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Effects of Selenium on DNA Methylation and Genomic Instability Induced by Drought Stress in Wheat (*Triticum aestivum* L.)

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Abstract: The main purpose of the study was to clarify the effect of selenium (Se) on DNA damage and DNA methylation in wheat (*Triticum aestivum* L.) plants exposed to polyethylene glycol (PEG)-induced drought stress under in vitro tissue culture. Random amplified polymorphic DNA (RAPD) and coupled restriction enzyme digestion-random amplification (CRED-RA) were utilized to explain the DNA damage grade and variations in DNA methylation patterns, respectively. The outcomes indicate that drought stress gives rise to a rise in RAPD profile variations (as DNA damage) and a decrease in genomic template stability (GTS) rate and DNA methylation changes. According to the RAPD data, the greatest GTS value was computed at 56.9% (5% PEG 6000), and the lowest GTS value was 41.2% (15% PEG 6000), demonstrating the adverse effects of PEG 6000. However, DNA damage can be reduced by treatment with sodium selenate (2, 4, and 6 µM of Na_2SeO_4) together with PEG (5%, 10%, and 15% PEG 6000)-induced water deficits. Moreover, according to CRED-RA analysis, PEG-induced DNA methylation rates were changed after treating different doses of Se. These data demonstrate that Se dose-dependently modulates both DNA damage and methylation alterations induced by drought in wheat.

Keywords: DNA damage**,** DNA methylation, drought stress, polyethylene glycol, selenium

1. Introduction

Cultivated crops are generally exposed to a term of atmospheric and soil water deficit during their sessile life cycle (de Oliveira et al., 2013). Drought is well recognized as a highly detrimental environmental stress due to its significant impact on crop productivity, resulting in annual losses that can amount to billions of dollars globally (Andrade et al., 2018). The global population is experiencing rapid growth and is projected to reach approximately 10 billion by 2050. This necessitates a 50% increase in food production compared to the rates observed in 2015 (Raza et al., 2024). Wheat (*Triticum aestivum* L.) is an important cereal crop that provides nutrition for about one-fifth of the global population (Gupta et al., 2024). Estimates indicate that developing countries will boost wheat production by 30% and the globe will require 70 million tons by 2050 to meet future demands (Sharma et al., 2015). While climate change threatens food security due to increased drought caused by high temperatures, incorporating drought-tolerant cereal crops into the planting model will help solve this problem (Yanagi, 2024).

Drought also is widely recognized for its ability to generate physiological, biochemical, and molecular alterations, hence causing negative effects on different parameters associated with plant growth and development (Cho et al., 2009; Si et al., 2009). Drought decreases cell elongation and expansion, which impacts root growth, therefore lowering nutrient absorption, resulting in growth retardation, reduced leaf water potential and net photosynthesis, and spikelet sterility (Begna, 2020). Drought also increases reactive oxygen species (ROS) and lipid and protein oxidation, which disrupts redox homeostasis and ion balance and alters the manufacture of osmotic regulators such as

proline, betaine, sorbitol, and mannitol (Geng et al., 2024). When a plant makes more ROS than its natural antioxidant defenses can handle, biochemical changes called oxidative stress happen. This could damage macromolecules like nucleic acid, cellular proteins, membrane lipids (lipid peroxidation), and other cellular parts (Bhat et al., 2015). Growth regulators vitamin E (i.e. a-tocopherol), methyl jasmonates, ascorbic acid, and triazole response to oxidative stress elicited (Liu et al., 2011; Fahad et al., 2016). Plants must undergo constant changes at the molecular level to adapt to various environments, and the development of regulatory mechanisms for stress tolerance is an extremely important endeavor. Epigenetic regulation, in the absence of alterations in DNA sequences, is believed to exert a substantial influence on the adaptive replies of plants to stressors (Sahu et al., 2013). These epigenetic mechanisms controls entail several chemical changes that affect gene expression and chromatin structure across the genome (Zhao et al., 2007).

Plants employ various epigenetic mechanisms, such as cytosine (DNA) methylation, histone modification patterns, and variants, as well as RNA-mediated alterations, to adapt and thrive in unfavorable environmental conditions (Pikaard and Scheid, 2014; Ashapkin et al., 2020). DNA methylation is a well-known modification for elucidating epigenetic changes in gene transcription and genome stability (Zhang et al., 2019a). DNA methylation is also tissue-specific. Typically, hypermethylation is linked with gene repression whereas hypomethylation is associated with gene transcription (Lu et al., 2007). In recent studies, it has been detected that abiotic stressors, including water scarcity, heavy metal exposure, drought, cold, high salinity, and osmotic change, can modulate gene transcription through epigenetic processes, including histone modifications and DNA methylation (Tan, 2010; Grativol et al., 2012; Kumar et al., 2012). Drought stress conditions usually tend to increase demethylation (interior and exterior cytosine methylation) (Sallam et al., 2019). Also, the degree of DNA methylation declined by way of salt concentration increases in cotton (Li et al., 2009). On the contrary, cytosine methylation increased in rapeseed plants exposed to salt stress, as well as rice and wheat plants exposed to heavy metals, cadmium (Cd) and lead (Pb) (Lu et al., 2007; Ge et al., 2012). Besides, epigenetic pathways, various variables inherent to the plant have been concerned with modulating the crucial responses to drought stress (Tan, 2010). These include vitamins, trace metals, selenium (Se), humic acid, phenolic acids, silicon, fatty acids, polyamines, and other compounds. It is expected that these substances will also counteract the adverse biological impacts of drought stress. Although Se has long been thought to be poisonous and unnecessary for plant life, Schwarz and Foltz (1957) found that small concentrations of selenium had several positive benefits and may be used instead of alfa-tocopherol. Some research has indicated that treatment of Se can encourage plant growth (Nawaz et al., 2016), alleviate oxidative stress caused by solar ultraviolet radiation (Pennanen et al., 2002), improve the rescue of chlorophyll from cold stress (Chu et al., 2010), raise the antioxidative activity of plants and decrease both the production of lipid peroxidation and the ROS ratio in the leaf tissue (Reis et al., 2015), rise photosynthesis, normalize the water content of plants in water lack (Proietti et al., 2013) and minimize the impacts of potentially toxic elements (Kumar et al., 2012). The appropriate exogenous Se dose has been shown to increase salt resistance, antioxidant, and osmoregulation ability (Kong et al., 2005; Yao et al., 2009). Subsequent studies evaluating the effects of drought-induced stress have corroborated the efficacy of this protection (Yao et al., 2009, 2012). Furthermore, the influence of Se on the degree of DNA methylation has been seen in human, animal, and plant species (Bocchini et al., 2018; Zhang et al., 2019b; Genchi et al., 2023; Jiang et al., 2023).

It can be difficult to analyze the responses of plants grown in the field or greenhouse conditions to the many different abiotic stressors because of the complex and dynamic character of these stresses (Pérez-Clemente and Gómez-Cadenas, 2012). In vitro tissue culture methods are in the process of developing novel cells, tissues, plants, and secondary metabolites to examine the potential impacts of stress by utilizing selective agents such as polyethylene glycol (PEG) or mannitol for drought stress simulation (Rao and Ftz, 2013). Polyethylene glycol, a chemical with a high molecular weight, is utilized as a stress agent for drought due to being non-penetrating and non-ionic inert osmotic properties that lower the water potential of nutritional solutions without being taken up or phytotoxic (Sahu et al., 2023). The use of PEG to produce water restriction (drought stress) is the most popular screening method for testing drought tolerance of crops at seed germination and early growth period (Awan et al., 2021). There has been little study to understand the effect of Se against drought-induced genetic and epigenetic changes in higher plants. The purpose of this study was to reveal whether Se affects DNA damage and DNA methylation in wheat (*T. aestivum* L.) under in vitro drought stress induced by PEG 6000.

2. Materials and Methods

2.1. Plant material

Mature embryos were obtained from the seeds of *T. aestivum* L. cv Kırık, which was previously determined to be sensitive to drought (Öztürk et al., 2014). The selected seeds were rinsed in 70% ethanol for 5 min, then rinsed out with sterile distilled water. Pre-sterilized mature seeds were then subjected to sodium hypochlorite (NaOCl, 5%) with drops of TWEEN-20 for 10 min. The mature seeds were then carefully cleaned in sterile distilled water and put in an incubator at 4 °C for one day. Explants of mature embryos were dissected under aseptic conditions.

2.2. Culture media and conditions

Isolated embryos were cultured on Murashige and Skoog (MS) (Murashige and Skoog, 1962) medium containing different amounts of PEG 6000 (0, 5, 10, and 15%) and sodium selenate (Na_2SeO_4) (0, 2, 4, and 6 M). Sucrose (20 g L^{-1}) was used as a carbohydrate source. The MS medium was arranged to pH 5.8 and solidified with 0.2% phytagel before sterilization by autoclaving for 20 min at 121 °C. These cultures were grown for 14 days under a 16 h photoperiod (62 µmol $m^{-2}s$) at 22±1 °C.

2.3. Isolation of genomic DNA (gDNA)

The molecular analysis employed an approach including the use of a bulk sample. After 14 days, plant tissue samples were collected from five randomly chosen plants for each application and kept at -80 °C. Total gDNA was isolated using the minor changes protocol of the hexadecylcetyltrimethylammonium bromide (CTAB) method. DNA concentration, purification, and quality were carried out following a previous study (Erturk et al., 2014).

2.4. Random amplified polymorphic DNA (RAPD) analysis

Forty 10-mer RAPD primers were analyzed with gDNA of no treatment (0% PEG 6000 + 0 µM Na_2SeO_4). Only fourteen different primers amplified polymorphic amplicons and were chosen. In a 20 µl volume, the following polymerase chain reaction (PCR) components were added: 25 ng template gDNA, 1 U Taq DNA polymerase, 10 pmol primers (10 bp primers), 2.5 mM magnesium chloride ($MgCl_2$), 400 µM deoxynucleoside triphosphates (dNTP), and 1X PCR buffer (10X).

The process of DNA amplification was conducted within a thermocycler using the following conditions: primary denaturation (first heated) at 95 ºC for 5 min; 42 cycles of (94 °C for 1 min, 36 °C for 1 min, and 72 °C for 2 min), a final extension step was performed at 72 °C for 15 min. PCR amplification products were electrophoresed directly on agarose gel (1%) containing 1X Tris-Borate-Ethylenediamine tetraacetic acid (TBE) buffer and ethidium bromide (EtBr) and imaged with ultraviolet (UV) light. Moreover, the sizes of the PCR product fragments were identified using a 1 kb DNA ladder.

2.5. Coupled restriction enzyme digestion-random amplification (CRED-RA) analysis

The gDNA samples obtained from each treatment were subjected to digestion using HpaII and MspI restriction endonuclease enzymes. These have diverse cut capabilities based on the statute of C in the methylation pattern. Both enzymes cut the 5 CG-C / CGG-3´ sequence, while HpaII is active when both C is unmethylated, and MspI is not active when external C is methylated. After checking restricted DNA on an agarose gel, the digestion product (1 µl) was amplified with six primers (selected in RAPD analysis). CRED-RA amplification and imaging conditions were the same as for RAPD studies.

2.6. Molecular analysis

Analysis of RAPD and CRED-RA bands was scored via the TotalLab (TL120) software (TotalLab Ltd., Newcastle upon Tyne, İngiltere). The genomic template stability (GTS) was assessed using the formula: 100-(100x a/n). In the given formula, the variable "a" denotes the number of polymorphic bands observed in the DNA profiles of the treated samples, whereas the variable "n" reflects the total number of bands present in the control DNA profiles. The appearance/disappearance or density difference of bands in the treated sample RAPD profiles in comparison to the un-treatment RAPD profiles were identified as polymorphism. The mean was calculated by comparing each application with the control. To compare the effect of each application, alterations in these values were computed as a percent of their untreated plants (set to 100%). To conduct CRED-RA analysis, the average percentage of polymorphisms (%) was calculated for each dosage application using the formula: 100 x a/n.

3. Results

The RAPD molecular technique was accomplished to assess the impact of Se and PEG 6000 treatments on the genomic DNA of wheat. Out of the 40 10-mer oligonucleotide primers that were subjected to RAPD analysis, only 14 exhibited distinct and consistent outcomes. Table 1 indicates a

Table 1. Molecular sizes (bp) of bands (+: appearance / -: disappearance) in RAPD profiles

Primers	Control	+/-	0% PEG 6000			5% PEG 6000				10% PEG 6000				15% PEG 6000			
			Na_2SeO_4			Na_2SeO_4				Na_2SeO_4				Na_2SeO_4			
			2 µM	4 µM	6 µM	0 µM	2 µM	4 µM	6 µM	0 µM	2 µM	4 µM	6 µM	0 µM	2 µM	4 µM	6 µM
OPW_13 (CACAGCGACA)	4	+	-	-	-	-	849 541	-	707	953 553	798 455	-	553	849	-	849 441	849 468
		-	4272	4272	4272	4272 638	4272 638	4272	638	-	-	4272 638	4272 638	4272 638	4272 638	638	-
OPW_18 (TTCAGGGCAC)	2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OPA_4 (AATCGGGCTG)	6	+	-	679	714	-	445	-	1000 419	1176	1055 428	-	706	697	-	1000 419	1055 601
		-	-	1362	801	801	-	801	1362 801	801	1362 801	1362 801	801	1362 801	1362 801	1362 801	1362 801
OPA_12 (TCGGCGATAG)	2	+	-	-	734	734	591	734	500	636	546	591	734	514	620	522	-
		-	-	-	958	958	958 698	958	958	-	-	958	958	958 698	958	958	958 698
OPY_11 (AGACGATGGG)	5	+	-	-	705	1093	-	655	-	1093	-	-	-	-	908	-	-
		-	1281 860	1281 860	1281 860 500	-	1281 860	500	-	-	860	1281	1281 860 158 162	1281 860 500 162 158	1281 1058 860	1281 860 1062 158	1281 860
OPY_13 (GGGTCTCGGT)	3	+	457 9614 63	463	463	-	-	-	896	518	-	-	-	890	476	-	-
		-	-	-	-	-	-	-	1334	-	-	-	1334 1014 788	1014	-	-	1334 1014 788
OPY_7 (AGAGCCGTCA)	7	+	719	2297 719	10000 2385 783	10000 2276 818 1195	10000	2408 10000	10000 965	10000 750	10000 2728 855	-	855	10000 2857 855	719	10000 2891 892	2432 719
		-	1195 3334 1320 8219 4911 591	591	591 8219	591 8219	4911 8219	8219	591 8219	4911 2759 1195 595	4911 591	1195 855 591	8219 91 1195	1195 591	8219 4911 591	591	8219 591
OPH_19 (CTGACCAGCC)	3	+	3027	-	-	-	3000	-	3012 2147	3019	3012	3012	-	2401	-	2299	2548
		-	1340	2340	-	-	-	-	1340	-	-	-	1340	1340	1340	1340	1340

+: Indicates appearance of a new band, -: Disappearance of a normal band

Table 1. (Continued)

Primers	Control	+/-	0% PEG 6000			5% PEG 6000				10% PEG 6000				15% PEG 6000			
			Na_2SeO_4			Na_2SeO_4				Na_2SeO_4				Na_2SeO_4			
			2 µM	4 µM	6 µM	0 µM	2 µM	4 µM	6 µM	0 µM	2 µM	4 µM	6 µM	0 µM	2 µM	4 µM	6 µM
OPY_1 (GTGGCATCTC)	5	+	-	-	2380	2438	-	2552	2715	2608	2715	-	-	-	1417	2000	-
		-	1487	-	-	1162	1162	-	1162	1162	1362 1162	1162	1362 1486 1487 1162 1537	1162	1362	1537 1162	1362 1486 1487 1162 1537
OPY_8 (AGGCAGAGCA)	8	+	910	-	1532	950	1038 849	1538	-	896	1511 881	832	881	-	1227 793	-	700
		-	4391 3789	1365 3789 4391	4391 3789 500	4391 3789 500	4391 3789 500	4391 3789 1038 500	4391 3789 500 1038	4391 3789 500	4391 3789	4391 3789 500	4391 3789 1365	4391 3789 1038 772	4391 3789 500	4391 3789 500 1038	4391 3789 500 1038 1365 1641 1457 2383
OPY_15 (AGTCGCCCTT)	2	+	898 726	730	730	1808 871 717	1768	730	2815 1728	927	1788 699	907 717	730	755	927	1728 704	1808 768
		-	-	-	-	-	-	-	-	1616	-	-	-	-	1616	-	-
OPW_6 (AGGCCCGATG)	5	+	558	-	-	2086	-	-	-	515	-	-	1638 583	465	-	-	-
		-	-	968	604	604	-	-	604	839	988 839 788 604	988 839 788 604	-	604	988 604	-	-
OPW_4 (CAGAAGCGGA)	3	+	-	780	-	-	-	-	-	1561	1253	-	-	-	-	-	-
		-	2726 1923	1923	1923	1923	2726	1923	2726	1923	2726	1923	2726 1923	2726 1923	1923 2726	2726	2726 1923
OPB_10 (CTGCTGGGAC)	3	+	1447 1324	1427 1295	1479 1243	1324	1418 1324 1130	1472 1353 1152	1402 465 1387 1173 100007 00	1257	1227 1152	1472 1243	-	1056	-	1295 1056	-
		-	-	-	-	297	-	-	-	1476 297	-	-	1500 1453 297	297	297	-	1500 1453 297

+: Indicates appearance of a new band, -: Disappearance of a normal band

comprehensive outline of all polymorphic bands in the RAPD profile. The banding patterns obtained from the utilization of the OPW-6/RAPD primer are displayed in Figure 1. According to RAPD, in total, 58 polymorphic bands were detected in control with 14 RAPD primers. The PCR amplification using RAPD primers produced 2-8 bands with an average of 4.14. Among these primers, a maximum of 8 bands pattern was produced in OPY_8, while 2 bands were amplified by OPW_18, OPA_12, and OPY_15. PEG 6000 and/or Se-treated plants resulted in significant alterations in RAPD fingerprints as compared to untreated bands (Table 1). These changes are defined by variances in the amount, size, and intensity of amplified DNA fragments for each of the 14 primers, as well as the removal of typical bands or the emergence of new bands (Table 1 and Figure 1). The amplified fragment lengths observed in the RAPD profiles varied from 158 (OPY-11) to 10000 (OPY-7 / OPB-10) bp. Tabassum et al. (2013) also obtained the largest band length obtained in our study, 10000 bp in the RAPD profile.

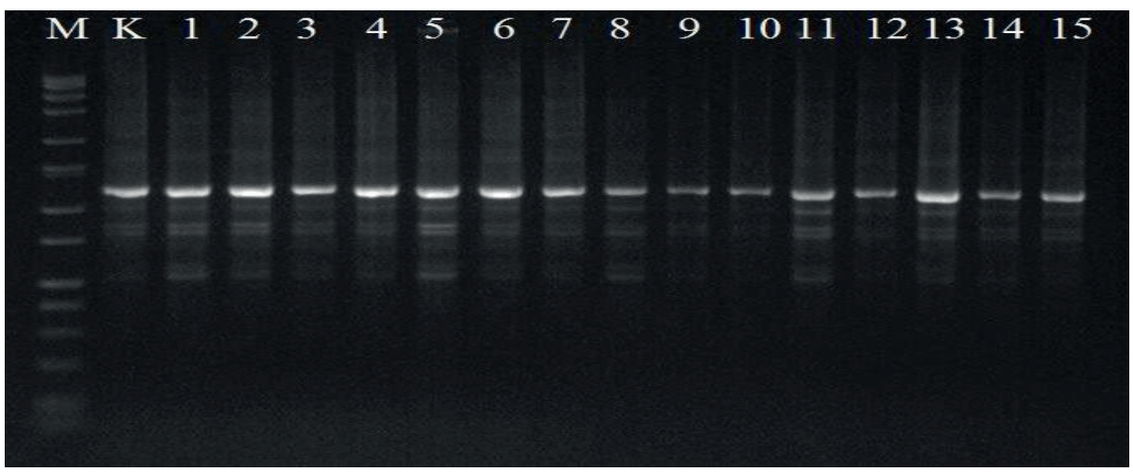

Figure 1. The amplification products formed against the RAPD primer of OPW 6

M: Marker, K(Control): 0 PEG 6000+0 µM Na_2O_4Se, 1: 2 µM Na_2O_4Se, 2: 4 µM Na_2O_4Se, 3: 6 µM Na_2O_4Se, 4: 5% PEG 6000, 5: 5% PEG 6000+2 µM Na_2O_4Se, 6: 5% PEG 6000+4 µM Na_2O_4Se, 7: 5% PEG 6000+6 µM Na_2O_4Se, 8: 10% PEG 6000, 9: 10% PEG 6000+2 µM Na_2O_4Se, 10: 10% PEG 6000+4 µM Na_2O_4Se, 11: 10% PEG 6000+6 µM Na_2O_4Se, 12: 15% PEG 6000, 13: 15% PEG 6000+2 µM Na_2O_4Se, 14: 15% PEG 6000+4 µM Na_2O_4Se, 15: 15% PEG 6000+6 µM Na_2O_4Se

After three doses of Se treatments, 39 normal bands were lost, and 31 new bands appeared compared to un-treatment plants. Polymorphic bands were noticed at each dose of PEG 6000 and/or Se for different 14 primers. Changes in RAPD patterns were also estimated as GTS values in relation to the profile reported in untreated plants. According to RAPD results, the highest GTS value was calculated at 56.9% (5% PEG 6000) and the lowest GTS value was 41.2% (15% PEG 6000) which shows the harmful impacts of PEG 6000 (Table 1). RAPD results indicated that GTS values were decreased with an increase in different PEG 6000 concentrations. However, an increase occurred in GTS value after Se treatment. When three doses of Se (2, 4, and 6 µM Na_2O_4Se) were applied together with drought (PEG 6000), especially the 4 µM dose of Se, reduced the polymorphism and increased the GTS value (Figure 2).

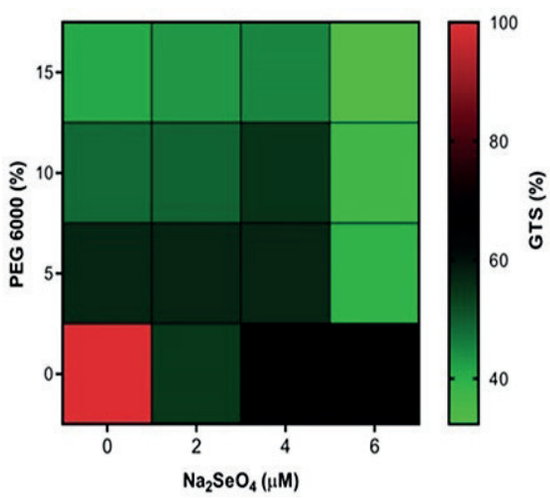

Figure 2. Genomic template stability % based on treatments

Using the CRED-RA approach, the influences of drought stress at various levels and the potential protecting role of Se on wheat plants were assessed in terms of DNA methylation. Among 14 RAPD primers, six oligonucleotides (OPA-4, OPB-10, OPW-13, OPW-18, OPY-1, and OPW-4) were utilized for the CRED–RA analysis to detect the impacts of Na_2SeO_4 treatments on DNA methylation (Figure 3). When comparing the PCR amplicons derived from untreated DNA, a concentration-related average polymorphism in DNA methylation was identified. Moreover, compared to the control, the MspI polymorphism rate of other Se and PEG 6000 treatments ranged from 17.2% to 59.2% while this value for HpaII ranged from 7.8% to 64.9% based on CRED-RA analysis. The highest methylation change was detected in 10% PEG 6000 application alone considering the polymorphism in the two enzymes. However, PEG-induced DNA methylation rates were changed after the treatment of different doses of Se. Moreover, it was determined that Se treatment in 0% PEG 6000 caused MspI and HpaII polymorphism, that is, methylation change (Figure 4).

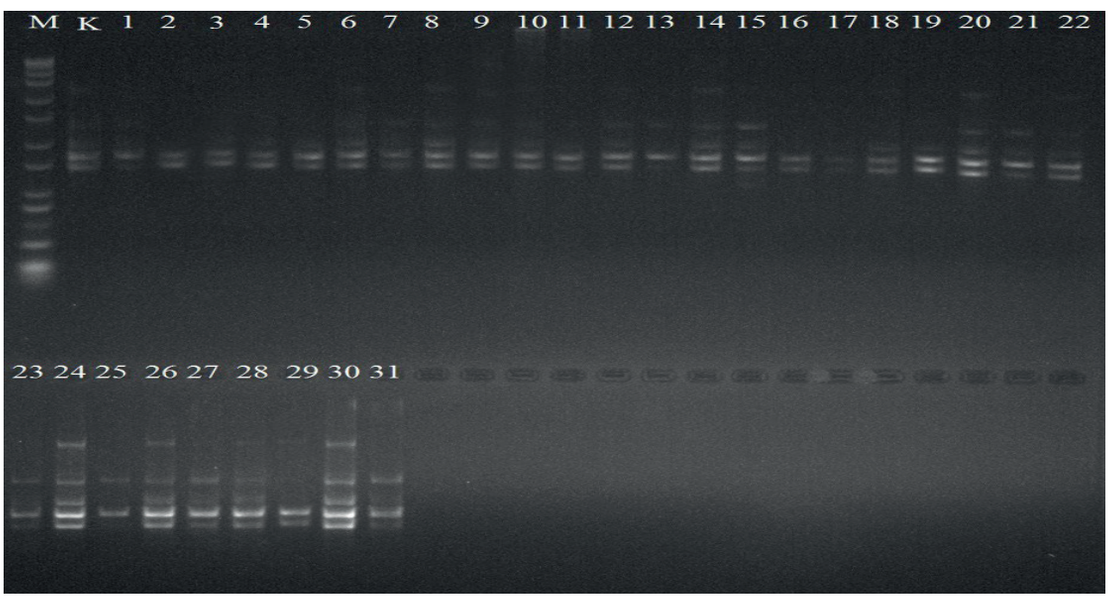

Figure 3. The amplification products formed against the CRED-RA primer of OPW 13

M: Marker, K(Control): 0 PEG 6000+0 µM Na_2O_4Se, 1: 2 µM Na_2O_4Se, 2: 4 µM Na_2O_4Se, 3: 6 µM Na_2O_4Se, 4: 5% PEG 6000, 5: 5% PEG 6000+2 µM Na_2O_4Se, 6: 5% PEG 6000+4 µM Na_2O_4Se, 7: 5% PEG 6000+6 µM Na_2O_4Se, 8: 10% PEG 6000, 9: 10% PEG 6000+2 µM Na_2O_4Se, 10: 10% PEG 6000+4 µM Na_2O_4Se, 11: 10% PEG 6000+6 µM Na_2O_4Se, 12: 15% PEG 6000, 13: 15% PEG 6000+2 µM Na_2O_4Se, 14: 15% PEG 6000+4 µM Na_2O_4Se, 15: 15% PEG 6000+6 µM Na_2O_4Se

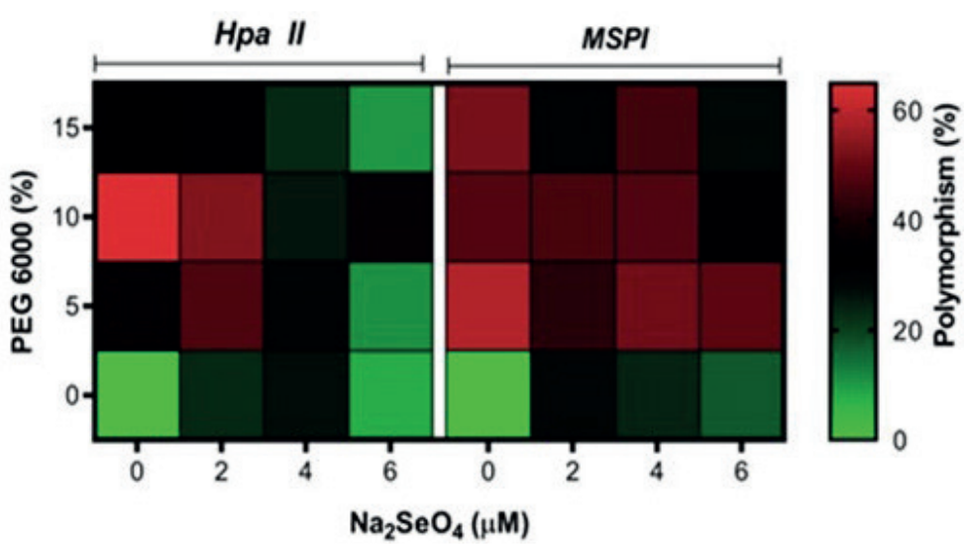

Figure 4. DNA methylation changes % based on treatments

4. Discussion and Conclusion

Drought stress is recognized as the most significantly limiting abiotic stress factor in wheat production worldwide. Heat surges caused by global climate change have already increased dryness in many drought-prone fields (Dhar et al., 2019). Prior researchers have reported that abiotic stresses such as salt, extreme temperature, heavy metals, and drought induce DNA damage (Martinez et al., 2018; Shim et al., 2018; Jaskulak et al., 2019). RAPD and CRED-RA techniques were successfully used to determine DNA damage caused by drought stress. The changes in the RAPD patterns by the treatment with different concentrations of PEG-induced water deficit indicate that drought stress causes DNA damage. DNA structural change may inhibit the polymerization of DNA and disrupt the effectiveness of the Tag DNA polymerase in PCR reactions (Liu et al., 2011). Although the molecular mechanisms underlying the genotoxicity of drought stress are unknown, it has been suggested that drought stress may trigger high levels of free radicals and ROS production (Pandey et al., 2023). Some ROSs do not interact with DNA, but they are precursors for the generation of hydroxyl radical (•OH). The reaction of •OH with DNA generates a multiplicity of products since it attacks purines, pyrimidines, and sugar-containing guanine residues to form 8-Hydroxyguanine (8-OHGua or the base part of 8-OHdG). Moreover, 8-OHdG in DNA predominantly gives rise to a transversion mutation (GC to TA) (Aydin et al., 2017; Kawai et al., 2018). When plants are subject to extreme environmental conditions, not only they may cause biochemical and genetic changes, but also epigenetic modification induced DNA methylation, nucleosome positioning, and histone modifications (Lämke and Bäurle, 2017). Particularly, these epigenetic changes may be mechanisms for plant adaptation and response to stress conditions. DNA methylation is a major epigenetic alteration that influences DNA structure and function in a variety of biological processes. DNA hypermethylation has been observed in pea-exposed water deficit (Yao et al., 2012). Our research findings corroborate prior studies by providing evidence that drought stress can induce comprehensive alterations in DNA methylation patterns throughout the genome (Banerjee and Roychoudhury, 2017; Sallam et al., 2019; Turhan et al., 2021).

Some studies have revealed that Se can enhance plant tolerance to stressful environments, including heavy metals stress, UV-B stress, salt stress, chilling stress, and drought stress (Golob et al., 2019; de Sousa et al., 2022; Rasool et al., 2023; Huang et al., 2024; Song et al., 2024). Our outcomes proposed that the protective impact of Se on the DNA damage of wheat subjected to drought depends on the Se dose. Treatment with 2 µM and 4 µM Se showed a curative effect on drought-related polymorphism of plants, whereas 6 µM Se treatment increased the polymorphism. High doses of Se can cause oxidative stress and impaired protein structure in plants (Gupta and Gupta, 2017). High doses of Se have been shown to reduce pro-oxidants in ryegrass seedlings (Bocchini et al., 2018). The protecting impact of Se on different stresses has been linked to the reduction of lipid peroxidation, the steadying of DNA methylation, and the decrease of oxidative stress generated by stress (Djanaguiraman et al., 2005; Kuznetsov et al., 2006; Taspinar et al., 2009). Furthermore, the antioxidative action of Se was followed by an enhancement in glutathione ascorbate peroxidase, superoxide dismutase activities, and peroxidase (Hartikainen, 2005; Jiang et al., 2017). Besides, Se-stimulated variations in the activities of oxidoreductase enzymes were described in rapeseed (Hasanuzzaman et al., 2012), ryegrass (Cartes et al., 2010), and rice (Kumar et al., 2014). This information leads us to believe that Se's antioxidant potential action might shield cells from the damaging effects of genotoxic stress and the instability of their genomes brought on by drought. Although the protective role of Se drought stress in higher plants has been previously established, its effect on DNA methylation in drought-stressed plants has not been fully elucidated. The potential impact of Se on DNA methylation may be attributed to its involvement in the mitigation of ROS induced by drought conditions (Bocchini et al., 2018). Additionally, it has been suggested that Se ions can modify DNA cytosine methylation patterns, resulting in the formation of methyl derivatives (Filek et al., 2008). Furthermore, it has been observed that ROSs function as signaling molecules, inducing epigenetic modifications without altering the underlying DNA sequence (Creppy et al., 2002; Marnett et al., 2003; Valinluck et al., 2004).

DNA methylation polymorphisms and genomic instability in response to PEG 6000-induced drought stress and sodium selenite were analyzed by the techniques of RAPD and CRED-RA in wheat. As a result of the study, highly significant genomic instability and methylation differences were found under drought stress. Moreover, Se treatment was effective in maintaining the molecular stability of wheat under drought stress while changes in DNA methylation which is considered a specific defensive mechanism for variable gene expression. These findings underscore the potential of Se supplementation as a sustainable strategy to improve drought tolerance in

crops and mitigate the adverse effects of water scarcity on agricultural productivity. However, further research is needed to elucidate the underlying mechanisms and optimize Se application protocols for different crop species and environmental conditions.

Ethical Statement

The authors declare that ethical approval is not required for this research.

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Declaration of Author Contributions

Conceptualization, Material, Methodology, Investigation, *Z. ŞAHİN*; Conceptualization, Material, Methodology, Investigation, Writing-Review & Editing, *G. AĞAR*; Visualization, Writing-Original Draft Preparation, Writing-Review & Editing, *E. YİĞİDER*; Data Curation, Formal Analysis, Writing-Review & Editing, *M. AYDIN*. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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