

## Investigation of DNA Methylation Level in Wheat Genome Exposed to Vanadium by Using CRED-RA Technique

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**ABSTRACT:** Vanadium (V) has become a serious pollutant due to its widespread use in industry. In this study, DNA methylation changes in *Triticum aestivum* L. 'Çetinel 2000' seeds were determined by CRED-RA (Coupled Restriction Enzyme Digestion-Random Amplification) analysis. DNA damage and genomic instability were determined by Simple Repeat Inter-Sequence Polymorphism (ISSR) analysis. Vanadium solutions (4.4 mM, 6.6 mM, 8.8 mM) and boric acid (BA) solutions (4 mM and 8 mM) were used. According to CRED-RA analysis, it was observed that V caused more changes in the DNA methylation level in wheat genome compared to BA application. All doses of V caused DNA damage and decreased genomic template stability (GTS). It was determined that the genomic stability was maintained in BA doses applied with V. Genomic stability was maintained at BA doses administered with V. As a result, BA may be an alternative to reduce genotoxic damage to V stress in plants.

**Keywords:** Boric acid, wheat, CRED-RA, DNA methylation, *Triticum aestivum* L., vanadium.

### Vanadyuma Maruz Kalan Buğday Genomunda DNA Metilasyon Düzeyinin CRED-RA Tekniği Kullanılarak Araştırılması

**ÖZ:** Vanadyum (V), endüstride yaygın kullanımı nedeniyle ciddi bir kirlenici haline gelmiştir. Bu çalışmada, *Triticum aestivum* L. 'Çetinel 2000' tohumlarındaki DNA metilasyon değişiklikleri CRED-RA (Çift Restriksiyon Enzimi Kesimi ve Rastgele Çoğaltım) analizleri ile belirlenmiştir. DNA hasarları ve genomik kararsızlık Basit Tekrarlı Diziler Arası Polimorfizm (ISSR) analizi ile belirlenmiştir. Vanadyum çözeltileri (4,4 mM, 6,6 mM, 8,8 mM) ve borik asit (BA) çözeltileri (4 mM ve 8 mM) kullanılmıştır. CRED-RA analizine göre, V uygulamasının buğday genomunda DNA metilasyon seviyesinde BA uygulamasına göre daha fazla değişikliğe neden olduğu görülmüştür. Tüm V dozları DNA hasarına sebep olarak genomik kararlılığın (GTS) azalmasına neden olmuştur. V ile birlikte uygulanan BA dozlarında genomik kararlılık korunmuştur. Sonuç olarak, BA'nın V stres koşulları altında bitkilerde genetik hasarı azaltmak için alternatif bir kimyasal olabileceği düşünülmektedir.

**Anahtar Kelimeler:** Borik asit, buğday, CRED-RA, DNA metilasyonu, *Triticum aestivum* L., vanadyum.

## INTRODUCTION

Abiotic stresses cause major losses each year due to reduced agricultural productivity and increased crop failure. Heavy metal stress, which is abiotic stress, damages plants and other living things. Contamination of soils with heavy metals due to increased industrialization adversely affects the living organisms in the environment. Vanadium (V) has an amount approximately twice that of copper (Cu) and equal to zinc (Zn), 10 times higher than lead (Pb), and is a ubiquitous element in the environment (Imtiaz *et al.*, 2015). V contaminates soil, water and atmosphere by spreading widely in different ways such as leakage, combustion, fertilizer usage and waste from industry (Kar *et al.*, 2004). Recently, V pollution has become a major environmental problem worldwide and its high dose disrupts the growth and productivity of plants (Imtiaz *et al.*, 2015). Vanadium levels higher than 2 ppm cause reduced absorption and use of essential elements in plants, chlorophyll degradation, root deformation, inhibition and degradation of enzyme activities (Aihemaiti *et al.*, 2020). Metal toxicity affects plants in different ways. Metals may exert their effects by targeting the structural, catalytic and transport regions of the cell. By causing oxidative damage, they cause the antioxidant defense system to deteriorate and the production of reactive oxygen species (ROS) to increase (Gill and Tuteja, 2010). Oxidative damage to DNA causes modifications such as single and double chain fractures, methylation and alkylation. Eventually, the accumulation of such phenomena can be found in different genotypes (Sharma *et al.*, 2009). These damages in DNA cause genomic instability. Binding of metals to DNA generally leads to breakage of hydrogen bonds that provide base pairing and deterioration of the double chain structure, but such changes in the conformation of nucleic acids may also vary depending on the type of metal ions bound. This toxicity, which occurs as a result of the interaction of DNA molecules and toxic agents and is carried to future generations, is known as genotoxicity (Bal *et al.*, 2011). Some genotoxic effects occur in the plant and these changes can be detected by ISSR (Inter Simple Sequence Repeats) and RAPD (Random Amplified Polymorphic DNA) molecular markers. While RAPD primers are

capable of capturing CG groups that are methylated in genomic DNA, ISSR replicates in the genome and the binding points of the primers have different characteristics (Reddy *et al.*, 2002).

Heavy metals that cause genotoxicity can also be linked directly to DNA, producing free radicals or reducing the antioxidant activity that removes free radicals. Heavy metals also cause epigenetic changes such as DNA methylation (Cansaran *et al.*, 2011). One of the important techniques used to detect DNA methylation is CRED-RA (Coupled Restriction Enzyme Digestion-Random Amplification). This technique can be useful in studies of variation between sequences and species. Boron (B), a mineral, is important in plant growth and development. Most studies on B show that it is one of the basic micro molecules for plants and the negative effects of deficiency or excess are species-specific (Kekec *et al.*, 2010). In B deficiency, morphological disorders occur in the root, stem and leaf tips of plant growth regions, and this is related to the synthesis and structural functions of B in the cell wall (Brown *et al.*, 2002). In B deficiency, problems are also observed in the formation of male organs in wheat and it was recorded that there was a parallel decrease in grain yield (Rerkasem and Jamjod, 1997). Studies have been carried out on the morphological, physiological and biochemical effects of V exposure on plants. However, no studies have been found on the detection of epigenetic changes at the molecular level.

The aim of this study was to determine;

- the level at which vanadium induces changes in DNA methylation and DNA damage in the wheat genome
- whether boric acid applications contribute positively to epigenetic change.

## MATERIALS and METHODS

### Plant material and abiotic stress treatment

The wheat variety “Çetinel 2000” seeds used for germination in the laboratory environment were provided from Atatürk University Faculty of

Agriculture, Department of Field Crops. The ammonium metavanate (204846 Sigma-Aldrich) form of vanadium and the boric acid (B6768 Sigma-Aldrich) form of boron were used. Vanadium has six oxidation states and since this form is most commonly used, this form is preferred as a heavy metal application (Crans *et al.*, 1998). Boron mineral is among the micronutrient elements required for agricultural products (Turkez *et al.*, 2012). So it was used as an ameliorating agent. The seeds were kept in 5% sodium hypochlorite (NaOCl) for 10 minutes and surface sterilization was performed. Then, after washing and drying at least 5 times with distilled water, seeds were planted on sterile petri dishes (12 cm diameter), containing two layers of sterile Whatman filter paper (average 25 seeds and 4 replicates). Only pure water was used for control. Wheat seedlings were treated with one of three solutions of V (4.4 mM, 6.6 mM and 8.8 mM) (Nawaz *et al.*, 2018) and two BA solutions (4 mM, 8 mM) (Çatav *et al.*, 2018). Petri dishes were kept at 25 ° C in the dark for 14 days to germinate. Plant samples were taken from the treated petri dishes and the control group and stored at -80 C until DNA isolation.

### DNA isolation

First leaf samples were collected and crushed with liquid nitrogen. Total DNA isolation method used by Li and Quiros (2001) was expanded and modified. The concentrations and purity of the DNA samples were measured with the NanoDrop-1000 spectrophotometer (OD 260/280) and then controlled by 1.2% agarose gel electrophoresis.

### ISSR PCR

ISSR reaction was made according to the method of Li *et al.* (2007). Twelve random ISSR primers were tested (Cui *et al.*, 2017). Eight of the ISSR primers giving polymorphic amplicons were used (Bakir and Agar, 2020). The ISSR-PCR reactions were carried out in a 20 µL volume, each reaction tube contained 50 ng gDNA, 1X PCR buffer (10X), 1.5 mM MgCl<sub>2</sub>, 300 µM dNTP, 1 U Taq DNA polymerase and 25 pmol primer. ISSR-PCR conditions were as follows: 94 ° C for 4 minutes,

35 cycles, respectively, at 94 ° C for 40 seconds, at the annealing temperature of each primer for 40 seconds, at 72 ° C for 2 minutes. Finally, the process was completed by holding at 72 ° C for 6 minutes. Samples extracted from the PCR instrument were stored at 4 ° C. PCR products were separated on 1.5 % agarose gel at 70 V for 150 min, and visualized under UV light.

### ISSR analysis

ISSR results were evaluated by comparing with control using TotalLab TL120 program (Arslan, 2020). Polymorphism (%) was calculated for each primer using the formula  $(100 * a / n)$ . "a" indicates the determined ISSR polymorphic bands for each application example. "n" indicates the total number of DNA bands obtained in the negative control group with the relevant primer. The polymorphism observed in the ISSR profiles of the sample groups was calculated according to the emergence of a new band or the disappearance of an existing band compared to the negative control group (Wu *et al.*, 2016).

### CRED-RA

CRED-RA analysis to determine DNA methylation was performed according to Leljok-Levanic *et al.* (2004). Genome methylation was analyzed using the *MspI* (Promega) and *HpaII* (Promega) restriction enzymes. Both enzymes recognize the CCGG sequence, however, *Hpa II* is only active when cytosines are not methylated. Whereas, *Msp I* cannot bind to DNA when the outer cytosine is methylated. First, the DNA was replicated by PCR with the ISSR method. Then, the DNA was cut with restriction enzymes and evaluations were made by replicating the PCR. For this process, 16,3 µl sterile water, 1µl DNA (1µg/µl), 0,2 µl BSA (10µg/µl), 2µl RE 10X Buffer, 0.5µl restriction enzyme were added to a 0.5ml tube with a final volume of 20 µl. For enzyme digestion, the tubes were incubated for 4 hours in a 37°C oven. Enzyme was deactivated in an oven at 65°C for 15 minutes. In order to visualize the products obtained, 4 µl sample was mixed with 1µl 6X loading buffer, and electrophoresed in 1% agarose gel at 70V. Gels were examined with a gel imaging system.

### CRED-RA analysis

Evaluation of PCR products was expressed as presence and absence of bands for each primer in each individual. According to the bands formed, it was determined whether or not the primers hybridized to the sample template DNA. TotalLab TL120 was used to evaluate these bands (Arslan, 2020). By determining the bands formed and disappearing in all CRED-RA gel images, the polymorphism value (%) was calculated from the formula  $100 \times a / n$ .

### RESULTS

In the genotoxicity part of the study, the genotoxic effects on the extracted genomic DNAs were investigated. ISSR technique, which is a molecular marker technique, was used while detecting the changes in DNA. Genotoxic changes in polymorphic bands obtained from 8 of 12 ISSR primer trials were evaluated. A decrease in the GTS (Genomic Template Stability) rate and an increase in the polymorphism rate were detected in V application depending on the dose. The molecular weight of the bands changing in the ISSR profiles is given in Table 1. GTS rates were determined as 79% at the 4.4 mM V dose, 74.2% at the 6.6 mM V dose and 72.9% at the 8.8 mM V dose. While the GTS rates in BA alone were 85.1% at a dose of 4 mM, it was determined as

86.3% at a dose of 8 mM (Table 1). When the effect of BA applied with vanadium on the GTS rate was examined, it was determined that BA caused a positive increase in the GTS rates depending on dose.

CRED-RA analysis results were given in Table 2. Eight oligonucleotide primers which gave specific and stable results in ISSR analysis were used for CRED-RA analysis. Compared with the PCR products obtained from the control DNA, BA and/or V treatments resulted in apparent changes in CRED-RA patterns (Figure 1 and 2). In addition, the total number of polymorphic bands are given in Table 3. DNA methylation occurred at all doses of the combined treatments. Methylation values were 19.9% and 11% for the 4 and 8 mM BA applications, respectively. The percentage of polymorphism occurring at V doses were determined as 40.1%, 42.7% and 44.2%, respectively. These rates show that increasing V stress increases the methylation rate. When the effect of V and BA were considered together, the percentage of polymorphism decreased. Regarding the combined applications with the highest dose of BA and highest dose of V (8.8 mM V+8 mM BA), the methylation value was the lowest (15.3%). However, the methylation value was the highest (22.4 %) with the lowest dose of BA and lowest dose of V (4.4 mM V+4 mM BA).

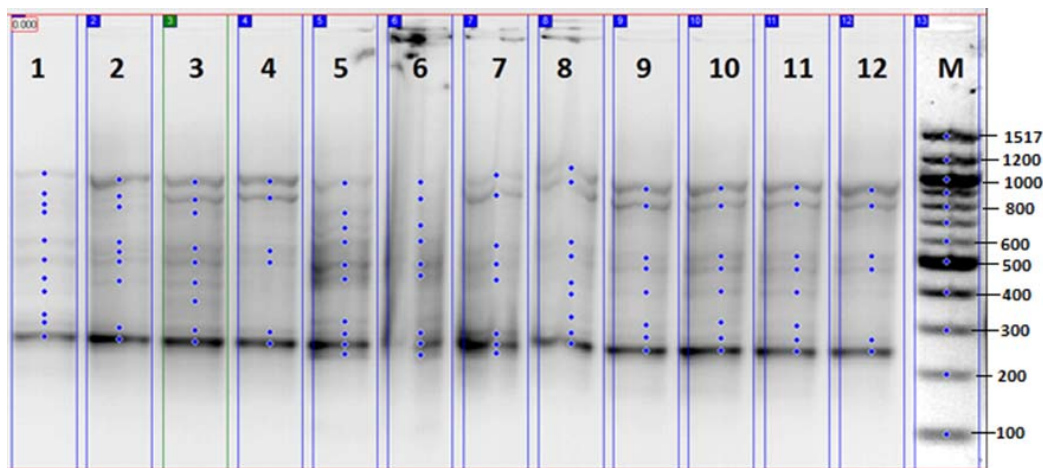


Figure 1. Amplification products of ISSR-895 primer<sup>§</sup>.

Şekil 1. ISSR-895 primerinin amplifikasyon ürünleri<sup>§</sup>.

<sup>§</sup>1: Control 2: 4,4 mM V, 3: 6,6 mM V, 4: 8,8 mM V, 5: 4 mM BA, 6: 8 mM BA, 7: 4,4 mM V +4 mM BA, 8: 4,4 mM V +8 mM BA, 9: 6,6 mM V +4 mM BA, 10: 6,6 mM V +8 mM BA, 11: 8,8 mM V +4 mM BA, 12: 8,8 mM V +8 mM BA.

Table 1. Molecular weights (bp) of bands changing in ISSR band profiles compared to control group in vanadium and boric acid (+: existing /-: lost) applications.

Çizelge 1. Vanadyum ve borik asit uygulamalarında kontrol grubuna göre ISSR bant profillerinde bantların değişen moleküler ağırlıkları (bp) (+: varolan/-: kaybolan).

Primers Primerler	Number of control bands/ Kontrol bantlarının sayısı	Existing/ lost Varolan/ kaybolan	Molecular weights of bands / Bantların moleküler ağırlıkları										
			4.4 mM V	6.6 mM V	8.8 mM V	4 mM BA	8 mM BA	4.4 mM V+ 4 mM BA	4.4 mM V+ 8 mM BA	6.6 mM V+ 4 mM BA	6.6 mM V+ 8 mM BA	8.8 mM V+ 4 mM BA	8.8 mM V+ 8 mM BA
ISSR-900	18	+	644, 258	1124, 750	1123	1123	1154	1211	1176, 928, 171	1325, 1165,		1072	1067
		-		180, 74				232, 118, 74		150, 74	535, 467, 280	91, 74	
ISSR-895	19	+	1482	1482	1482	1046, 511, 169	554	1482	1482	1482			
		-	794, 231, 172, 74	934, 794, 174	538, 172, 74		931, 794	931, 828, 794, 172	931, 828, 544, 172	570, 172, 74	761, 538, 172	828, 231, 172	595, 397, 172, 74
ISSR-876	21	+		1855, 925, 515, 263	648, 394				2151, 1877, 953,				
		-	1573, 807	1622, 1247	1622, 1360, 1040	1717, 1483, 1247	1717, 1483	1717, 1483, 302	1717, 1483, 302	338, 302	1483, 1139, 860, 435, 649	807, 435	1360, 1240, 807, 302
ISSR-868	9	+		1675		1675	1675		1492		1265	1478	1403
		-	898	1026, 500	1026, 630, 350	1478			1000, 650		1000, 674		1000
ISSR-808	10	+	1024	1046	1012	1012	1012	1352	1015	1362	1406	1406, 1172,	1340, 907,
		-	600	503	544				424	653, 418	1209	788, 421	505
ISSR-811	10	+	1215	1215	456		409, 317,	512, 340, 175	542,	512, 215,		512, 284	
		-	1083, 822, 704	1083, 956, 822, 708	1387, 825	1387, 1083, 822	775	1215, 1083, 821	1387, 1083	1083, 778	1215, 1083, 956	1387, 1083, 956	
ISSR-873	9	+			644	519	611						870
		-		426	595, 256		1022	426, 182	182	595	543	554	265
ISSR-880	8	+	990,	990,	990, 619, 443					982, 344			
		-	1341, 678, 505, 344	625	1341			1341		1341	1341, 645	1341	1341, 1019
GTS%	100		79	74.2	72.9	85.1	86.3	80.4	82.9	76.2	79.7	74.3	79.9

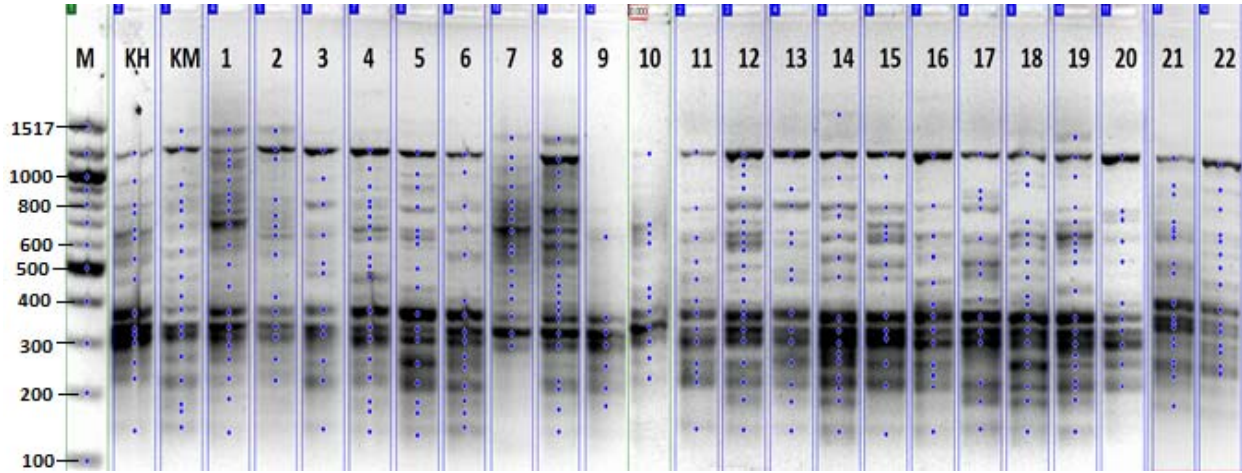


Figure 2. Amplification products of ISSR-895 primer after digestion with CRED-RA enzymes<sup>§</sup>.

Şekil 2. ISSR-895 primerinin CRED-RA enzimleri ile kesildikten sonra oluşan amplifikasyon ürünleri<sup>§</sup>.

<sup>§</sup> M: Marker, KH: Control sample cut with the *HpaII* enzyme, KM: the control sample cut with the *MspI* enzyme, 1: 4,4 mM V (H), 2: 4,4 mM V (M), 3: 6,6 mM V (H), 4: 6,6 mM V (M), 5: 8,8 mM V (H), 6: 8,8 mM V (M), 7: 4 mM BA (H), 8: 4 mM BA (M), 9: 8 mM BA (H), 10: 8 mM BA (M), 11: 4,4 mM V +4 mM BA (H), 12: 4,4 mM V +4 mM BA (M), 13: 4,4 mM V +8 mM BA (H), 14: 4,4 mM V +8 mM BA (M), 15: 6,6 mM V +4 mM BA (H), 16: 6,6 mM V +4 mM BA (M), 17: 6,6 mM V +8 mM BA (H), 18: 6,6 mM V +8 mM BA (M), 19: 8,8 mM V +4 mM BA (H), 20: 8,8 mM V +4 mM BA (M), 21: 8,8 mM V +8 mM BA (H) 22: 8,8 mM V +8 mM BA (M)

Table 2. % Polymorphism according to CRED-RA results.

Çizelge 2. CRED-RA sonuçlarına göre % polimorfizm.

Primers/ Primerler	Boric acid/Borik asit (mM)	0 mM V		4.4 mM V		6.6 mM V		8.8 mM V	
		H <sup>§</sup>	M <sup>§</sup>	H	M	H	M	H	M
ISSR-900	0	-	-	21	27.7	15.7	33.3	0	44.4
	4 mM	21	11.1	5.2	11.1	15.7	16.6	26.3	11.1
	8 mM	15.7	11.1	15.7	16.6	26.3	16.6	47.3	16.6
ISSR-895	0	-	-	33.3	42.1	20	31.5	20	36.8
	4 mM	46.6	15.7	13.3	21	6.6	26.3	13.3	21
	8 mM	33.3	10.5	13.3	15.7	13.3	15.7	20	21.0
ISSR-876	0	-	-	29.4	23.8	5.88	28.5	35.2	42.8
	4 mM	64.7	28.5	11.7	38	41.1	33.3	17.6	19
	8 mM	47	14.2	0	19.0	23.5	19	11.7	19
ISSR-868	0	-	-	18.1	33.3	27.2	44.4	9.0	55.5
	4 mM	54.5	22.2	18.1	22.2	18.1	11.1	54.5	22.2
	8 mM	63.6	11.1	18.1	0	36.3	11.1	54.5	0
ISSR-808	0	-	-	80	50	40	60	20	40
	4 mM	60	10	80	20	140	20	120	20
	8 mM	80	10	140	20	120	20	140	20
ISSR-811	0	-	-	66.6	50	0	50	50	40
	4 mM	83.3	50	16.6	20	0	40	33.3	10
	8 mM	66.6	20	0	30	33.3	50	66.6	10
ISSR-873	0	-	-	18.1	44.4	9	44.4	9.0	44.4
	4 mM	36.3	22.2	9	22.2	18.1	9	0	22.2
	8 mM	54.5	11.1	9	11.1	18.1	0	0	11.1
ISSR-880	0	-	-	0	50	20	50	40	50
	4 mM	0	0	20	25	80	0	20	12.5
	8 mM	0	0	20	25	80	0	60	25
%Polymorphism/ %Polimorfizm	0	-	-	33.3	40.1	17.2	42.7	22.9	44.2
	4 mM	45.8	19.9	21.7	22.4	39.9	19.5	35.6	17.2
	8 mM	46.7	11	27	17.1	43.8	16.5	50	15.3

<sup>§</sup>H: *HpaII*, M: *MspI*

Table 3. Polymorphic band numbers in vanadium and boric acid application according to CRED-RA results.  
Çizelge 3. CRED-RA sonuçlarına göre vanadyum ve borik asit uygulamasında polimorfik bant sayıları.

Primers Primerler	Total number of polymorphic bands/ Toplam polimorfik bant sayısı												Polymorphism%/ Polimorfizm											
	Number of control bands Kontrol bantlarının sayısı						Total number of polymorphic bands/ Toplam polimorfik bant sayısı						8.8 mM V		6.6 mM V		4.4 mM V		8 mM BA		4 mM BA		8 mM BA	
	H	M	H <sup>§</sup>	M <sup>§</sup>	H	M	8.8 mM V	8.8 mM V	4 mM BA	4 mM BA	8 mM BA	8 mM BA	H	M	H	M	H	M	H	M	H	M	H	M
ISSR-900	19	18	4	5	3	6	0	8	4	2	3	2	21.0	27.7	15.7	33.3	0	44.4	21.0	11.1	15.7	11.1	15.7	11.1
ISSR-895	15	19	5	8	2	6	3	7	7	3	5	2	33.3	42.1	20.0	31.5	20.0	36.8	46.6	15.7	33.3	10.5	33.3	10.5
ISSR-873	11	9	2	4	1	4	1	4	4	2	6	1	18.1	44.4	9.0	44.4	9.0	44.4	36.3	22.2	54.5	11.1	54.5	11.1
ISSR-880	5	8	0	4	1	4	2	4	0	0	0	0	0	50.0	20.0	50.0	40.0	50.0	0	0	0	0	0	0
ISSR-868	11	9	2	3	3	4	1	5	6	2	7	1	18.1	33.3	27.2	44.4	9.0	55.5	54.5	22.2	63.6	11.1	63.6	11.1
ISSR-876	17	21	5	5	1	6	6	9	5	6	4	3	29.4	23.8	5.88	28.5	35.2	42.8	29.4	28.5	23.5	14.2	23.5	14.2
ISSR-811	6	10	4	5	0	5	3	4	5	5	4	2	66.6	50.0	0	50.0	50.0	40.0	83.3	50.0	66.6	20.0	66.6	20.0
ISSR-808	5	10	4	5	2	6	1	4	3	1	4	1	80.0	50.0	40.0	60.0	20.0	40.0	60.0	10.0	80.0	10.0	80.0	10.0

§H: Hpa II, M: Msp I







## DISCUSSION

The effect of different doses of vanadium heavy metal on DNA damage in the seeds of the wheat "Çetinel 2000" variety was investigated by the ISSR method and their effect on DNA methylation was analyzed with the CRED-RA technique. In ISSR analyses, polymorphism was observed in parallel with the increase in vanadium dose compared to the control group. Polymorphism was detected in the appearance of new bands, disappearance of bands or decreases/increases in band density. Studies based on ISSR-PCR are important to examine genomic DNA variation. DNA damage and mutagenic effects can be evaluated with the ISSR technique (Sorrentino *et al.*, 2017). It has been determined that the polymorphism caused by heavy metals in ISSR profiles caused a decrease in the GTS rates depending on the dose increase. The GTS percentages showed that V caused DNA damage. Vanadate inhibits the activity of protein-tyrosine phosphatases, causing genotoxicity. Since these enzymes gather meiotic spindle threads together at the control point during meiosis, inhibition of protein-tyrosine phosphatases leads to genotoxicity (Stankiewicz *et al.*, 1995). In addition, vanadium inhibits DNA and RNA synthesis through vanadium accumulation in nucleic acid regions by disrupting cellular metabolism (Evangelou, 2002). Turkez *et al.* (2012) found that boric acid effectively reduced the genotoxicity of cadmium, lead, arsenic and mercury, which act as clastogens and aneugenes. Clastogenicity and oxidative stress of DNA cause strand breaks. This last function can explain the protective effects of boric acid on metal-induced genotoxicity. Our research parallels these studies. According to ISSR results, as V concentration increased, changes in ISSR bands and increases in genomic instability indicated that V was genotoxic. Additionally, in our study, it was determined that as the ratio of three different doses of vanadium and boric acid increased, the preservation of genomic stability increased and polymorphism decreased. This has shown that boric acid has a healing effect against stress. The CRED-RA technique is a powerful technique used

to examine the methylation status in the genome. In various studies with different plant species, the CRED-RA technique has been used to explain methylation situations (Cai *et al.*, 1996). There is no study on the effect of vanadium on DNA methylation in plants. However, there are studies on the effects of other heavy metals and abiotic stress factors on DNA methylation. The effects of lead on DNA methylation in paddy and wheat plants were studied and hypermethylation was observed (Ge *et al.*, 2002). Parallel to this, nickel also caused hypermethylation depending on the dose increase (Ellen *et al.*, 2009). Yang *et al.* (2016) stated that DNA methylation increased in boron deficiency in *Coccinia grandis* roots. Taspınar *et al.* (2018) found that aluminum caused DNA hypermethylation in *Zea mays* seedlings by CRED-RA technique. In this report, we present our results showing that V exposure can trigger methylation in *T. aestivum*. According to the results of CRED-RA we obtained in our study, it was observed that vanadium application alone caused a change in DNA methylation in proportion to increasing doses, and boric acid application eliminated this effect of vanadium.

## CONCLUSION

DNA methylation polymorphisms in response to V were assessed using a straightforward technique, CRED-RA, in an agronomically invaluable plant species. In conclusion, it was observed that V had genotoxic effects in this study, and it was once again proven that the ISSR technique can be used as a test in genetic toxicology. The protective effect of boric acid on metal-induced genotoxicity is thought to be related to the antioxidant defense mechanism by reducing genotoxicity (Bakir and Agar, 2020). Boric acid applied to the plant under stress conditions may have a healing effect by inhibiting DNA methylation. Although the protective role of boric acid is thought to be effective, other mechanisms should be investigated. There is a need to investigate whether BA plays a role in DNA methylation. In addition, other epigenetic mechanisms beside DNA methylation should be studied.

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