Transposon Studies on *Colchium chalcedonicum*

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ABSTRACT

Colchicum chalcedonicum is one of the endemic plants in Turkey. The aim of this study was the investigation of the retrotransposon *SIRE1, Sukkula* and *Nikita* presence and insertion patterns in *C. chalcedonicum.* The plant samples were collected from the botanic garden of the Istanbul University. DNA isolation was performed from leaves by using modified CTAB/SEVAG protocol. Retrotransposon movements were investigated using *SIRE1, Sukkula* and *Nikita* primers by Inter Retrotranposon Amplified Polymorphism PCR technique (IRAP-PCR). Polymorphism percentages (%) were calculated based on Jaccard Similarity Index. We observed that polymorphism ratios of *SIRE1, Sukkula* and *Nikita* retrotransposons among all samples were 0-40%, 0-100% and 0-60%, respectively. This is the first report to demonstrate three barley ─ *SIRE1, Sukkula* and *Nikita*─ retrotransposons presence and movements in *C. chalcedonicum* which is belonged to *Colchium* family, thus these IRAP primers may be used in further characterization and diversity studies of *Colchicum* family.

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Introduction

Ecology of Turkey is very favourable for rich flora, because of its geographical location, topographic features, environmental and climate convenience at the cross section of three phytogeographic regions. Approximately 900 geophyte taxa (bulbs, tubers and rhizomes plants) grow naturally in Turkey [1]. Geophytes mostly consist of Araceae, Liliaceae, Primulaceae, Iridaceae, Geraniaceae, Orchidaceae, Ranunculaceae, Amaryllidaceae families that some of them have medical and economical properties [2-4]. *Colchicum* family, which is belonged to Liliaceae, are presented as 39 species in Turkey that one of the endemic species of *Colchicum* is *Colchicum chalcedonicum* which is native to Asia, Europe and Africa. *C. chalcedonicum*, which is also called as Kadikoy (Chalcedon) crocus, was first collected by Aznavour [5]. *C.*

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chalcedonicum has usually 4 leaves, long-oval shapes corms under the soil, its chromosome number is 2n=50, and grows dry stones and rocky places [6-8].

Eukaryotic genomes comprise an abundance of repeated DNA which can move from one location of the genome to another, are also defined as transposable elements (TEs) can move within or between genomes [9]. More than 50 years ago, TEs were first identified by geneticist Barbara McClintock [10]. Nowadays, it is known that transposons are found in almost all organisms. Different types of TEs have been described that important difference between TE types is the presence of the reverse transcriptase (i.e. the transcription of RNA into DNA). Therefore, TEs are categorized into two groups based on their transposition mechanism and structural features: the retrotransposons (class I) and the DNA transposons (class II) [9].

Plants mostly contain retrotransposons more than 80% of in their genomes such as maize, wheat and barley [11, 12]. Retrotransposons use RNA to move new chromosomal locations that this mechanism is also called as "copy and paste" mechanism. Additionally, retrotransposons are subdivided into two groups; (1) long terminal repeats (LTR) retrotransposons, and (2) non-LTR retrotransposons. These repeats have a role in the insertion of the TEs which are also defined as "footprints" when the TEs are excised [13]. During speciation and evolution, TEs enlarged a large percentage of genome volume as demonstrated in plants [14], *Drosophila* or primates [15-17]. Due to the transposition event such as insertions, excisions, duplications or translocations, TEs can produce genetic variations [18-22]. Some studies showed that DNA transposons can alter the expression by insertion of specific regions in the genome such as introns, exons or regulatory elements. Moreover, TEs can be reorganized the genome by the mobilization of non-transposon DNA. In addition, TEs act as recombination substrates trigger recombination between two sequences of a transposon placed in the same or different chromosomes, which could be the origin for several types of chromosome alterations. Hence, TEs can be resulted in the loss of genomic DNA by internal deletions [23-25].

SIRE1 is plant specific LTR retrotransposon belonging to the sirevirus class of the *Ty1-Copia* retrotransposon family have their own genome structure among LTR retrotransposons according to possessing a putative *envelope-like* (*ENV-like*) gene immediately downstream of the reverse transcriptase gene [13]. Each copy of *SIRE1* is appx. 11 kb, making *SIRE1* one of the largest retroelement in soybean, additionally, *SIRE1* is active in the other plant species such as barley [26, 27]. Another LTR retrotransposon, which was first identified in barley by Shirasu, is *Sukkula* retroelement is approximately 5 kb, containing reverse transcriptase in appx. 3.5 kb central domain which is found to be conserved as in primary sequence and secondary structure.

However, *Sukkula* includes no open reading frames (ORFs) encoding typical retroelement proteins. According to these features of *Sukkula*, a novel group of retrotransposons, *la*rge *r*etrotransposon *d*erivatives or LARDs, have been described that they are member of the *gypsy* class of LTR retrotransposons, are similar to TRIMs (Terminal-repeat Retrotranposons in Miniature) in their lack of a protein-coding domain [28]. *Nikita* was the 4th TE reported in barley has been used to determine polymorphism in polyploids, genetic variability, comparison of different retrotransposon-based marker techniques and hybrids [29-33]. For genome diversification in plants, active retrotransposons are mostly considered as major contributors because of their transposition and accumulation potentials in the genome [34-36].

Molecular marker techniques have become an important tool in molecular plant breeding [37]. Inter Retrotranposon Amplified Polymorphism (IRAP) is a molecular marker technique based on retrotransposon movements. Currently IRAP molecular markers are widely used to investigate polymorphism among individuals, species identification and environmental effects on individual's genetic diversity in plants. Retrotransposons integrate a daughter copy, which causes new joints between genomic DNA and the conserved LTRs; therefore, they can be used as markers. IRAP PCR neither requires restriction enzyme digestion or ligation to produce the marker bands is based on polymerase chain reaction amplification [38]. IRAP PCR technique determines retrotransposon insertional polymorphisms by amplifying the portion of DNA between two retroelements. One or two primers pointing outwards from an LTR are used, thus IRAP PCR amplifies the tract of DNA between two nearby retrotransposons. The result of IRAP PCR products are called as 'the fingerprints', from amplification of hundreds to thousands of target sites in the genome [37, 38].

The aim of this study was to identify well-studied plant LTR-retrotransposons, including *SIRE1*, *Sukkula* and *Nikita* in *C. chalcedonicum.* For this purpose, *SIRE1*, *Sukkula* and *Nikita* insertion patterns were investigated using IRAP analysis method and results were analysed by Jaccard Similarity Index technique. This is the first report to demonstrate *SIRE1*, *Sukkula* and *Nikita* retrotransposons insertion patterns in *C. chalcedonicum.*

Materials and Methods

Plant material

C. chalcedonicum leaves used in this study were kindly provided from Erdal Uzen and the specimen was deposited in the Botanic Garden of Istanbul University.

DNA extraction

Genomic DNA extraction was performed with some modifications using CTAB/SEVAG protocol. A total amount of 100 mg of each sample were homogenized by grinding in liquid nitrogen. For total DNA extraction, 100 mg grounded samples were incubated with 500 ml CTAB buffer solution at 65ºC for 1 h. Then, the samples were centrifuged for 15 min at 16,000 x g. Afterwards, the aqueous phase was transferred to fresh tubes by pipette, then 200 µl poteinase K (25 mg/ml) and 5 µl RNase A solution (10 mg/ml) were added and incubated at 65 ºC for 30 min. Next, the samples were centrifuged for 10 min at 16,000 x g. The supernatants were transferred to fresh tubes including equal volume of SEVAG (phenol:chloroform:isoamyl alcohol, 25:24:1), the samples were mixed and centrifuged for 10 min at 16000 x g. ~500 ml of aqueous phase was transferred to fresh tubes which consisted of equal volume of chloroform. Afterwards, the samples were centrifuged for 10 min at 16000 x g. The aqueous phase of samples was mixed with 2 volumes of CTAB precipitation solution and incubated at room temperature for an hour. Following centrifugation was carried out for 5 min at 16000 x g. The pellet of the samples was dissolved in 350 ml 1.2 M NaCl solution, then 350 ml chloroform were added and centrifuged for 10 min at 16000 x g. The aqueous phase was transferred to tubes containing 0.6 volumes of isopropanol and incubated at -20ºC for 30 min. The samples were centrifuged for 10 min at 16000 x g. Afterwards, the pellets were washed with 70% ethanol and dissolved in 30 ml of sterile dH_2O. Degradation of the isolated DNA was assessed by using 1% agarose gel electrophoresis. Spectrophotometric analysis of the DNA quantity and quality were measured at 230, 260 and 280nm by NanoDrop 2000c UV-Vis spectrophotometer (Thermo Scientific, 2000c) For IRAP analysis, the DNA's concentrations were equalized to 20 ng/µL.

IRAP PCR analysis

SIRE1, Sukkula and *Nikita* insertion patterns were investigated using modified IRAP-analysis. The primer sequences used in this study was shown in Table 1. *SIRE1, Sukkula* and *Nikita* IRAP-PCR analysis were carried out using a thermal cycler in a total volume of 20 µL, containing 4 µL of sterile dH_2O, 2 µL of primer (1 µM/µL), 4 µL of 20 ng/µL template genomic DNA (80 ng/µl) and 10 µL of 2X Illustra™ Hot Start Master Mix (GE Healthcare, Sigma). The values given in parentheses were the final concentrations. PCR conditions were as follows: initial denaturation at 94°C (3 min), followed by 40 cycles of denaturation at 94°C (30 s), annealing at 57ºC for *SIRE1*, at 55ºC for *Sukkula* and at 51°C for *Nikita* (30 s) and extension at 72°C (3 min). The reaction was completed by additional extension at 72°C for 10 min.

Table 1 List of primers used in this study.

No	*Primer Name*	*Sequence (5'→3')*	*References*
1	*SIRE1*	GCAGTTATGCAAGTGGGATCAGC	[38]
2	*Nikita*	CGCATTTGTTCAAGCCTAAACC	[40]
3	*Bagy2* F	CTCGCTCGCCCACTACATCAACCGCGTTTATT	[41]
4	*Bagy2* R	ATCATTCCCTCTAGGGCATAATTC	[41]

Evaluation of polymorphism results

The percentages of polymorphism (%) were calculated using Jaccard similarity index [42]. In brief, bands result of IRAP PCR were scored as a binary value: '1' for presence and '0' for absence; the binary matrix (1/0) was then utilized to calculate the similarity between the different individuals using Jaccard's similarity index. Moreover, band profiles were also analysed by GelJ v.2.0 to construct the phylogenetic tree, UPGMA (unweighted pair-group method with arithmetic mean) clustering method with Jaccard's Similarity Index was utilized to cluster the subjects according to band distances on gel images [43].

Results

Retrotransposons ─*SIRE1*, *Sukkula* and *Nikita*─ movements in *C. chalcedonicum* have been analysed using IRAP-PCR technique. IRAP-PCR results were electrophoretically separated in a 2% agarose gel (see Fig 1). *SIRE1* and *Sukkula* IRAP band profiles were ranged from 100 to 400 bp. However, IRAP band profiles of *Nikita* were ranged from to 300 to 2000 bp.

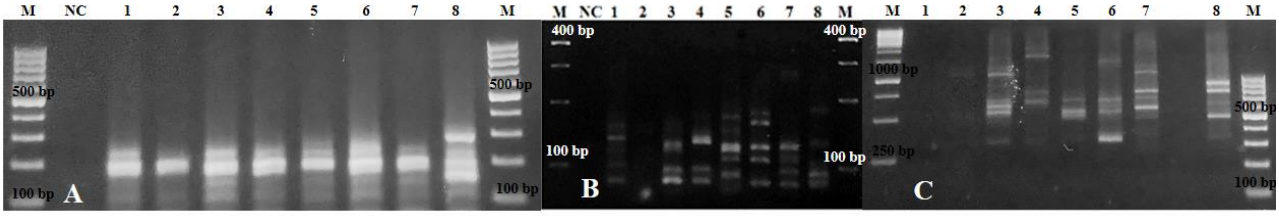

Fig 1 Demonstration of IRAP PCR products separated in 2% agarose gel. **A:** *SIRE1* results. **B:** *Sukkula* results. **C:** *Nikita* results. M: 100 bp marker and 1 kb marker. NC: no template control. 1-8: PCR products obtained from leaves of individual seedlings

The resulting IRAP-PCR amplification products demonstrated that polymorphism ratios of *SIRE1*, *Sukkula* and *Nikita* retrotransposons among all samples were 0-40%, 0-100% and 0-60%, respectively (see Table 2 and 3).

Table 2 Polymorphism percentages (%) of *SIRE1* and *Sukkula*. **A:** *SIRE1*; **B:** *Sukkula*

A	1	2	3	4	5	6	7	8	**B**	1	2	3	4	5	6	7	8
1	-								1	-							
2	25	-							2	**100**	-						
3	17	0	-						3	10	10	-					
4	0	0	0	-					4	22	22	11	-				
5	0	0	0	17	-				5	0	0	10	22	-			
6	0	20	0	**40**	14	-			6	0	0	33	9	8	-		
7	0	0	0	17	0	14	-		7	0	0	9	9	18	17	-	
8	14	0	29	0	0	0	0	-	8	9	9	0	22	20	8	18	-

Table 3 Polymorphism percentages (%) of *Nikita*

C	3	4	5	6	7	8
3	-					
4	25	-				
5	14	0	-			
6	25	0	**60**	-		
7	0	10	0	0	-	
8	9	0	0	0	8	-

As a result of GelJ analysis, two main clusters have been observed for all retrotransposons ─*SIRE1*, *Sukkula* and *Nikita*─ used in this study. For *SIRE1* clustering based on UPGMA dendrogram, first group consisted of 4 subjects (No: 3, 4, 5 and 10), and second group contained the other four ─6, 7, 8 and 9─ subjects. However, first cluster of *Sukkula* only comprised two subjects 3 and 4; the other 6 subjects were found in the second cluster. Additionally, clustering of *Nikita* was only resulted in 6 subjects thus, first cluster contained 4 subjects (No: 4, 5, 6 and 7) and the 2nd group consisted of 2 subjects (No: 8 and 10) (see Fig 2).

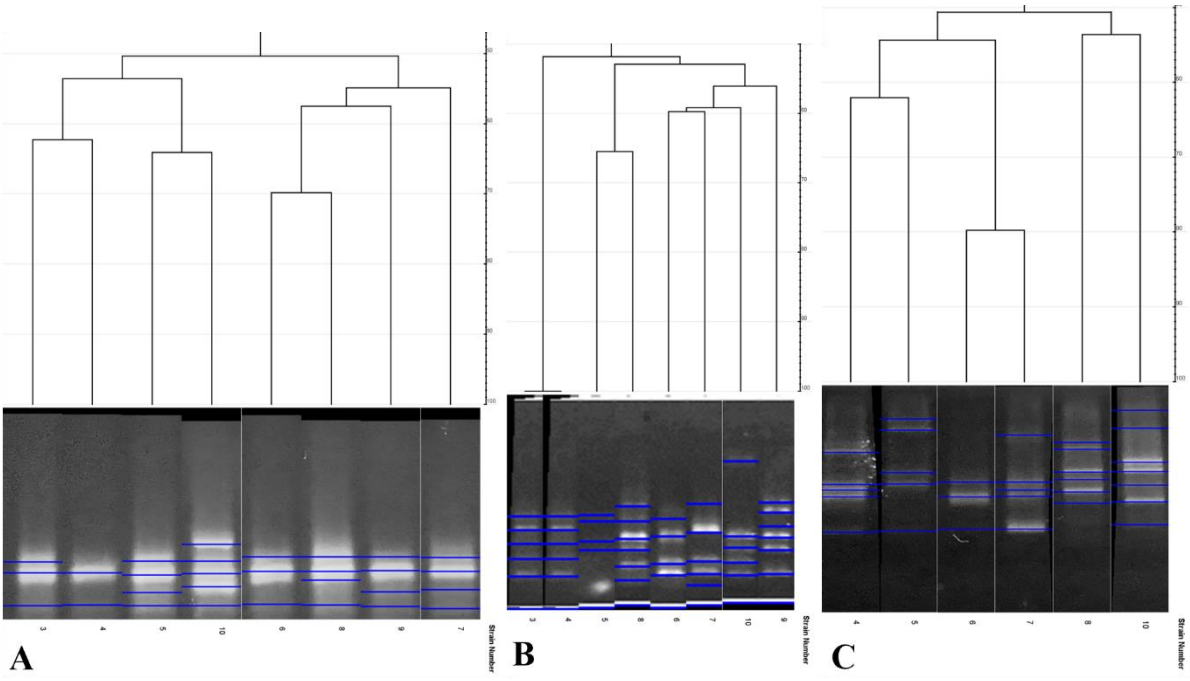

Fig 2 Clustering of subjects based on IRAP PCR amplification using *SIRE1*, *Sukkula* and *Nikita* specific primers (GelJ v.2.0 analysis). **A:** SIRE1 clustering. **B:** Sukkula clustering. **C:** Nikita clustering. 1-8: PCR products obtained from leaves of individual seedlings

Discussion

Transposable elements are widespread and dynamic elements of the genome, especially, they consist of the 80% of the genome in cereals [44]. According to their transposition mechanism, TEs can alter gene products and gene expression profiles [45]. In addition, TEs have considered as an important motive for genome evolution and speciation due to their dynamic feature [30]. Retrotransposons are usually utilized to determine genetic relationships between varieties and related species based on their variation capacity between species [46-48]. The aim of this study was to determine the presence and insertion patterns of the *SIRE1*, *Sukkula* and *Nikita* retrotransposons in *C. calcedonicum.* Polymorphism percentages (%) were calculated as 0-40%, 0-100% and 0-60% for *SIRE1*, *Sukkula* and *Nikita*, respectively, according to IRAP PCR results due to Jaccard Similarity Index. Two main clusters have been observed for all three retrotransposon types. ─ *SIRE1*, *Sukkula* and *Nikita*─

Several plant species, which are widespread and endemic, are placed in Anatolia is the richest regions in Turkey [49]. Moreover, Turkey has the extensive distribution of *Colchicum* family consist of 39 species that 15 of them endemic [50-52]. However, chromosome number was only reported associated with *C. chalcedonicum* genome. Our study is the first report demonstrating

TE presence and insertion patterns in *C. chalcedonicum* genome. TEs are widespread in the plant kingdom, thus they participate of several common features, both structural and mechanistic, with mobile elements from other eukaryotes [53]. We observed that IRAP primers of *SIRE1*, *Sukkula* and *Nikita* retrotransposons were studied in *C. chalcedonicum* genome, indicating that these retrotransposons were presence and active in *C. chalcedonicum.* Moreover, genetically active TEs could be distinguished through genome [54, 55].

Numerous studies have reported the number, diversity, and distribution of transposable elements within plant genomes [56-59]. *SIRE1*, which is a family of *copia/Ty1*-related retrotransposons in the soybean genome, is most closely related to *opie-2* from maize. Both *Glycine max* and *Glycine soja* comprise of *SIRE1* elements, suggesting this retrotransposon family was present before soybean domestication [60]. However, our studies demonstrated that *SIRE1* is still active in soybean, indicating this family may proceed to be proliferated in the soybean genome (unpublished data). *SIRE1* retrotransposon family was also studied in barley and human genomes by Cakmak et al. [27; unpublished data]. In barley, Cakmak et al. [27] have found polymorphism rates between 0–64% among all samples. Also, polymorphism percentages (%) were observed as 0-40% in our study for *SIRE1* IRAP primer. Our results indicated that *SIRE1* IRAP primer is suitable for further characterization and diversity studies.

Several studies have been performed for genetic characterization and diversity analysis using *Sukkula* retrotransposon [61-63]. However, *Sukkula*, which means "shuttle" in Finnish, have been reported the 2nd most active retrotransposon in the barley genome [29]. Moreover, *Sukkula* have been proposed to be associated with intraspecies variations by Kartal-Alacam et al. [63], which demonstrated the polymorphism rates up to 61% and 70% using IRAP, and iPBS, respectively. Moreover, *Sukkula* movements have been observed in human that polymorphism percentages (%) were found to be 8–100% among all samples; 10–91% in 12 female subjects and 13–100% in 12 male subjects [64]. In our study, we observed 0-100% polymorphism rate, suggesting that *Sukkula* retrotransposon is favourable to perform *Colchicum* characterization and diversity studies.

Although *Nikita* retrotransposon have been reported as the fourth most active retrotransposon in barley [29], studies associated with *Nikita* is quite limited. Bayram et al. [65] have only observed some polymorphic bands (~550 and 650 bp) in callus culture, indicating that tissue culture conditions may be responsible for the movement of the *Nikita.* Additionally, *Sukkula* and *Nikita* IRAP primer transferability have been demonstrated in *Pimpinella anisum* L. by Marakli [66]. Interestingly, polymorphism rate of *Nikita* in this study was calculated as 0-60%,

suggesting that *Nikita* IRAP primer may also be used in further characterization and diversity studies.

Conclusion

C. calcedonicum is an endemic and valuable plant for Turkey. Comparison of three IRAP primers ─ *SIRE1*, *Sukkula* and *Nikita*─ used in this study demonstrated that *Sukkula* IRAP primer is favourable for *Colchicum* family according to polymorphism range of molecular marker technique.

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