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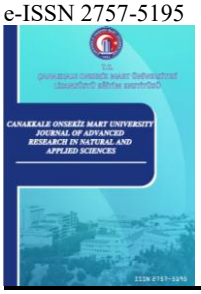

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Comparative Retrotransposon Analysis in Wheat

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Research Article

Abstract − The presence of retrotransposons is associated with polyploidy, especially in wheat, and may cause an increase in genome size. In this study, the evolutionary information was aimed to reveal based on the comparison retrotransposon movements between bread and einkorn wheat. For that reason, the transposition of *BARE1*, *Sukkula* and *Nikita* retrotransposons in bread and einkorn wheat were analysed by using IRAP-PCR molecular marker method. Both monomorphic and polymorphic bands in each wheat species have been demonstrated. IRAP-PCR products of *Sukkula* retrotransposon was showed as 10 bands in bread wheat, but no bands could be determined in einkorn wheat. *Nikita* retrotransposon was demonstrated as 6 bands in bread wheat, 14 bands in einkorn wheat. Polymorphism rate was calculated as 81% for *Nikita* between bread wheat and einkorn wheat. However, the presence of *BARE1* were not observed in both species. The obtained findings suggest that *Nikita* retrotransposon contributes to genome obesity, especially in bread wheat. The failure of *Sukkula* retrotransposon detection in einkorn wheat indicates that *Sukkula* may be inserted in the genome of bread wheat by horizontal gene transfer during wheat domestication events. These results may contribute understand the organization of wheat genome during domestication.

Keywords − *BARE1, Bread Wheat, Einkorn Wheat, Nikita, Sukkula.*

1. Introduction

Wheat, especially durum and bread wheat, is a one of the main foods used as human nutrition in the world-wide. Wheat genome evolution has been under the impact of hybridization and polyploidization events. Wheat varieties consist of 13 diploid and 18 allopolyploid species. Archaeological evidence points out einkorn wheat (*Triticum monococcum*), which is diploid, have been domesticated in the Karacadağ mountains of Turkey, during the Pre-Pottery Neolithic period (Heun et al., 1997). However, bread wheat (*Triticum aestivum*), which was rooted in two main domestication events, is an allohexaploid (AABBDD). First domestication event was resulted with allotetraploid (AABB) durum wheat is rooted in the part of Fertile Crescent region in Turkey on 2.5–4.5 MYA. The second domestication event is allohexaploidization of bread wheat (Dubcovsky & Dvorak, 2007).

In plants, repetitive DNA is mostly derived from the proliferation of retrotransposons play significant roles in the evolution of almost all organisms. Most of the retrotransposons are inactive in the genome under normal condition, although they can be induced by some environmental conditions such as biotic and abiotic stresses (Arvas et al., 2021). Especially in wheat, polyploidy has been associated with the presence of retrotransposons that they can cause an increase in genome size (Hartley & O'neill, 2019). *BARE1* is the most common and

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active Long Terminal Repeat (LTR)-retrotransposon, especially in somatic tissues (Marakli, Yilmaz & Gozukirmizi, 2012). Another barley retrotransposon is *Nikita* has been widely used in different studies such as genetic diversity and determination of polymorphism patterns in polyploids (Bayram et al., 2012). Moreover, sequence analysis indicated *Sukkula* sequences are mostly conserved in barley. In plants, active retrotransposons play important roles for genome diversification due to transposition and accumulation potentials. Especially, barley-specific retrotransposons have been studied and their transferability exhibited that these retrotransposons provide valuable information about species diversification during the evolutionary time (Marakli et al., 2019).

Well-studied barley retrotransposons, including *BARE1*, *Nikita* and *Sukkula* were aimed to identify in bread and einkorn wheat in this study. For this purpose, the presence of *BARE1*, *Nikita* and *Sukkula* was investigated by using IRAP-PCR technique and results were analysed by Dice similarity coefficient. This is the first report to demonstrate *Sukkula* and *Nikita* insertions in wheat genome and is expected to provide insight into the effects on genomic variations.

2. Materials and Methods

2.1. Plant Growth Conditions

T. aestivum and *T. monococcum* plant seeds were provided from Directorate of Trakya Agricultural Research Institute in Turkey. Surface sterilization of wheat seeds performed with 70% ethanol at 2 minutes, 0.1% $HgCl_2$ at 20 minutes, rinsed with dH_2O at 10 minutes, 20% commercial bleach at 15 minutes (with 3 drops of TWEEN® 20) (Unilever Industry and Trade Turkish Joint Stock Company, Istanbul; Sigma, P1379, Merck SA, Argentina) and then seeds were washed with sterile distilled water for three times. Afterward, seeds were placed as 7 seeds in each petri dish containing MS medium (Caisson, Smithfield, USA). Both wheat seeds were germinated for ten days at 25 ±2°C, 16h light/8h dark period under controlled conditions in a growth chamber (Miprolab MK500, Ankara). $HgCl_2$ and TWEEN® 20 were not used in surface sterilization of bread wheat, rest of protocol performed as same.

2.2. Genomic DNA Extraction

Six seeds were randomly selected among both bread wheat and einkorn wheat. Genomic DNA extraction was performed the manufacturer's protocol by using the HiPurA® Plant Genomic DNA Miniprep Purification Kit (Himedia, Einhausen, Germany). DNA concentration was evaluated by UV spectrophotometry (Thermo Scientific, AZH1705428). DNA integrity was observed on agarose gel (1%) electrophoresis.

2.3. IRAP-PCR

Four of *T. aestivum* plants' DNAs were mixed and six of *T. monococcum* plant's DNA were mixed and used as template of IRAP-PCR method (Biorad, Dubai). Primers of *Sukkula*, *Nikita*, and *BARE1* retrotransposon used in IRAP-PCR showed at Table 1. IRAP-PCR was performed with Ex Taq™ DNA Polymerase Perfect Mix (Takara, RR039B, Saint Germain en Laye, France).

Table 1
Primer sequences used in IRAP-PCR.

Primer	Sequences	Reference
Sukkula	5'GTCGGGCTACGGCTGCAAGG 3'	Leigh et al., 2003
Nikita	5'CGCATTTGTTCAAGCCTAAACC 3'	Rodriguez et al., 2006
BARE1	5'ATCATTCCCTCTAGGGCATAATTC 3'	Schulman et al., 2004

IRAP-PCR was carried out under the following order: 95°C for 10 min, followed by 40 cycles of 95°C for 30 sec, 55 and 61 °C used as annealing temperature for 30 sec for *Nikita* and *Sukkula*, respectively, and 72°C for 3 min. Final extension was at 72°C for 10 min. For *BARE1* primer, gradient PCR was conducted at annealing temperatures 59-65°C. Obtained products were evaluated by using 2% agarose gel.

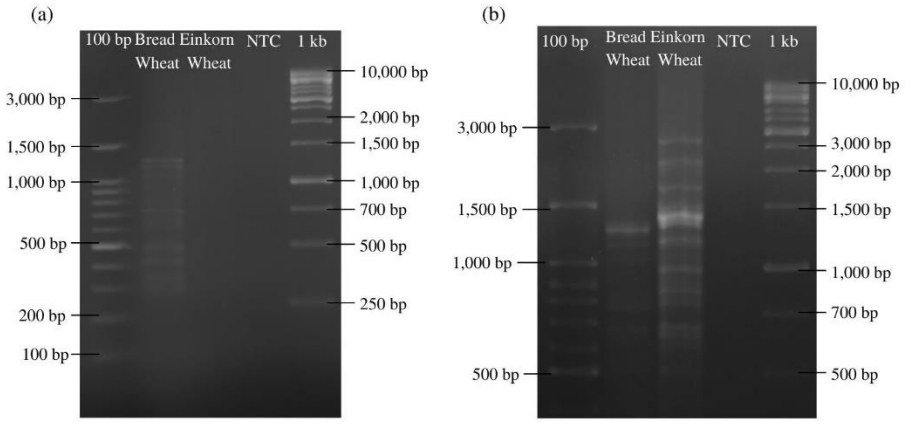
2.4. Polymorphism Analysis

The Dice Similarity Coefficient was used the evaluation of the polymorphism rates of samples (Dice, 1945; Nei & Li, 1979). Also, the GelJ v.2.0 program was used to cluster the samples (Heras et al., 2015).

3. Results and Discussion

Wheat is a dominant food is consumed in may diets by billions of people. Two main varieties dominate the current global wheat production; one is the durum wheat and bread wheat the other. Nowadays, einkorn wheat is mostly used for improving to pesticide and disease resistance, biotic and abiotic stress tolerance. Additionally, the improvement of agriculturally significant crops has accelerated by genomic studies. However, studying with wheat (*Triticum* spp.) is challenging due to its large size and the genome complexity (Appels et al., 2018). We conducted retrotransposon analysis in bread wheat and einkorn wheat to understand the wheat genome evolution and domestication footsteps. In this study, most abundant retrotransposons − *Sukkula*, *Nikita* and *BARE1*− in barley were selected and their transposition events were investigated by using IRAP-PCR. According to our results, Sukkula and Nikita presence were demonstrated in wheat at first time. However, we do not able to show *BARE1* existence in both wheat varieties.

According to the electrophoresis results, 10 bands were detected in *T. aestivum*, ranging from 200 to 1,500 bp for *Sukkula* (Figure 1a), but band patterns were not observed in *T. monococcum*. Analysis of *Nikita* band profiles totally showed 30 bands: 6 monomorphic and 9 polymorphic bands ranging from 500 to 3,500 bp (Figure 1b). For *Nikita*, polymorphism rates were calculated as 0–81% in between bread and einkorn wheat (Figure 1c). Also, the phylogenetic tree of *Nikita* was clustered for bread wheat and einkorn wheat shown in Figure 1d.



	T. aestivum	*T. monococcum*
T. aestivum	-	-
T. monococcum	81	-

Figure 1: Result of IRAP-PCR analysis. (a) IRAP-PCR products of *Sukkula* on 2% agarose gel, (b) IRAP-PCR products of *Nikita* on 2% agarose gel, (c) polymorphism rates of Nikita in two wheat species, (d) clustering of wheats based on the results of *Nikita* IRAP-PCR. NTC: no template control.

Retrotransposon amplification events are the main reason of 'genome obesity' in plants and shape the genome structure. Retrotransposon accumulation and polyploidization are not entirely independent events, however, both two mechanisms affect one another has also been great influence for crop breeding and domestication. The wheat genome has also undergone massive amplification and accumulation of transposons. Interestingly, transposon content was found to be very similar between sub-genomes of bread wheat. Moreover, transposon analysis demonstrated no evidence for explosion of transposon amplification in bread wheat genome after polyploidization events (Wicker et al., 2018). On the other hand, our findings suggest that *Nikita* contributed the genome obesity in bread wheat. *Nikita* has been reported as the fourth most active retrotransposon in barley, while *BARE1* was found the most active retrotransposon (Leigh et al., 2003). Active retrotransposons are mostly useful to study plant diversification due to their transpositions and accumulation potentials in the genome. Many reports exhibited polymorphism among species through retrotransposon movements according to their activities in the genome (Marakli, 2019).

Therefore, we selected *Nikita* to evaluate polymorphism rates in both wheat varieties. According to our results, *Nikita* was demonstrated in both bread wheat and einkorn wheat, indicating that *Nikita* is present and active in wheat genome. Moreover, polymorphism percentage was found as 81% in our study, suggesting that two wheat varieties had different pathways during the long evolutionary time. Retrotransposon transpositions and amplification can be activated by stress conditions, reduced DNA methylation, or after genome rearrangements which can lead the escape from host silencing mechanisms (Ito & Kakutani, 2014).

Sukkula was first identified in barley genome at Mlo locus. Interestingly, *Sukkula* means "shuttle" in Finnish because of these elements are non-autonomous belonging to large retro-transposon derivatives or LARDs (Shirasu et al., 2000). Additionally, some studies also indicated the presences in different genomic regions based on selection and "host control" pressures in a very long evolutionary time (Rebollo, Romanish & Mager, 2012). In our study, we demonstrated the *Sukkula* existence only in bread wheat, not in einkorn wheat. These findings suggested that *Sukkula* elements insertions may be occurred during the domestication of wheat. As it is known, domestication is a key event together with allopolyploidization to shape the wheat genome (Avni et al., 2017). However, further genome analysis is needed to determine in which domestication time *Sukkula* has inserted the wheat genome. Additionally, both barley and wheat have emerged the same regions at Fertile Crescent. Both these two species have evaluated in close regions may lead up to the horizontal gene transfers.

In some cases, insertion and amplification potentials of a transposon families are shared by related species. Interestingly, a transposon family in one species can be observed with a high copy number, while some transposon families in close relatives can be present with a low copy number (Estep, DeBarry & Bennetzen, 2013). Studies suggest that *BARE1* in barley is the most active retrotransposon (Marakli, Yilmaz & Gozukirmizi, 2012). However, we did not able to detect *BARE1* transposition events in both bread wheat and einkorn wheat. One of the main reasons why *BARE1* cannot be detected can be the absence of *BARE1* in wheat genome. Also, another reason can be the lack of recognition sites of primer due to the deterioration of the LTR sequences. Further analysis is required to reveal main reason why *BARE1* primer did not study in wheat genome.

4. Conclusion

Whole-genome sequences analysis in plants highlight the major roles of retrotransposon in evolution of wild and domesticated plant species such as einkorn wheat and bread wheat. The existence of barley retrotransposons -*Nikita* and *Sukkula*- was observed in the wheat genome at first time, although the presence of *BARE1* could not be detected in this study. Such information will contribute to reveal the effects of the retrotransposons on wheat genome organization during the domestication events.

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Author Contributions

Elif Karlik: Conceived and designed the analysis.

Bekir Ahmet Ilgar: Collected data and performed the analysis.

Seray Altintas: Collected data and wrote the paper.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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