Study of the soybean specific retrotransposon *(SIRE1)* in wheat (*Triticum aestivum* L.) genotypes grown *in vivo* and *in vitro*

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Transposon mobility and polymorphism ratios were examined in leaf samples obtained from a wheat plant (*Triticum aestivum* L.) *in vitro* callus culture, *in viv*o seeds and regenerants . The callus mass was cultured in Murashige and Skoog nutrient medium for 10 and 20 days. The seeds, and regenerating leaves were grown in the soil for 10 and 20 days and leaf samples were obtained. Soybean-specific *SIRE1* retrotransposons were detected in seeds, callus, and regenerating leaves of wheat, and different polymorphism results were obtained in each sample. The mobility of retrotransposons was determined in Azamatli 95, Barakatli 95, and Gobustan wheat genotypes using the IRAP method. *SIRE1* IRAP analyses resulted in 432 bands ranging from "300 to 2500" bp. It has been observed that 244 bands are polymorphic, and 188 bands are monomorphic. This work is the first epigenetic study conducted on wheat (*in vitro* callus, *in vivo* seed and rejenerant leaf) plant in Azerbaijan.

Keywords: *Wheat, in vitro, seed, callus, regenerant leaf, epigenetics, transposons, SIRE1, IRAP*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important staple food crop cultivated in very different environments, serving as a food source for 30% of the human population (Food Outlook, 2017; Kaur et al., 2016). Conventional breeding approaches have not been enough to improve grain quality, therefore new breeding strategies are needed. For this purpose, the identification of agronomically important genes has gained interest for improvement. Despite of complexity and enormous size of the wheat genome (Walkowiak et al., 2020).

New scientific achievements have been performed as a result of cooperation between traditional *in vitro* cell selection methods and molecular analyses. In biotechnology, *in vitro* plant cells are a convenient model for studying the genetics of the plant organism and many physiological-biochemical processes. Also, cell, and tissue culture serve as a model system for studying plant resistance to various negative environmental factors: abiotic (salinity, acidic environment, low temperature, etc.) or biotic factors (pathogens) does. Both abiotic and biotic factors have caused a number of epigenetic changes within the cell. Epigenetics is defined as heritable changes in gene expression that occur without any change in DNA sequence. Epigenetics is being investigated in many prokaryotic, and eukaryotic organisms in developmental, physiological, neurological, cytogenetic, genetic, evolutionary, pathological, and medical fields. (Willbanks et al., 2016). Histone modifications, RNA-induced silencing, and DNA methylation are epigenetic mechanisms (Lucibell et al., 2022). In addition to these mechanisms, mobile genetic elements, transposable elements or transposons, plays an important role in the evolution of almost all organisms (Schulman et al., 2004). These transposable repetitive sequences move from one chromosomal site to another through transposition. Most retrotransposons are normally inactive in the genome although they can be induced by some environmental conditions such as biotic and abiotic stresses (Arvas et al., 2021). Transposons, found in all eukaryotes and some prokaryotes except for a few species of the genus Plasmodium, form a large part of eukaryotic genomes (Willbanks et al., 2016). Transposons make up about 50-90% of plant genomes and 3-45% of animal genomes. They form more than 75% of the maize genome. In wheat, in particular, polyploidy has been associated with the presence of retrotransposons causing an increase in genome size (Hartley et al., 2019). Insertion polymorphisms caused by transposons are identified using molecular techniques. Molecular markers are nucleotide sequences that contain certain characteristics. The molecular marker

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technique is generally based on the polymerase chain reaction (PCR) principle and allows the calculation of polymorphism ratios between samples. A variety of PCR-based markers have been extensively investigated and used in research. As mentioned above, retrotransposons belonging to class I transposons were considered in this study. Retrotransposons are highly ideal targets for establishing molecular markers due to their suitable sequence characteristics. And a variety of transposon-based molecular markers have been established (Gozukirmizi et al., 2015). These include Inter-Retrotransposon Amplified Polymorphism (IRAP) (Kalendar et al., 2006), Retrotransposon-Microsatellite Amplified Polymorphism (REMAP), Inter-Primer Binding Site Amplification (IPBS), Sequence-Specific Amplification Polymorphism (S-SAP), Retrotransposon-Based Insertion Polymorphism (RBIP), Inter-SINE Amplified Polymorphism (ISAP), Inter-MITE Polymorphism" (IMP), RAPD-Retrotransposon Amplified Polymorphism (R-RAP), Inverse Sequence Tagged Repeats (ISTR) and Transposable display (TD) (Gozukirmizi et al., 2015). These methods are used for target gene identification, genetic variation, linkage, genome mapping, DNA fingerprinting, phylogenetics, systematics, somaclonal variation studies, transgenic studies, developmental biology, mutagenesis, and other purposes.

This study aims to investigate the movements of *SIRE1* retrotransposons and then estimate the polymorphism ratios between samples. At the initial stage of the research, a collection of models was created, and at the next stage, the processes of clarifying the morphological features and molecular methods of epigenetic changes were reflected. Important results were obtained as a result of a comparison of callus cells obtained from immature embryos of wheat with mother plant (F1 generation seed) and regenerating plant under *in vitro* conditions using IRAP molecular marker. In this way, the activity of retrotransposons was monitored on the 10th and 20th days of seed, callus, and regenerant samples of 3 wheat genotypes (Azamatli, Barakatli-95, and Gobustan).

MATERIALS AND METHODS

Plant growth conditions. Azamatli 95, Barakatli 95, and Gobustan wheat genotypes were obtained from the Research Institute of Crop Husbandry (Absheron). 10-day-old and 20- day-old control (seed), callus, and regenerating plant leaf samples obtained from the plants were used during the study. For control, seeds of wheat genotypes were planted, and 10-day-old and 20-day-old leaf samples were obtained. For calli production, embryos extracted aseptically from immature wheat genotypes were cultured in Murashige and Skoog (Murashige et al., 1962). 2,4-Dichlorophenoxyacetic acid (2,4 D) was used at a concentration of 2 mg/l for the induction of callus formation and the proliferation of callus culture in subsequent subcultivations. The acidity of the environment was in the range of pH 5.6 - 5.8. Cultivation of callus tissue was carried out at 26^0C±1 temperature and 70% humidity. Callus mass was subcultivation every 30 days. 10-day-old and 20-day-old old callus cells were used after the formation of cells. Regenerative plants were grown from callus cells. Regenerative callus was cultured in an acclimatization chamber under light conditions with the addition of indolyl acetic acid (IAA) to the medium. After obtaining regenerant seeds from callus, samples were taken on 10-day-old and 20-day-old regenerant leaves obtained from them.

Molecular analyses

Genomic DNA isolation. Genomic DNAs (gDNA) were isolated from seeds, callus, and regenerating leaves of 3 wheat genotypes (Kidwell and Osborn, 1992). Spectrophotometric quantification purity of the isolated gDNA was measured in a spectrophotometer (NanoDrop 2000C) and diluted to 20 ng/µl for the PCR reaction. Moreover, the quality of gDNA was also evaluated in electrophoretic analyses. The experiment was repeated twice by using two independent biological replicates.

IRAP-PCR analysis of SIRE1 retrotransposon and calculation of polymorphism. For IRAP PCR analyses, a primer with the sequence 5" CAGTTATGCAAGTGGGATCAGCA 3" (Chesnay et al., 2007) of the *SIRE1* retrotransposon was used. The PCR mixture consisted of 10 mL of Taq 2 x Master Mix Red, 2,5 mL of 25 mmol/L $MgCl_2$, 3 mL of 20 ng/mL template g DNA, 1 mL of nuclease-free dH_2O, 2 mL of primer (10 mmol/L). PCR samples were amplified at 95ºC for 3 minutes, 95ºC for 30 seconds, 50ºC for 30 seconds, 72ºC for 3 minutes, and 72ºC for 10 minutes. PCR products were run on a gel dissolved in 1% agarose 1xTAE buffer for 90 minutes at 120 V. After staining with ethidium bromide for at least 180 min, they were visualized under a UV transilluminator. Band sizes were determined by comparison on a 1000 bp (1 kb) DNA ladder. Based on the IRAP-PCR results, PCR bands were counted and Jaccard Similarity Coefficient was used to calculate polymorphism among samples (Jaccard, 1908).

RESULTS AND DISCUSSION

Polymorphism values of *SIRE1* retrotransposons were examined in 10-day-old and 20-day-old seed (F1 generation), callus, and regenerant variants of Azamatli 95, Barakatli 95, and Gobustan wheat cultivars. *SIRE1* IRAP analyses resulted in 432 bands ranging from "300 to 2500" bp (Fig.1). Of these, 244 bands were identified as polymorphic, and 188 bands were identified as monomorphic. Polymorphism coefficients calculated by the Jaccard coefficient using these band profiles are listed in Table 1.

In the 10-day-old control leaf (seed) samples belonging to three cultivars, we detected 0-50% polymorphism. The highest polymorphism rate was detected in Barakatl 95 and Gobustan samples. In callus samples, this ratio was 0-67%, and the highest value of polymorphism was observed in Gobustan callus (67%). Similarly, the highest polymorphism ratio was obtained in Gobustan regenerants (44%).

When compared to Azamatli 95 (10-day-old) seed and callus samples, seed and regenerant, and callus and regenerant, there were 90%, 43%, and 80% polymorphism rates observed. For Barakatli-95, these rates were 75% for seed and callus samples, 56% for seed and regenerant, and 80% for callus and regenerant leaf. In addition, There were 63%, 56%, and 22% polymorphism ratios were determined for seed and callus, seed and regenerant leaf, and even callus and regenerant in Gobustan.

In the 20-day-old seed samples belonging to three cultivars, we detected 0-90% polymorphism. And the highest rate was recorded in Barakatli-95 samples (90%). In callus samples, this ratio was 13-83-67%, and the highest value of polymorphism was observed in Gobustan callus (83%). Unlike seeds and callus, no polymorphism was detected in regenerant samples.

25% polymorphism between seed and callus of Azamatli 95 (20-day-old) and no polymorphism was determined between seed and regenerant leaf. When comparing callus and regenerant, 25% polymorphism was recorded. No polymorphism was detected when comparing seed and callus and callus and regenerant of Barakatli 95 genotype. 25% polymorphism was detected between seed and callus, as well as between seed and regenerant, and between callus and regenerant in the range of 0-58%. The highest rate of polymorphism was determined in samples from Gobustan (58%).

Comparing 10 and 20-day samples, 63% polymorphism was determined in Azamatli-95 seed, 67% in Bararkatli 95, and 78% in Gobustan. İn callus samples, 89% polymorphism was found in Azamatli-95, 88% in Barakatli-95, and 67% in Gobustan. In regenerant samples, 55% polymorphism was found in Azamatli 95, 58% in Barakatli 95, and 50% in Gobustan.

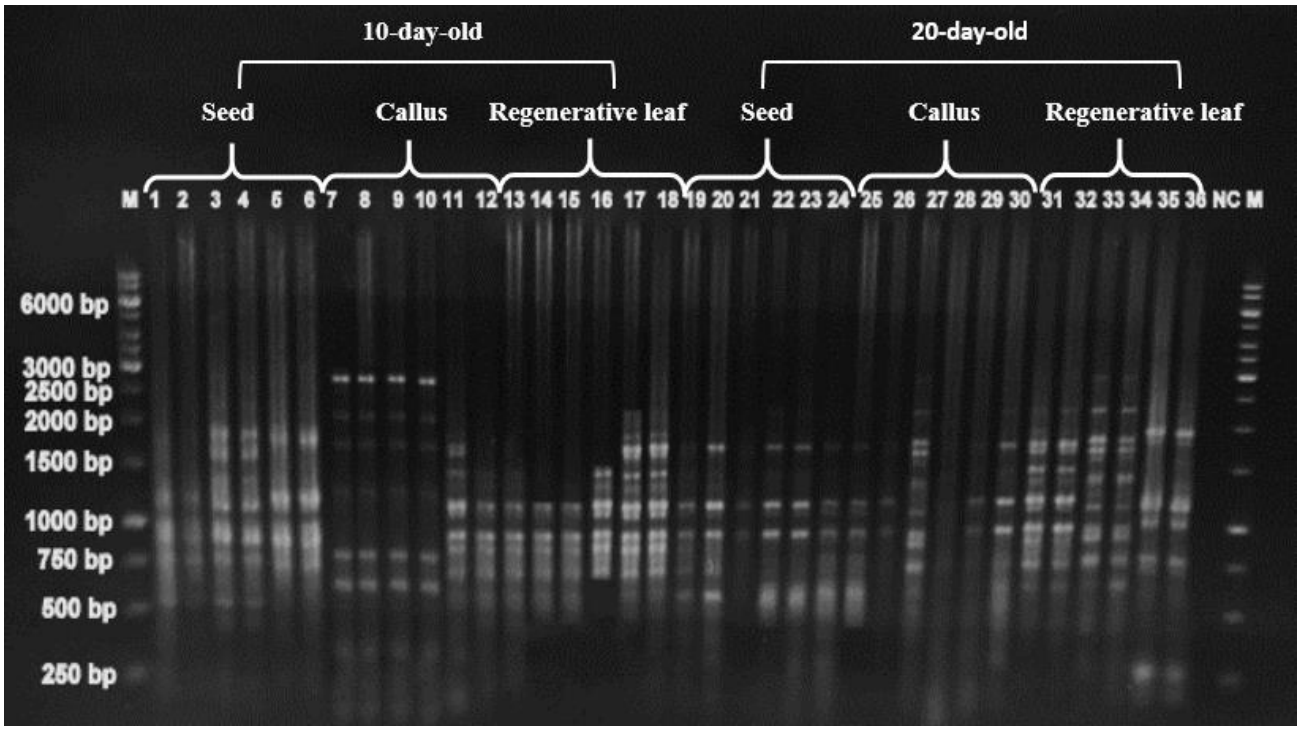

Fig. 1. *SIRE1* IRAP-PCR results. M - marker; 1-2, 7-8, 13-14, 19-20, 25-26, 31-32- Azamatli 95; 3-4, 9-10, 15-16, 21-22, 27-28, 33-34 – Barakatli 95; 5-6, 11-12, 17-18, 23-24, 29-30, 35-36 - Gobustan. 1-6 - 10-day-old-seed; 7-12 - 10-day-old-callus; 13-18 - 10-day-old regenerating leaves; 19-24 - 20-day-old-control seeds; 25-30 - 20-day-old-callus; 31- 36 - 20-day-old regenerating leaves. NC - negative control.

Table 1. *SIRE1* retrotransposon polymorphism ratios among groups calculated with Jaccard's coefficient

			10-day-old																		20-day-old																	
			Seed (control leaf)						Callus						Regenerant leaf						Seed (control leaf)						Callus						Regenerant leaf					
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
10-day-old	Seed	1	0																																			
		2	0	0																																		
		3	50	50	0																																	
		4	50	50	0	0																																
		5	40	40	50	50	0																															
		6	40	40	50	50	0	0																												1		
	Callus	7	90	90	75	75	78	78	0																													
		8	90	90	75	75	78	78	0	0																												
		9	90	90	75	75	78	78	0	0	0																											
		10	90	90	75	75	78	78	0	0	0	0																										
		11	56	56	11	11	56	56	67	67	67	67	0																									
		12	43	43	33	33	63	63	73	73	73	73	22	0																								
	Rejenerant laf	13	43	43	33	33	63	63	73	73	73	73	22	0	0																							
		14	20	20	56	56	50	50	80	80	80	80	44	29	29	0																						
		15	20	20	56	56	50	50	80	80	80	80	44	29	29	0	0																					
		16	57	57	44	44	57	57	70	70	70	70	33	14	14	43	43	0																				
		17	56	56	11	11	56	56	67	67	67	67	0	22	22	44	44	33	0																			
		18	56	56	11	11	56	56	67	67	67	67	0	22	22	44	44	33	0	0																		
20-day-old	Seed	19	50	50	22	22	50	50	64	64	64	64	11	13	13	38	38	25	11	11	0																	
		20	63	63	33	33	63	63	73	73	73	73	22	25	25	50	50	38	22	22	13	0																
		21	67	67	67	67	67	67	89	89	89	89	70	78	78	71	71	75	70	70	80	90	25															
		22	63	63	50	50	63	63	70	70	70	70	55	73	73	67	67	82	55	55	64	73	57	14														
		23	63	63	50	50	63	63	70	70	70	70	55	73	73	67	67	82	55	55	64	73	57	14	14													
		24	63	63	50	50	78	78	70	70	70	70	55	60	60	67	67	70	55	55	64	73	57	38	38	14												
	Callus	25	63	63	50	50	78	78	70	70	70	70	55	60	60	67	67	70	55	55	64	73	57	38	38	14	14											
		26	67	67	67	67	67	67	89	89	89	89	70	78	78	71	71	75	70	70	80	90	25	57	57	57	57	25										
		27	75	75	42	42	75	75	45	45	45	45	33	50	50	67	67	58	33	33	42	50	73	45	45	45	45	73	9									
		28	83	83	78	78	83	83	88	88	88	88	80	75	75	86	86	71	80	80	78	75	80	88	88	71	71	80	82	33								
		29	50	50	56	56	71	71	90	90	90	90	60	67	67	57	57	78	60	60	70	80	40	43	43	43	43	40	64	83	20							
		30	67	67	40	40	67	67	60	60	60	60	45	64	64	70	70	73	45	45	55	64	63	25	25	25	25	63	36	75	50	13						
	Rejenerant	31	64	64	27	27	64	64	58	58	58	58	18	36	36	55	55	45	18	18	27	36	73	45	45	45	45	73	25	82	64	36	9					
		32	64	64	27	27	64	64	58	58	58	58	18	36	36	55	55	45	18	18	27	36	73	45	45	45	45	73	25	82	64	36	9	9				
		33	67	67	33	33	67	67	50	50	50	50	25	42	42	58	58	50	25	25	33	42	75	50	50	50	50	75	17	83	67	42	17	17	8			
		34	67	67	33	33	67	67	50	50	50	50	25	42	42	58	58	50	25	25	33	42	75	50	50	50	50	75	17	83	67	42	17	17	8	8		
		35	75	75	60	60	89	89	91	91	91	91	50	56	56	63	63	67	50	50	60	56	71	67	67	67	67	71	55	86	57	70	55	55	58	58	17	
		36	63	63	33	33	78	78	83	83	83	83	22	25	25	50	50	38	22	22	33	25	78	73	73	60	60	78	50	75	67	64	36	36	42	42	38	0

CONCLUSION

In conclusion, it should be noted that *SIRE1* retrotransposons were examined in all 3 genotypes of the wheat plant using the IRAP method. In these studies, a band of 300-2500 bp of *SIRE1* retrotransposon specific to soybean plants was detected. Of these, 244 bands were identified as polymorphic, and 188 bands as monomorphic. The polymorphism results suggest that different results were obtained both within genotypes and between seed, callus, and regenerating leaf samples. Thus, the highest polymorphism value in 10-day-old seeds, callus, and regenerant leaves was found in the Gobustan wheat variety. This suggests that Azamatli 95 and Barakatli 95 varieties have shown more durability. When we look at the 20-day-old samples, we can see that the highest rates were in Barakatli 95 seed samples, in the calli of Gobustan and no polymorphism was detected in the regenerants. Azamatli 95 genotype had more preservative results during the studies on 10 and 20-day seed, callus, and regenerating leaf samples. This study may allow studying the mobility of retrotransposons according to the growing conditions of plants both at different developmental stages. The most important point discovered in this study is the discovery of the *SIRE1* retrotransposon specific to the soybean plant in the seeds, callus, and regenerating leaves of the wheat plant. Furthermore, studies such as these indicate that there is much more to be explored among plants and, more broadly, living things.

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In vivo **və** ***in vitro*** **şəraitlərdə yetişdirilən buğda** ***(Triticum aestivum*** **L.*)*** **bitkisində spesifik soya** ***(SIRE1)*** **retrotranspozonunun tədqiqi**

Günay İlman qızı İsmayılova

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Buğda (*Triticum aestivum* L.) bitkisinin *in vitro* kallus kulturası, *in vivo* toxum və regenerantlarından əldə edilən yarpaq nümunələrində transpozonların mütəhərrikliyi və polimorfizm dərəcələri tədqiq edilmişdir. Kallus kütləsi Murashige ve Skoog qida mühitində 10 və 20 gün ərzində kultivasiya olunmuşdur. Toxumlar və regenerant yarpaqlar isə torpaqda 10 və 20 gün ərzində böyüdülərək yarpaq nümunələri əldə edilmişdir. Soya bitkisinə xas olan *SIRE1* retrotranspozonları buğdanın toxum, kallus və regenerant yarpaqlarında aşkar edilmiş və hər bir nümunədə fərqli polimorfizm nəticələri alınmışdır. IRAP metodu ilə Əzəmətli-95, Bərəkətli-95 və Qobustan buğda genotiplərində retrotranspozonların mütəhərrikliyi müəyyən edilmişdir. *SIRE1*-in IRAP analizlərinə əsasən 300-2500 n.c. aralığında 432 zolaq aşkarlanmışdır, onlardan 244-ü polimorf, 188-i isə monomorf zolaqdır. Bu iş Azərbaycanda buğda bitkisinin, *in vitro* kallusu, *in vivo* toxum və regenerant yarpaqları üzərində aparılan ilk epigenetik tədqiqat işidir.

Açar sözlər: *Buğda, in vitro, toxum, kallus, regenerant yarpaq, epigenetika, transpozonlar, SIRE1, IRAP*