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Determination of plant-specific retrotransposons in chicken

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Abstract: Mobile genetic elements are also known as transposons, comprising a significant proportion of plant and animal genomes. Retrotransposons, a class of transposons, move copy and paste mechanism and, therefore, cause genome obesity. Moreover, the transferability of retrotransposons within and between different kingdoms has provided valuable information for evolutionary relationships in addition to detailed knowledge about genomes. We identified barley-specific retrotransposons (*Nikita* and *Sukkula*) and determined polymorphism ratios of these retrotransposons in a chicken genome for the first time to gain new insights for retrotransposons found in different genomes. Transposon sequences belonging to chicken and barley genomes were also investigated to understand the evolutionary relationships. For this purpose, the samples from Turkish native chicken Gerze were analyzed using a retrotransposon-based molecular marker, IRAP (inter-retrotransposon amplified polymorphism). As a result of the analyses, there was no polymorphism among the samples in *Sukkula* analysis, while *Nikita* showed 0%–60% polymorphism. Moreover, *in silico* analyses also presented that these two retrotransposons are closely related to other chicken retrotransposons. Obtaining results could offer a practical approach for chicken genome analyses, especially for the identification of genes to obtain desired traits.

Key words: Gerze chicken, inter-retrotransposon amplified polymorphism (IRAP), mobile genetic elements, *Nikita, Sukkula*

1. Introduction

Transposable elements (TEs) constitute a major part of the genome in vertebrate genomes [1]. They are classified as Class I (RNA transposon or retrotransposon) and Class II (DNA transposon), moving through the "copy and paste" or "cut and paste" mechanisms, respectively [2]. TEs have gained more importance due to their role in genetic variation, evolution, and contribution to speciation [3]. Therefore, there are many studies related to the identification of TEs and their roles in gene expression and gene expression regulations [3–5] because of the effect of retrotransposons by changing the dynamic functions of the host DNA [6].

Sequencing genomes belonging to bacteria, plants, and animals have increased due to improving NGS technologies [7–9]. Chicken (*Gallus gallus*) is one of them with ~1.2 billion base pairs. The genome size of chicken is approximately one-third of most mammalian including the human genome. On the other hand, TEs content is remarkably low (~10%) in the chicken genome [10–12].

The chicken, the first genome sequenced domestic animal, is also one of the primary model organisms that bridge the evolutionary gap between mammals and other vertebrates [13]. During domestication, the chicken's genetic structure has been changed and differentiated by natural and artificial selections [14]. The domestic chicken (*Gallus gallus domesticus*) originated from the red junglefowl (*Gallus gallus*) in Southeast Asia, the gray junglefowl (*Gallus sonneratii*) in Southwest India, and the Sri Lankan junglefowl (*Gallus lafayetii*) in Sri Lanka. The domestic chicken is transported from China and India to Europe via Russia and Anatolia via migration and trade routes [15]. Since Anatolia is a gateway of domestic chicken spreading to the rest of the world, it is crucial to study Turkish chicken breeds for retrotransposon analyses on the chicken genome. Gerze chicken, as one of the critical native genetic resources for Turkey, is very suitable for such research.

The studied chicken-specific transposons in this presented study were CR1, ALVE and ART-CH groups belonging to ERV. The CR1 (chicken repeat 1) element, a short interspersed repetitive DNA element belonging to the non-LTR class [10]. Another retrotransposon ALVE (avian leukosis virus subgroup E- ALV-E) is characterised in *ev* loci. Furthermore, ART-CH (avian retrotransposon from the chicken genome) is found in 50 genomic copies in the chicken genome, showing homology to the avian leucosis and sarcoma virus (ALSV) [16].

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Many studies determine specific retrotransposons in different genomes, reporting evolutionary relationships among species [17,18]. In this study, we aimed to identify barley-specific retrotransposons (*Nikita* and *Sukkula*) via IRAP (Inter-Retrotransposon Amplified Polymorphism) molecular marker technique in the chicken genome for the first time. Moreover, *in silico* analyses were also performed to figure out the evolutionary relationships between *Nikita*-*Sukkula* and chicken-specific retrotransposons.

2. Material and methods

2.1. Obtaining samples and genomic DNA isolation

In this study, the samples from Gerze chicken population under conservation of the Republic of Turkey Ministry of Agriculture and Forestry Gerze Directorate were used for *Nikita* and *Sukkula* retrotransposon movements. Ondokuz Mayıs University Animal Experiments Local Ethics Committee permitted this study with the decision number of 06.11.2012/1. For this purpose, blood samples from 9 Gerze chickens were collected and genomic DNAs (gDNAs) from samples were isolated by using the salting-out method [19]. Qualitative and quantitative of gDNAs were evaluated on 1% agarose gel and a NanoDrop spectrophotometer (Thermo, USA), respectively. The experiment was replicated three times.

2.2. *Nikita* and *Sukkula* IRAP-PCR analyses

IRAP analysis was performed according to Kalendar and Schulman [20]. PCR assays were performed in the T100 Thermal Cycler (BIO-RAD, USA). Amplification of the reactions was optimized in a final volume of 25 µL containing 6.5 µL ultrapure water, 12.5 µL PCR master mix (DreamTaq Green PCR Master Mix, Thermo Scientific), 2 µL of primer (0.8 µM), and 4 µL of template genomic DNA (3.2 µM). Final concentrations were given in parenthesis. Primer sequences are 5'ACCCCTCTAGGCGACATCC3' for *Nikita* and 3'GGAACGTCGGCATCGGGCTG5' for *Sukkula* [21]. PCR conditions were as follows: one initial denaturation at 95 °C for 3 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 54°C for 30 s and extension at 72 °C for 30 s. The final elongation step was performed at 72 °C for 5 min. The amplification products and molecular weight marker (100 bp DNA Ladder, Solis BioDyne) were resolved on 1% agarose gel in 1X Tris–Acetic Acid–EDTA at 90 V for 90 min and photographed on a Gel Documentation System (BIO-RAD). IRAP-PCR band profiles were evaluated visually, and polymorphism ratios were calculated by Jaccard's coefficient in all samples [22].

2.3. Multiple alignment and phylogenetic tree analyses

Different transposons (*ALVE, CR1, ART-CH-O, ART-CH-L, ART-CH-D, ART-CH-B, ART-CH-R, ART-CH-H, ART*) belonging to the chicken genome as well as barley-specific *Nikita* and *Sukkula* were retrieved from NCBI (The National Center for Biotechnology Information – www.ncbi.nlm.nih.gov) (Table 1). Evolutionary relationships among transposons were evaluated by multiple alignment analyses and phylogenetic tree construction. Clustal Omega (http:// www.ebi.ac.uk/Tools/msa/clustalo) and MEGA X programs were used for this purpose.

Multiple alignment results were used to construct a phylogenetic tree via MEGA X [23]. The program was adjusted as neighbour-joining (NJ) method [24] with p-distance model [25] and even bootstrap test (1000 replicates) for this analysis [26].

3. Results and discussion

3.1. Barley-specific *Nikita* and *Sukkula* retrotransposons identified in chicken genome

Due to the interesting nature and their abundance in the genome, retrotransposons are an excellent source for investigations of genome variations by using molecular markers. Therefore, different retrotransposon-based molecular markers such as REMAP (REtrotransposon-Microsatellite-Amplified Polymorphism), RBIP (Retrotransposon-Based Insertion Polymorphism), SSAP (Sequence-Specific Amplified Polymorphism) and iPBS (inter-Primer Binding Site), etc. have been developed not only genome variation but also explanation of disease and pathways related to biotic/abiotic stress in different genomes [27,28]. In this study, *Nikita* and *Sukkula*

Table 1. Transposons' accession numbers.

Name	Accession number	Name	Accession number
Nikita	AF474072.1:c22229-21152	***ART-CH-L***	DQ500090.1
Sukkula	AF453665.1	***ART-CH-H***	DQ500091.1
Sukkula	AH014393.2:c86082-81112	***ART-CH-D***	DQ500089.1
ALVE	KJ908690.1	***ART-CH-B***	DQ500088.1
CR1	U88211.1	***ART-CH-R***	DQ500092.1
ART-CH-O	DQ500087.1	***ART***	L25262.1

retrotransposons were identified by using IRAP method relying on amplifying the DNA segments between two nearby retrotransposons using outward-facing primers [20]. Due to low cost, reproducibility, high polymorphism, and informativity, this technique has been used for genetic assessment for several plant species because retrotransposons constitute up to 80% of plant genomes [29–31].

We studied 9 different chicken gDNAs to analyze the retrotransposons' movements using the IRAP technique. The experiment was replicated three times, and consistent results were obtained. Therefore, findings were given in one replicate. For the first time, these retrotransposons were identified in the chicken genome (Figure 1). Both monomorphic and polymorphic bands were observed in *Nikita*, while polymorphism was not detected in *Sukkula*.

Therefore, *Nikita* IRAP-PCR analyses resulted in 45 scorable bands including 40 monomorphic and 5 polymorphic bands ranging from 100 to 800 bp. Monomorphic bands were indicated as positive (+) and polymorphic bands as negative (-) in Table 2.

According to these band profiles, we calculated polymorphism rates in all samples when compared to each other by using Jaccard's coefficient. Polymorphism ratios were between 0%–60% in samples (Table 3).

The transferability of TEs among species has provided valuable results to figure out evolutionary relationships. In our previous studies, different retrotransposons were identified in different plant genomes [17,18,32]. Many studies showed that the polymorphism rates of the same retrotransposons could be variable in different organs [33], among different individuals in the same species [31],

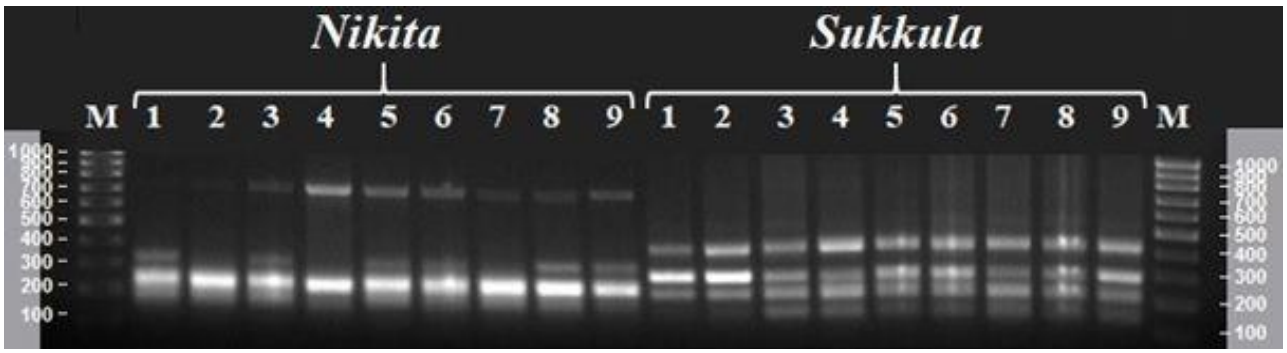

Figure 1. IRAP-PCR results of *Nikita* and *Sukkula.* M, marker; 1-9; different chicken samples.

Table 2. Band profiles of *Nikita* retrotransposon.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
+	4	4	5	3	5	5	4	5	5
-	1	1	0	2	0	0	1	0	0

Table 3. *Nikita* polymorphism percentages (%).

%	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	-								
2	40	-							
3	20	20	-						
4	**60**	25	40	-					
5	20	20	0	40	-				
6	20	20	0	40	0	-			
7	40	0	20	25	20	20	-		
8	20	20	0	40	0	0	20	-	
9	20	20	0	40	0	0	20	0	-

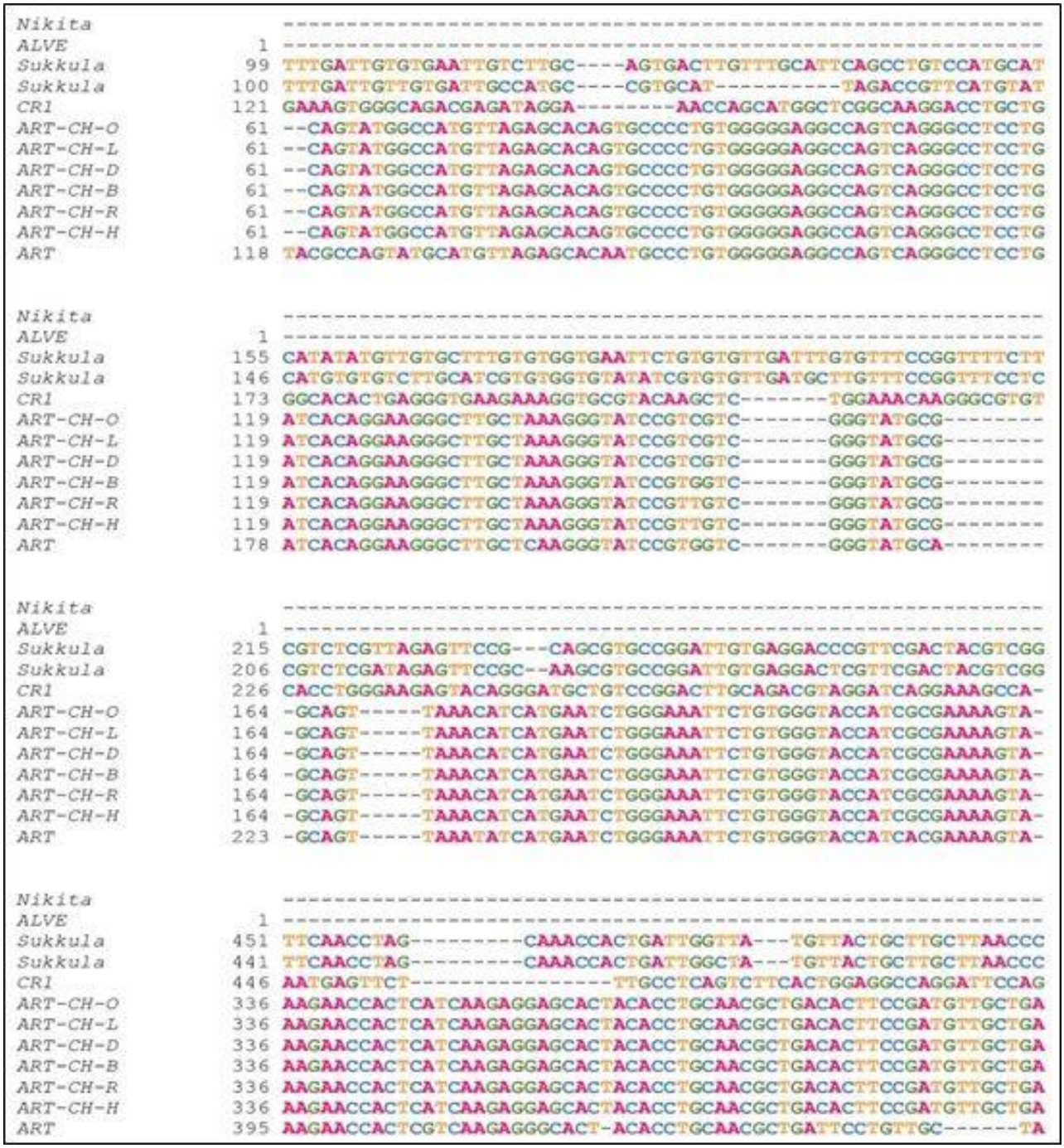

Figure 2. Multiple sequence alignment of chicken- and barley-specific transposons. Every four nucleotides are presented in different colors.

and also different species [34] in normal and or stress conditions. Supporting these reports, we observed that there was no polymorphic band in *Sukkula* retrotransposon while *Nikita* polymorphism ratios were 0%–60% among chicken samples. Similar to our study, Saraswathi et al. [35] also used *Nikita* and *Sukkula* retrotransposon in the germplasm collection of *Musa*. They reported that different primer pairs together with *Nikita* and *Sukkula* showed polymorphic band profiles among samples. This is an expected result, because retrotransposon movements could be affected by many different situations including epigenetic and environmental conditions.

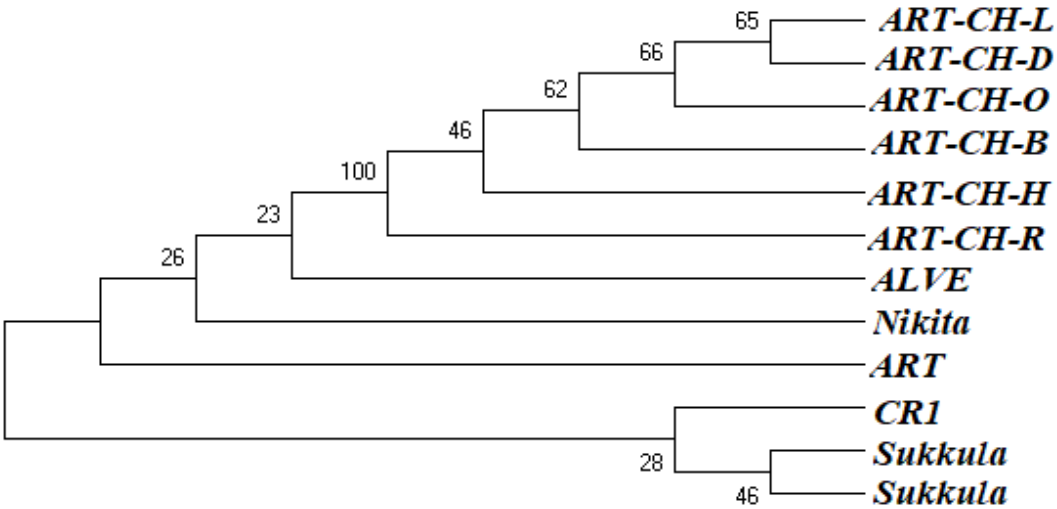

Figure 3. Phylogenetic analysis of chicken- and barley-specific transposons' sequences. Bootstrap percentages are indicated at the branch points.

Although transposons' movements across families and even phyla within the same kingdoms have been identified in different studies, there is still little information about transposon transfer between kingdoms Animalia and Plantae [36]. The sequenced genomes have gained significant importance to identify the horizontal transfer of transposon (HTT) between animal and plant genomes. Gao et al. [37] performed one of these investigations, reporting one of the first potential examples of HTT between animals and flowering plants by performing sequencing, phylogenetic, and evolutionary analyses.

3.2. Sequence similarities were detected among transposons' sequences

We also surveyed the evolutionary relationships among *Nikita*, *Sukkula,* and chicken-specific retrotransposons using *in silico* screening. For this purpose, a total of 12 transposons consisting of chicken- and barley-specific retrotransposons were analyzed to identify relationships among them. Figure 2 indicated part of the alignment analysis. We revealed that *Sukkula* and other sequences shared more similarities in clustal analysis.

The phylogenetic tree was revealed by analyzing 12 nucleotide sequences with a total of 502 positions (Figure 3). *Sukkula* and *CR1* sequences were found in the first clade. Moreover, two groups consisting of *ART* in one group and *Nikita, ALVE, ART-CH-R, ART-CH-H, ART-CH-B, ART-CH-O, ART-CH-D,* and *ART-CH-L* in another group were observed in the second clade.

Meyerowitz [38] concluded that approximately 1600 Mya, plants and animals diverged from a common ancestor. Sequencing results have provided valuable proof related to the horizontal transfer of retrotransposons between two kingdoms [36,37]. Concordant with this, we observed that CR1 element resembles *Sukkula* retrotransposon whereas *Nikita* retrotransposon is similar to other chicken retrotransposons.

In conclusion, domestic animals have been commonly studied to identify roles in the gene(s) especially related to phenotypic traits [39]. Transposons are one of the main evolution drivers affecting genes' expression. Therefore, increasing knowledge about genomes in these animals makes it possible to apply different methods to get desired traits. Here, we performed IRAP-PCR analyses to investigate the existence of barley-specific retrotransposons and *in silico* analyses to understand evolutionary relationships but no sequencing experiment was performed. Therefore, sequencing of native chickens' genomes will also give us detailed information between plants and chickens.

Ethical standards

Ondokuz Mayis University Animal Experiments Local Ethics Committee gave permission for this study with the decision number of 06.11.2012/1.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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