019), SCAR (sequence characterized amplified region) (Zhang et

al., 2010), SNP (single-nucleotide polymorphism) (Zhu et al., 2016) and SRAP (sequence-related amplified polymorphism) (Zhang et al., 2012).

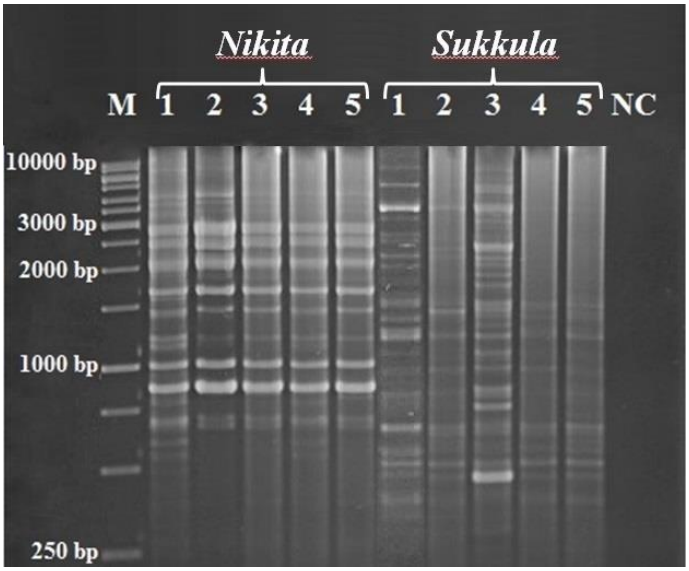


**Figure 1.** *Nikita* and *Sukkula* IRAP-PCR results of *Cucumis sativus* L. M, marker; NC, negative control. Numbers indicate five biological replicates.

Another important plant species, watermelon, also analysed in this presented study in terms of retrotransposon analyses. Both *Nikita* and *Sukkula* analyses showed that there were homomorphic band profiles ranging from 250 and 10.000 bp in length in five biological replicates (Figure 2).

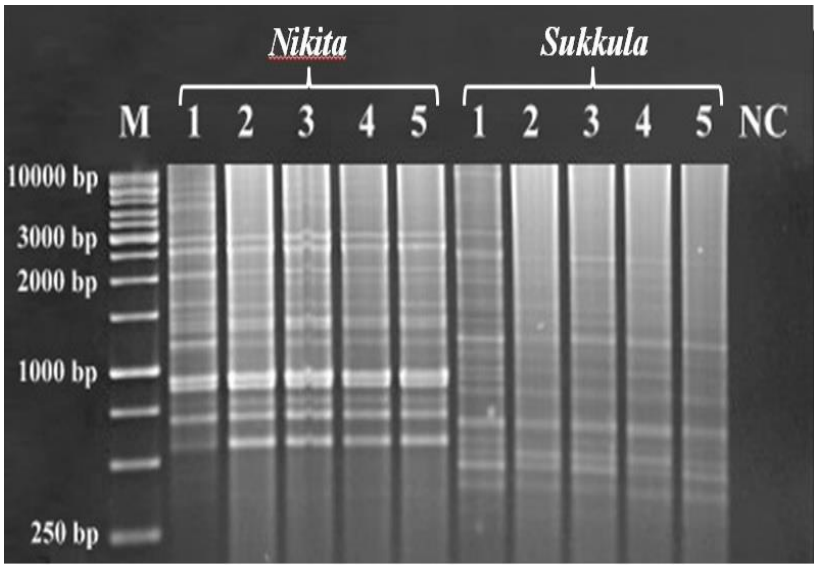


**Figure 2.** *Nikita* and *Sukkula* IRAP-PCR results of *Citrullus lanatus* L. M, marker; NC, negative control. Numbers indicate five biological replicates.

Similar to other economically important plants, there is increasing effort to improve watermelon varieties in terms of fruit quality and even against diseases (Kyriacou et al., 2018). Hence, genome analyses including sequencing, developing molecular markers, transcriptome analyses etc. have gained importance (Sun et al., 2019; Jiang et al., 2019; Pandey et al., 2019). Transposons are also valuable sequences to figure out watermelon genome in detail. Fort his reason, Guo et al. (2013) investigated LTR retrotransposons in watermelon and another species in its family, cucumber. They concluded that more than 4.5 million years, LTR retrotransposons accumulated much faster in watermelon than cucumber (Huang et al., 2009). They also reported that difference in their genome sizes may show the differential LTR retrotransposon accumulation.

Genetic diversity of melon has been analysed by using many molecular markers including RAPD (Random Amplified Polymorphic DNA) (Karimi et al., 2016), SSR (Simple Sequence Repeat) (Carvalho et al., 2017) and DArTseq (Diversity Arrays Technology) (Zaitoun et al., 2018). However, there is no report to investigate transposon by using IRAP-PCR. In this study, similar to cucumber and watermelon, we observed that there is no polymorphism among five biological replicates in terms of *Nikita* and *Sukkula* retrotransposon movements (Figure 3).

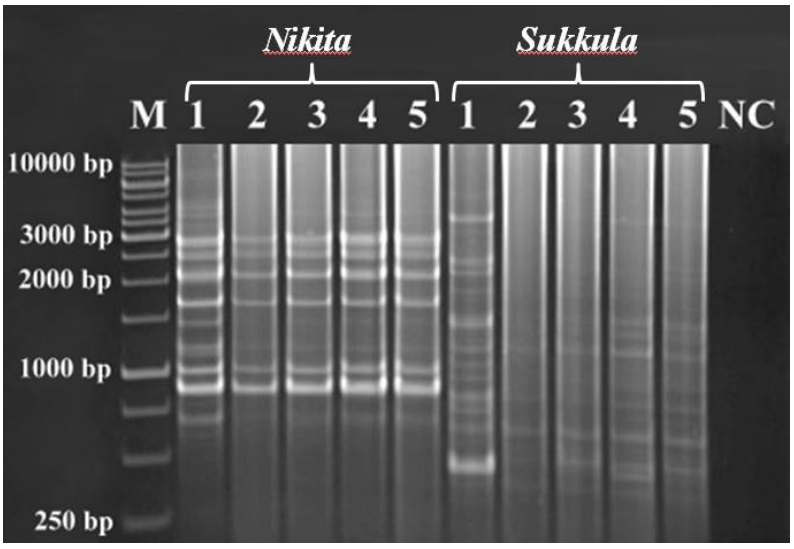


**Figure 3.** *Nikita* and *Sukkula* IRAP-PCR results of *C. melo* L. M, marker; NC, negative control. Numbers indicate five biological replicates.

Garcia-Mas et al. (2012) analysed the reference genome sequence of melon. They found that 19.7% of the sequence correspond to conservative annotation of the most recent

transposable elements. These sequences could be related to different pathways. To support this opinion, Martin et al. (2009) reported that sex determination gene induced by upstream transposon insertion.

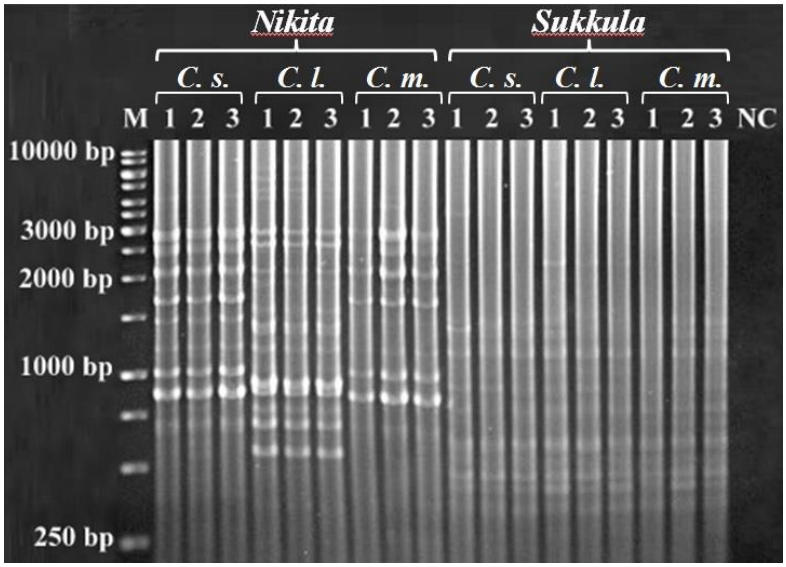


**Figure 4.** *Nikita* and *Sukkula* IRAP-PCR results of cucumber, watermelon and melon. *C.s. Cucumis sativus* L.; *C.l., Citrullus lanatus* L.; *C.m, C. melo* L., M, marker; NC, negative control. Numbers indicate three biological replicates.

Our results showed that cucumber, watermelon and melon showed similar *Nikita* and *Sukkula* band patterns (250-10.000 bp) (Figure 4). These findings were supported by Guo et al. (2013). They investigated syntenic relationships among these three species, reporting 3,543 orthologous covering 60% of watermelon genome. Transposons are found different percentages in plant genomes and important evolution drivers by changing organisation of plant genomes (Kazazian, 2004). Meyer and Purugganan (2013) reported 60 genes related to plant domestication and breeding and concluded that 15% of them comprise transposable element insertions which have important effects. Therefore, there are many reports to determine polymorphism among species, role of transposon effects on biotic/abiotic stresses and evolutionary relationships (Yilmaz et al., 2018; Lanciano and Mirouze, 2018; Kalendar et al., 2019; Marakli et al., 2019). Furthermore, retrotransposons are valuable sources of molecular markers because of ubiquity, abundance, dispersion, and dynamism (Kalendar et al., 2011). There are many reports to study different plant species belonging to same family to understand the role of transposon in species determination, organ differentiation and disease resistance in terms of retrotransposon movements. Therefore, obtaining results have provided

more detailed information about plant genomes. Here, barley-specific *Nikita* and *Sukkula* retrotransposons were identified for the first time. Results could be used for understanding the evolutionary relationships among species belonging to Cucurbitaceae family.

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