Available online: June 02, 2020

Commun.Fac.Sci.Univ.Ank.Ser. C Bio.
Volume 29, Number 1, Pages 161-175 (2020)
ISSN 1303-6025 E-ISSN 2651-3749
https://dergipark.org.tr/en/pub/communc/issue/51836/663035

ANKARA UNIVERSITY FACULTY OF SCIENCES 1948 COMMUNICATIONS SERIES C

EFFECTS OF β-ESTRADIOL ON DNA METHYLATION CHANGES AND GENOMIC STABILITY IN *TRITICUM AESTIVUM* L. EXPOSED SALT

MURAT AYDIN, SEMRA YAĞCI, GÜLERAY AĞAR, AND
MAHMUT SINAN TAŞPINAR

ABSTRACT. Salt stress is considered one of the most important agricultural problems because of causing yield loss. Although it is well known that salinity damages to DNA and results in DNA methylation changes in plants, there is no report investigating the effect of mammalian hormones on plants under salinity stress. Therefore, the present study was aimed at investigating DNA damage levels (Genomic Template Stability) and DNA methylation changes in *Triticum aestivum* L. cv Kırik subjected to salinity stress and determine whether β-estradiol has any effect on these changes. RAPD (Randomly Amplified Polymorphic DNA) and CRED-RA (Coupled Restriction Enzyme Digestion-Random Amplification) were used to define the DNA damage levels and changes in the pattern of DNA methylation, respectively. The results showed that salinity stress led to an increase in RAPDs and CRED-RA profiles changes. Furthermore, salinity stress was a reduction in genomic template stability (GTS) and DNA methylation changes. The effects caused by salinity stress were decreased after treatment with different concentrations of β-estradiol. The results of this study have clearly shown that β-estradiol could be used effectively to protect wheat seedlings from the destructive effects of salinity stress in molecular levels.

1. INTRODUCTION

Abiotic stress causes economic losses because of reductions in productivity of agricultural crops. Salt stress is one of the major abiotic stresses for plants. The destructive effects of salinity on plants can be observed at the whole-plant level as the death of plants [1-3]. As a result of being exposed to salinity stress, plant growth

Received by the editors: December 22, 2019; Accepted: April 26, 2020.
Key word and phrases: Mammalian sex hormone, DNA damage, epigenetic change, wheat

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Communications Faculty of Sciences University of Ankara Series C: Biology

and development are negatively affected. The effects of salinity plant devastation are related to osmotic stress, stress of soil solution, ionic equilibrium, specific ion effects, an increased amount of toxic ions, oxidative stress, the occurrence of genetic damage and epigenetic modification or a combination of all these factors [2-4].

Salt stress causes biochemical and physiological changes at the cellular and molecular levels, such as an increase in the plant stress hormone abscisic acid (ABA) and reactive oxygen species (ROS) levels, damaging cells and tissues by disturbing cellular structures, DNA methylation (e.g. cytosine methylation) and histone modification (e.g., acetylation, methylation and phosphorylation) changes, also plays a crucial role in regulation gene expression in plant [5,6]. In addition, several studies in recent years have demonstrated that environmental stresses such as water, cold, drought, salt, osmotic insults alter gene expression by DNA methylation and histone modification. Some studies have reported that the level of global DNA methylation decreases as the salt concentration is increased [7-9]. By contrast, salt stress has resulted in cytosine hypermethylation in rape, *Arabidopsis thailana*, and pea plants. It has been reported that different concentrations of salinity caused DNA methylation changes in *Jatropha curcas* L. Cytosine methylation plays an integral role in regulating gene expression at both transcriptional and posttranscriptional levels [10]. Surprisingly, methylation in the transcribed regions of endogenous genes is unexpectedly constitutes a common adaptation mechanism against stress in plants [11].

Plant growth regulators may help to improve the methods to increase the resistance of plants to adverse environmental conditions [12]. It has been reported that plant hormones modulate plant responses to oxidative stress generated by salinity [13,14]. On the other hand, a few studies have demonstrated that exogenous mammalian sex hormones (MSHs) such as progesterone, β-estradiol and androsterone have positive effects on plant growth and development. Moreover, they stimulate the activities of oxidative enzymes and synthesis reactions, reduce hydrogen peroxide (H_2O_2) content and lipid peroxidation (MDA) levels by inducing the activities of antioxidant enzymes, increase protein and nucleic acid contents and affect the inorganic constituents of plants under non-stress conditions [15,16]. Erdal [15], first recorded that MSH treatment stimulated superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX) and nitrate reductase (NR) activities and decreased in the lipid peroxidation (MDA) level, superoxide (O_2^-) production and H_2O_2 content following salt treatment. The same researcher demonstrated that MSH treatment generated a preventive effect against salt stress which promote the amount of dry weight, sugar, proline, protein, chlorophyll and glutathione (GSH)

[2]. Furthermore, according to the results of other studies in chickpea seedlings, the sodium, potassium and calcium content were increased by MSH treatment while the chloride content was reduced. Those results are critical for defusing salt stress because MSHs prevented the change in the K/Na and Ca/Na ratios [17, 18].

Previous results have suggested that MSH treatment modulates negative effects by salt stress in plants. However, the effect of β-estradiol on genetic and DNA methylation changes against salt stress has not been elucidated. The main aim of the present study is to determine whether β-estradiol has any protective effect against the adverse effects of salt stress in wheat

2. Materials and Methods

2.1. Plant material and treatment conditions

T. aestivum L. cv. Kırik seed samples known to be sensitive to salinity were obtained from the Department of Field Crops, Faculty of Agriculture, Ataturk University (Turkey). After sterilization with 1% w/v of sodium hypochlorite for 10 minute and washing with sterilize dH2O, sterilized seeds were soaked in solutions containing 0 (dH_2O), 10^{-8}, 10^{-9}, and 10^{-10} M β-estradiol at 25±1ºC for 24 hours. 20 pretreated seeds placed in each petri dishes with two layers of Whatman number 1 filter paper. NaCl (Sodium chloride) solutions (0, 100 mM and 200 mM) were added to each petri dishes. Petri dishes were kept in 22±1°C under 16-h light/8-h dark light conditions for 14 days for seed germination. Each treatment was replicated three times. Each petri dish was evaluated as a repeat. Bulk sample strategy was applied for molecular analysis. Three seedling were randomly taken from each repetition and a total of nine plants were used for each treatment. Samples were stored at -80ºC for DNA extraction.

2.2. Genomic DNA isolation

Genomic DNAs (gDNAs) was extracted from seedlings using the method described by Arslan et al. (2019) [19] and stored at -20ºC for further use. The quality and concentration of the gDNAs were measured using a Nano-Drop (ND-1000) spectrophotometer and electrophoresis in a 0.8% (w/v) agarose gel.

2.3. RAPD technique

13 primers (OPA-13, OPY-11, OPY-13, OPY-7, OPH-19, OPY-1, OPY-8, OPY-15, OPB-8, OPW-4, OPW-7, OPB-10 and OPW-5) were used in RAPD-PCR reactions (Table 1). PCR amplifications were carried out in thermocycler (SensoQuest GmbH, Göttingen, Germany) in a total volume of 25 µl, containing 50 ng gDNA, 10× PCR buffer (10 mM Tris–HCl, 50 mM KCl, pH=8.3), 400 µM dNTP, 10 pmol primer, 2.5 mM $MgCl_2$ and 1 U Taq DNA polymerase. The amplification profile composed of an initial denaturation at 95°C for 5 min, followed by 38 cycles at 94°C for 1 min, 36°C for 1 min 72°C for 2 min and a final extension of 15 min at 72°C.

2.4. CRED-RA technique

Genomic DNA sample from each treatment were separately digested with HpaII and MspI endonucleases. After checking digestion on agarose gel, 1µl of each digestion product were amplified with 8 random primers (OPY-11, OPY-7, OPY-13, OPH-9, OPW-6, OPB-8, OPW-4 and OPW-5). Amplification and visualization conditions for CRED-RA are the same as described for RAPD analysis.

2.5. Electrophoresis

The PCR products (7 µl) were mixed with 6x gel loading buffer (3 µl) and subjected to agarose (1.5% w/v) gel electrophoresis in 0.5x TBE (Tris-Borate- EDTA) buffer at 80 V for 120 min. Amplification products separated by gel electrophoresis were stained in ethidium bromide solution (2 µl Etbr/100ml of 1x TBE buffer) for 40 min. The amplified DNA products were detected using the Bio Doc Image Analysis System and analyzed using the UVI-soft analysis package (Cambridge Electronic Design Ltd, Cambridge, UK).

2.6. Molecular Patterns Analysis

RAPD patterns were evaluated using the Total Lab TL120 computer software. Genomic template stability (GTS, %) was calculated as follows: GTS = 100-(100x a/n), a in formula is the average number of polymorphic bands detected in each treated sample, and n is the number of total bands in the control. Polymorphisms in RAPD profiles included disappearance of a normal band and appearance of a new band compared with the control. The average was calculated for each experimental

group. To compare the sensitivity of each parameter, changes in these values were calculated as a percentage of their control (set to 100%) [19]. The average number of polymorphisms (%) was calculated for each dose to realize CRED–RA analysis. To calculate the number of polymorphisms (%), the following formula was used 100x a/n.

3. Results

In total, thirty-two oligonucleotide primers with 60-70% GC content were used for analyzing the PCR products of the *T. aestivum* L. cv Kırik genome, and only thirteen provided specific and stable results (Table 1). Compared with the PCR products obtained from the control DNA, β-estradiol and/or NaCl treatments resulted in apparent changes in RAPD patterns. These changes are characterized by variation in band intensity, loss of normal bands or appearance of new bands (Table 1). GTS was used for comparing the changes in RAPD profiles. GTS values decrease with increasing concentration of NaCl. This value was determined in 45.1% and 37.8% for 100 mM and 200 mM NaCl, respectively. Moreover, the application of β-estradiol did not cause any change in GTS. When the effect of β-estradiol and NaCl were considered together, it was determined that β-estradiol increased in GTS ratio in both NaCl concentrations. This increasing occurred in parallel with the β-estradiol dose increase. When the lowest dose of NaCl and highest dose of β-estradiol (H3+S1) were applied together, the GTS value was the highest (56.3%), and when the lowest dose of β-estradiol and highest dose of NaCl (H1+S2) were applied together, the GTS value was the lowest (46.7%) (Table 1).

CRED-RA analysis results were given in Table 2. Eight oligonucleotide primers which gave specific and stable results in RAPD analysis were used for CRED-RA analysis. Compared with the PCR products obtained from the control DNA, β-estradiol and/or NaCl treatments resulted in apparent changes in CRED-RA patterns (Figure 1). DNA methylation occurred at all doses of the combined treatments. Methylation value was 74.1% and 51.4% for 100 and 200 Mm NaCl applications, respectively. The highest methylation value was 42.2%, and the lowest was 27.9% in β-estradiol applications. Regarding the combined applications with the lowest dose of NaCl and highest dose of β-estradiol (H3+S1), the methylation value was the lowest (14.3%) with the lowest dose of β-estradiol and highest dose of NaCl (H1+S2) methylation value was the highest (38.7%).

Table 1. Molecular sizes (bp) of appeared (+)/disappeared bands (-) in RAPD profiles based on other treatments vs. control[1].

Primer	C	+/-	S1	S2	H1+S1	H2+S1	H3+S1	H1+S2	H2+S2	H3+S2
OPA_13	13	-	1438, 1284, 1184, 414, 322	1438, 1284, 1184, 934, 745,495, 414,322	1438, 1284, 1023, 745, 414,322	1438, 1284, 1023, 745, 414, 322	1438, 1284, 934,745, 414,322	1438, 1284, 1023, 745, 495,322	1184, 745, 495,322	1438, 1284, 1023, 745, 414,322
		+	1084,934	1084					849	
OPY_11	11	-	1387,715, 584	1687, 1387, 1062, 843, 438,352, 200	1687, 1062, 843,352	1062	1687, 1387, 1062	1387, 1062, 843,352, 200	1387, 1062, 438,352, 200	1687, 1387, 438,352, 200
		+				1237			922	
OPY_13	6	-	1121,800, 609,481	1121, 800, 481	928, 481	928, 481	800, 609	1121, 800	1121, 800, 481	1121, 800, 481
		+	692,322	322	652,288	556, 280	692	156	343	329,160
OPY_7	10	-	1300,1118, 388	1300, 1050, 689,388	1300,1 231, 1118, 1050, 541	1300, 1231, 970,541	1300, 1231, 970,541	1300, 1231, 1118, 1050, 754,388	1300, 1231, 388	1300, 1231, 388
		+	600	856				813		
OPH_19	10	-	693,	1220, 620, 491	1220, 693,	1220, 1073, 693	1220, 693,	1220, 1073, 947,776, 543	1220, 543, 387	1220, 620
		+	870		308		849,308			
OPY_1	9	-	671	1476, 952, 671	1476, 1269, 1138, 1076, 831	1476, 1269, 831	1476	1476, 831	1476, 1269, 952,459	1476, 1269, 1138, 831, 671,459
		+		568	533,353			788	1030	600
OPY_8	8	-	1353, 565	1353, 1233, 565	1353, 1233, 565	1353, 866	866, 565	1353, 565	1353, 565	1353, 565
		+	950,630	1166	805,484	1086,61	1093,48, 700		978, 653	
OPY_15	9	-	632,	764,632	764	1183, 764, 680	680	1183, 680, 632	1183, 764, 680,632	1183 680
		+	984,544	585,509	832534, 494,984	1458, 972, 867,529, 303	984,927, 504	849,566 240	914, 800, 240,572	800,372, 256

[1]**: C (Control):** 0 M β-estradiol + 0 mM NaCl, **S1:** 100 mM NaCl, **S2:** 200 mM NaCl, **H1:** 10^{-10} M β-estradiol, **H2:** 10^{-9} M β-estradiol, **H3:** 10^{-8} M β-estradiol, **H1+S1:** 10^{-10} M β-estradiol + 100 mM NaCl, **H2+S1:** 10^{-9} M β-estradiol + 100 mM NaCl, **H3+S1:** 10^{-8} M β-estradiol + 100 mM NaCl, **H1+S2:** 10^{-10} M β-estradiol + 200 mM NaCl, **H2+S2:** 10^{-9} M β-estradiol + 200 mM NaCl, **H3+S2:** 10^{-8} M β-estradiol + 200 mM NaCl

TABLE 1. (Continued).

Primer	C	+/-	S1	S2	H1+S1	H2+S1	H3+S1	H1+S2	H2+S2	H3+S2
OPB_8	11	-	1493,1313 1246,776	1493,1426, 1386,1313	1493,1426	1493,1426, 1386	1493,1426, 1386,1313,	1493,1426, 1386,1313, 1246,1153, 1053,984	1493,1426, 1386,1313, 1246	1493,1426, 1386,1313, 1246,1153, 1053,984
		+	861, 644, 541	876,845, 640,577, 510,443, 386	876,657, 615567, 472	876,657, 667,552, 447	858, 625, 577,472	867,833, 663,572, 500,424, 379,337	900,849, 707,611, 552,485, 421,389, 327	867,833, 663,572, 500,424, 379,337
OPW_4	12	-	1491, 1275, 1125, 886, 780, 674, 224	1491, 1275, 1125, 886, 674, 224	1275,575	1275,780	1491,1275, 780,481	1491,1275, 1125,886, 363	1491,1275, 1125,886	1491,1275, 1125,886
		+					982			
OPW_7	9	-	1376,1123, 983,762	1376,1123, 762	1123,983, 912,762	983,912, 762,	1376,983,	1376,1123, 983,762	1376,1195, 983,912, 762	1376,1195, 983,912,
		+	716	716,600, 574, 489, 400	834,708	826692, 581,489	658,356	617	850,634	640
OPB_10	11	-	1081,973, 800, 644, 516 300, 230	1081,973, 800,644, 516,300	644	644	800, 644	1081,973, 800,644 516,230	1081,973, 800,644, 516	1081,973, 800, 644, 516
		+	1300, 738, 590,178	1300,1172 738,590	1618,1445, 717	1618,1500, 1236,731	738	738,178	738,	909,724
OPW_5	10	-	1283,1058, 853,729, 600, 396, 296,	1283,1058, 853,729	1283,853, 396,296	729,296	1283,600, 396,296	1283,853	1283,1108, 853	1283,729
		+	917, 900, 574	917,574	992,523	1475,1183	671,567		548	
GTS%	**100**		**45.1**	**37.8**	**48.6**	**51.9**	**56.3**	**46.7**	**47.2**	**49.5**

TABLE 2. CRED-RA pattern analysis results[1].

Primer	Total bands				Total polymorphic bands						Polymorphism (%)			
	Control		HpaII		MspI		Hpa II		Msp I		Hpa II		Msp I	
	HpaII	MspI	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1
OPY-11	5	5	5	5	5	4	4	3	4	3	80	60	80	75
OPY-7	10	8	6	10	5	9	4	5	3	5	66.6	50	60	55.5
OPY-13	5	8	5	5	5	5	2	3	3	3	40	60	60	60
OPH-19	2	5	4	3	7	5	2	1	3	2	50	33.3	42.8	40
OPW-6	5	5	3	3	3	3	3	2	3	1	100	66.6	100	33.3
OPB-8	3	5	3	7	4	8	4	4	2	3	100	57.1	50	37.5
OPW-4	4	4	2	4	2	4	2	2	2	2	100	50	100	50
OPW-5	9	8	4	6	5	5	5	3	5	3	100	50	100	60
Total	**43**	**48**	**34**	**43**	**36**	**43**	**26**	**23**	**25**	**22**	-	-	-	-
Average	-	-	-	-	-	-	-	-	-	-	**79.5**	**53.3**	**74.1**	**51.4**

[1]: Abbreviations were listed in Table 1.

TABLE 2. (Continued).

Primer	Total bands								Total polymorphic bands						Polymorphism (%)					
	Control		HpaII			MspI			Hpa II			Msp I			Hpa II			Msp I		
	HpaII	MspI	H1	H2	H3	H1	H2	H3	H1	H2	H3	H1	H2	H3	H1	H2	H3	H1	H2	H3
OPY-11	5	5	5	4	5	7	3	5	2	1	0	2	0	0	40	20	0	28.5	0	0
OPY-7	10	8	9	9	11	11	7	10	3	2	1	3	4	2	33.3	22.2	9	27.2	57.1	20
OPY-13	5	8	4	4	9	1	6	7	2	3	4	7	2	1	50	75	44.4	100	33.3	14.2
OPH-19	2	5	2	3	3	4	4	5	2	1	1	1	1	0	100	33.3	33.3	25	25	0
OPW-6	5	5	5	4	4	5	5	4	0	1	1	0	2	1	0	25	25	0	40	25
OPB-8	3	5	6	7	5	2	7	8	3	4	2	5	2	3	50	57.1	40	100	28.5	37.5
OPW-4	4	4	5	3	4	5	3	2	1	1	2	1	1	2	20	33.3	50	20	33.3	100
OPW-5	9	8	10	10	6	8	9	11	3	2	3	3	5	3	30	20	50	37.5	55.5	27.2
Total	**43**	**48**	**46**	**44**	**47**	**43**	**44**	**52**	**16**	**15**	**14**	**22**	**17**	**12**	-	-	-	-	-	-
Average	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**40.4**	**35.7**	**31.4**	**42.2**	**34**	**27.9**

Primer	Total bands								Total polymorphic bands						Polymorphism (%)					
	Control		HpaII			MspI			Hpa II			Msp I			Hpa II			Msp I		
	HpaII	MspI	H1S1	H2S1	H3S1	H1S1	H2S1	H3S1	H1S1	H2S1	H3S1	H1S1	H2S1	H3S1	H1S1	H2S1	H3S1	H1S1	H2S1	H3S1
OPY-11	5	5	5	6	7	6	4	7	0	2	2	1	1	2	0	33.3	28.5	16.6	25	28.5
OPY-7	10	8	6	10	7	6	6	9	4	2	5	2	2	1	66.6	20	71.4	33.3	33.3	11.1
OPY-13	5	8	5	5	6	6	6	8	2	3	1	2	2	0	40	60	16.6	33.3	33.3	0
OPH-19	2	5	2	4	2	3	4	4	0	2	0	2	1	1	0	50	0	66.6	25	25
OPW-6	5	5	5	5	5	5	5	5	1	0	0	2	0	0	20	0	0	40	0	0
OPB-8	3	5	4	2	3	4	5	4	2	1	2	1	0	1	50	50	66.6	25	0	25
OPW-4	4	4	3	3	5	3	3	4	1	1	1	1	1	0	33.3	33.3	20	33.3	33.3	0
OPW-5	9	8	13	10	12	12	8	12	2	2	3	5	2	3	15.3	20	25	41.6	25	25
Total	**43**	**48**	**43**	**45**	**47**	**45**	**41**	**53**	**12**	**13**	**14**	**16**	**9**	**8**	-	-	-	-	-	-
Average	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**28.1**	**33.3**	**28.5**	**36.2**	**21.8**	**14.3**

Primer	Total bands								Total polymorphic bands						Polymorphism (%)					
	Control		HpaII			MspI			Hpa II			Msp I			Hpa II			Msp I		
	HpaII	MspI	H1S2	H2S2	H3S2	H1S2	H2S2	H3S2	H1S2	H2S2	H3S2	H1S2	H2S2	H3S2	H1S2	H2S2	H3S2	H1S2	H2S2	H3S2
OPY-11	5	5	3	5	7	3	6	7	2	0	0	2	1	1	66.6	0	0	66.6	16.6	14.2
OPY-7	10	8	5	13	7	7	10	8	4	4	0	3	2	1	80	30.7	0	42.8	20	14.2
OPY-13	5	8	7	8	5	4	8	5	0	3	2	0	0	1	0	37.5	40	0	0	20
OPH-19	2	5	5	5	5	6	5	6	0	3	3	1	0	2	0	60	60	16.6	0	33.3
OPW-6	5	5	4	5	4	5	5	6	2	0	2	2	0	1	50	0	50	40	0	16.6
OPB-8	3	5	7	2	4	5	8	3	2	2	2	2	3	1	28.5	100	50	40	37.5	33.3
OPW-4	4	4	3	3	3	3	3	3	2	1	0	2	1	0	66.6	33.3	0	66.6	33.3	0
OPW-5	9	8	7	9	7	8	8	6	2	3	0	3	6	1	28.5	33.3	0	37.5	75	16.6
Total	**43**	**48**	**41**	**50**	**42**	**41**	**53**	**44**	**14**	**16**	**9**	**15**	**13**	**8**	-	-	-	-	-	-
Average	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**40**	**36.8**	**25**	**38.7**	**22.8**	**18.5**

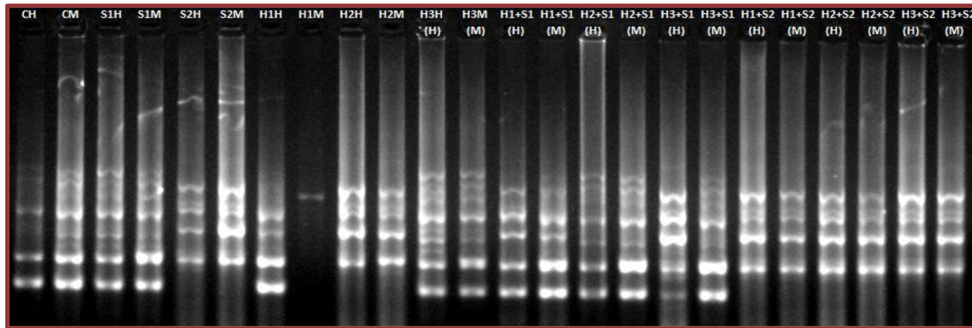

FIGURE 1. CRED-RA profiles based on treatments for primer OPY-13

4. DISCUSSION

Different abiotic stresses affect plant growth and productivity and cause economic losses. Salinity stress is one the most abiotic constraints for plant that also causes physiological drought indirectly. Recently, many studies showed that salt stress negatively affects plant growth and development, causing biochemical and physiological damages such as changes in dry weight, the levels of lipid peroxidation (MDA), ROS and chlorophyll and also the amount of proline, protein, sugar and enzyme activities in higher plants [2]. Several researchers reported that salt stress decreased the SOD, POX, CAT, APX, GSH and NR activities whereas it increased the O_2^-, H_2O_2 and MDA levels [2, 20].

ROS are permanently produced in cells even under optimal conditions in plants. Under non-stress conditions, plants maintain the balance between ROS production and activity of antioxidants. However, salt stress, like other abiotic stresses can upset the balance by increasing ROS production which caused to damage macro molecules such as DNA, protein and lipids. Previous studies have demonstrated that abiotic stresses such as drought, salt, water deficit and heavy metals cause DNA damage using different molecular techniques [21, 22]. In our study, we used RAPD to prove that salt stress caused to DNA damage in the wheat seedlings, a finding that was sign of GTS reduction. The differences in the DNA profiles observed in the present study were clearly dependent on extensive DNA damage (e.g. single and strand

breaks, modified bases, oxidized bases, DNA-protein cross-links, point mutations, complex chromosomal rearrangements, mutations in some oligonucleotide priming sites, large deletions and homologous recombination) induced by salt stress. The molecular mechanism responsible for the genotoxicity of salt stress suggested that salt stress could stimulate the release of free radicals and ROS such as O_2^-, hydroxyl radical (HO·), and H_2O_2 species [23-28]**.** Much of reactive oxygen does not appear to interact with DNA but they are precursors for hydroxyl radicals. The reaction of HO· with DNA generates a multitude of products because it attacks sugar, pyrimidines and purines, including guanine residues to form 8-hydroxydeoxyguanosine. In this instance, plants must develop a set of bio-defences to cope with these sources of damage by differential expression of several hundred genes and protein function in response to the different stresses. One of the molecular mechanisms by which plants could silence or super-activate the selected DNA templates is epigenetic modifications that change gene expression without changing DNA sequences [12,29]. Recently, several studies have demonstrated that salt stress alters gene expression through DNA methylation and histone modification [30-32]. Zhong et al. [33] reported that salt stress caused DNA methylation in *T. aestivum*. Similarly, Zhao et al. [34] suggested that demethylation positively contributed to salt tolerance and hypermethylation had a negative effect on salt tolerance in cotton. Lu et al. [21] suggested that both de novo methylation and demethylation events can help to plant adaptation under salt stress. The present study showed that different levels of salinity treatment caused DNA methylation changes in the whole genome. These changes can contribute to improve tolerance in plants under salt stress as well as its role in the control of plant development [33].

Exogenous applications in different stress conditions may help to increase tolerance in plants. Recently, some studies have emphasized that treatment with MSH may help increase to plant tolerance [2,17,18,35-37]. The studies have demonstrated that exogenous application of MSH (such as progesterone, β-estradiol and androsterone) substantially improved plant growth and development, augmented protein and nucleic acid contents, stimulated oxidative enzyme activities, and reduced H_2O_2 content and the MDA level under non-stress conditions [17].

Erdal and Dumlupinar [36] showed that MSH also affected the inorganic constituents of plants. The same researchers demonstrated that MSH treatment significantly decreased the Na content in chickpea seeds and barley leaves. In addition, other studies have shown that although MSH treatment increased the Na content in chickpea seedlings MSH treatment also increased K and Ca contents and decreased the Cl content [36,39]. Moreover, Erdal et al. [37] reported that MSH treatment together with salt stress increased the dry weight, sugar, proline, protein, chlorophyll, and GSH contents, as well as SOD, POX, CAT, APX and NR activities and reduced the MDA level, O_2^- production and H_2O_2 content compared with salinity alone.

Both earlier studies and our results suggest the protective role of MSH against stress related to osmo-protection, osmotic adjustment, carbon storage, radical scavenging and high antioxidant activities. According to the present findings, it is possible that the antigenotoxic effect of β-estradiol on salt stress might be related to its radical scavenging and high antioxidant activities. In the literature, no report is available the role of β-estradiol in salinity exposed wheat seedlings with regard to DNA methylation changes and genomic stability. To our knowledge, the present study represents the first report indicating the effects on DNA methylation changes of β-estradiol under non stress and salt stress conditions in wheat.

The contribution effect of β-estradiol against DNA methylation changes may be related to its effect on the transcription and translation processes of specific genes, improving the plant resistance under stress conditions. Many researchers have reported that the soluble protein content increases under salinity stress [2,39,40]. Therefore, β-estradiol could have adaptive significance for plants grown under salt stress. Based on the above findings, it is concluded that β-estradiol application to may be useful for large-scale agricultural benefit, and we intend to carry out such an investigation in the near future.

Acknowledgment. The authors thank the contributes of Atatürk University.

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Current Address: MURAT AYDIN, Ataturk University, Faculty of Agriculture, Department of Agricultural Biotechnology, Erzurum 25240, Turkey
E-mail: maydin@atauni.edu.tr
ORCID: https://orcid.org/0000-0003-1091-0609

Current Address: SEMRA YAĞCI, (Corresponding author): Atatürk University, Faculty of Science, Department of Biology, Erzurum 25240, Turkey
E-mail: semrayagci_89@outlook.com
ORCID: https://orcid.org/0000-0003-2939-5264

Current Address: GÜLERAY AĞAR, Atatürk University, Faculty of Science, Department of Biology, Erzurum 25240, Turkey
E-mail: gagar@atauni.edu.tr
ORCID: https://orcid.org/0000-0002-8445-5082

Current Address: MAHMUT SİNAN TAŞPINAR, Ataturk University, Faculty of Agriculture, Department of Agricultural Biotechnology, Erzurum 25240, Turkey
E-mail: taspinar@atauni.edu.tr
ORCID: https://orcid.org/0000-0001-6399-2703