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PUTRESCINE AS A PROTECTIVE MOLECULE ON DNA DAMAGE AND DNA METHYLATION CHANGES IN WHEAT UNDER DROUGHT

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ABSTRACT. The world suffers with the agricultural drought stress which leading to decreasing crop production, and also adversely affecting cereals on morphological, physiological, biochemical and molecular levels. However, exogenous treatment of some osmotically active materials like putrescine has been regarded as a good preventive against these harmful effects of drought. But there is a lack of information on putrescine has any effects on DNA damage and DNA methylation in crops. The current study was goal to determine DNA damage levels and DNA methylation changes in *Triticum aestivum* cv. Karasu 90 subjected to different concentrations of drought (-2, -4, -6 bar PEG) and whether putrescine (0.01, 0.1, 1 mM) has any ameliorative effect on these changes is determined with RAPDs and CRED-RAs techniques. In addition, total oxidant status (TOS) and total antioxidant status (TAS) values were investigated based on drought and putrescine treatments. The findings showed that drought stress caused DNA damage and DNA methylation changes. However, these effects decreased after putrescine treatments. Putrescine has been shown to decrease oxidative damage caused by drought via increasing antioxidant status in drought stress. According to results, it was concluded that putrescine could be preferred for its force to protect wheat DNA from the damaging effects of drought and the demethylation positively contributed to drought stress tolerance.

1. INTRODUCTION

Drought, which is a major abiotic stress globally, brings on extensive limits on crop productivity due to its unsuitable influences on plant morphology, physiology and also biochemistry, preventing growth and development [1]. Moreover, long-term drought induces oxidative stress by increasing the production of reactive oxygen species (ROS). ROS are constantly synthesized as byproducts in the chloroplast, mitochondria and peroxisome parts of the plant under normal conditions but increasing in stress conditions and they can damage the phospholipids of cell membranes, chlorophyll, proteins and nucleic acids [2]. In particular, irreparable oxidative stress-related damages to the DNA strand give rise to instability in the genome [3]. Plants have antioxidant defense organization to prevent oxidative damage caused by ROS. Antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathion peroxidase (GPX), catalase (CAT), etc., play a role in the direct removal of ROS and inhibit uncontrolled oxidation steps [4].

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Drought also alters gene expression via epigenetic modifications like DNA methylation and histone modifications [5-7]. It has been presented that water stress induces cytosine methylation in crops like wheat [8], pea [9], rice [10] etc. in many researches. Considering the worse effects of drought especially on the wheat which is the world's most grown and consumed crop, it has been inevitable to investigate the impact of DNA methylation on wheat. Furthermore, various DNA methylation patterns indicated in tolerant and sensitive wheat genotypes under drought stress [8].

Plants improve some strategies that are at morphological, anatomical, biochemical and molecular levels to avoid or tolerate the stresses which allow them to adapt and defense themselves from stress so as to cope up all these stresses [11]. One of them is phytohormones. Plant hormones play an important role in the regulation of plant responses to the environment [12]. Many researchers reported that plant hormones regulate plant responses to oxidative stress elicited by different stress factors [13, 14]. One can understood from these papers that osmotic, cold and drought stress caused to increase of ABA, salicylic acid and polyamine levels. Polyamines (putrescine, spermidine, spermine and cadaverine) are important growth regulating molecules known to participate in a wide variety of developmental events, including flowering, senescence, root development, organogenesis and embryogenesis [15, 16]. Plants exposed to abiotic stress raise polyamine levels to help regulate themselves tolerance to stress. Polyamines provide tolerance to stress as bounding to RNA and DNA guard DNA from enzymatic degradation, oxidative damages, mechanical shearing. Moreover, Polyamines stabilize RNA, to counteract of ribosomal dispersion [17]. It was the first indicate by Ruiz-Herrera et al. (1995) [18] that the impact of polyamines on cytosine-DNA methyltranferases was quite selective and this effect related to both the binding and activity of the methylases by polyamines. However, the protective effect of polyamines against DNA damage and DNA methylation changes in plants subjected to drought stress has not been elucidated.

The main of present study was to see whether putrescine has any protective effect against genetic and DNA methylation variations in *Triticum aestivum* cv Karasu 90 in drought stress. We used RAPDs to investigate the genetic damage and CRED-RAs to access the differences in methylation level and changes of pattern of DNA methylation. Also, total oxidant status (TOS) and total antioxidant status (TAS) were determined in drought stress and putrescine treatments.

2. MATERIAL AND METHOD

2.1. Plant material and treatment conditions

Karasu 90 (*Triticum aestivum* L.), which is a drought-sensitive cultivar, was used as plant material in this study. The equal seeds were surface-sterilized with 0.5% sodium

hypochlorite solution a 5 minutes and afterward rinsed several times with sterile distilled water. Sterilized seeds were soaked in various doses of putrescine [0 (distilled water), 0.01, 0.1 and 1 mM] (Sigma, 51799) for 24 h at 25±1 °C in darkness as pretreatment. The solutions were then carefully removed and the seeds were dried for 1h in laminar flow cabinet (Esco Airsystem, Singapur). Replicates of 25 seeds were sown in 12 cm diameter sterile petri dish with two layers of filter paper saturated with solution of different osmotic potentials (0, -2, -4 and -6 bar) which were created with PEG 6000 (Sigma Aldrich, USA) according to Michel and Kaufmann's equation [19]. The dishes were kept at 25 ± 1°C in 16 h photoperiod. Each treatment was replicated three times. Afterwards 10 days of germination, young leaves were harvested randomly from ten plants for each treatment and snap frozen in liquid nitrogen.

2.2. Genomic DNA isolation

The genomic DNA was obtained from young leaves using the method specified by Taspinar et al. (2017) [20] and stored at -20 ºC for later on use. The quality and quantity of isolated DNA were measured using a Nano-Drop (Qiagen, Qiaxpert Instrument, Germany) spectrophotometer and 1% (w/v) agarose gel with ethidium bromide staining.

2.3. RAPD and CRED-RA procedures

13 oligonucleotide primers (Sentegen Biotechnology, Türkiye) (OPA-4, OPA-12, OPH-16, OPH-18, OPH-19, OPB-10, OPY-1, OPY-7, OPY-13, OPW-4, OPW-6, OPW-13 and OPW-18) amplified polymorphic amplicons and used in RAPD-PCR reactions. For CRED-RA analysis, genomic DNA sample from each treatment were separately digested with HpaII (New England Biolabs, USA) and MspI (New England Biolabs, USA) endonucleases according to manufacturer’s instruction. Digestion was checked on 1% (w/v) agarose gel and after 1µl of each digestion product were amplified with 8 RAPD primers (OPA-4, OPB-10, OPH-18, OPY-1, OPY-13, OPY-15, OPW-4 and OPW-13). PCR amplifications (SensoQuest GmbH, Germany), electrophoresis (Bio-Rad, USA) and procedures for each technique were carried out according to Taspinar et al. (2017) [20].

2.4. Determination of TOS and TAS

TOS and TAS values for treatments were measured with Rel Assay brand commercial kits (Rel Assay Kit Diagnostics, Turkey).

2.5. Analysis

Molecular analysis (RAPD and CRED-RA) were carried out with Total Lab TL120 computer software. Genomic template stability (GTS, %) for RAPD and the average of polymorphisms

(%) for CRED-RA were obtained according to Taspinar et al. (2017) [20]. To determine Polymorphism Information Content (PIC) and Discriminating Power (D) values, Botstein et al. (1980) [21] and Prevost and Wilkinson (1999)'s [22] articles were used. A data matrix was created from RAPD gels by assigning 1 to present bands and 0 to absent bands. The data matrix was used to compute pairwise Jaccard similarity coefficients among all the drought and putrescine treatments (NTSYS-pc, ver. 1.8). Cluster analysis (UPGMA,SAHN in NTSYS) was performed on the matrix of Jaccard coefficients [23]. All data obtained from TAS and TOS parameters were analyzed by one way ANOVA using SAS PROC GLM (SAS version 9.4, SAS Institute Inc., Cary, NC). Treatment means were compared using the Fisher's least significant difference (LSD) at $p<0.05$.

3. RESULTS

3.1. RAPD

3.1.1 Levels of GTS

Totally, 32 oligonucleotide primers with %60-70 GC content were tested with untreated DNA (0 mM putrescine + 0 bar PEG6000) and only thirteen gave specific and stable results (TABLE 1). A total of 92 bands were obtained in control treatment. Among these 10 bands were occurred in OPH-19 (FIGURE 1) and 5 bands in OPH-16. Each primer produced 25 (OPH-19)– 2 (OPB-10) polymorphic bands in all treatments out of control. Molecular sizes of bands ranged from 2432 (OPH-16) to 57 (OPW-13). Compared to control, putrescine and/or PEG6000 treatments led to prominent variations in RAPD patterns. These changes reveal as loss of bands available in control or appearance of new bands. GTS was used for comparing the changes in RAPD profiles. GTS values tended to decrease with increasing concentration of PEG6000 treatments. The values were calculated as 33% in -2 bar, 28.6% in -4 bar and 19.1% in -6 bar PEG6000 treatments. Besides, putrescine treatments had very high GTS values compared to stress treatments. 75.4% was in 1 mM put, 68.6% in 0.1 mM put and 64.6% in 0.01 put were determined. Also in combined treatments the lowest value was 43.5% in -6 bar PEG6000 + 0.01 mM putrescine treatment and the highest value was 60.3% in -2 bar PEG6000 + 1 mM putrescine treatment (Table 1).

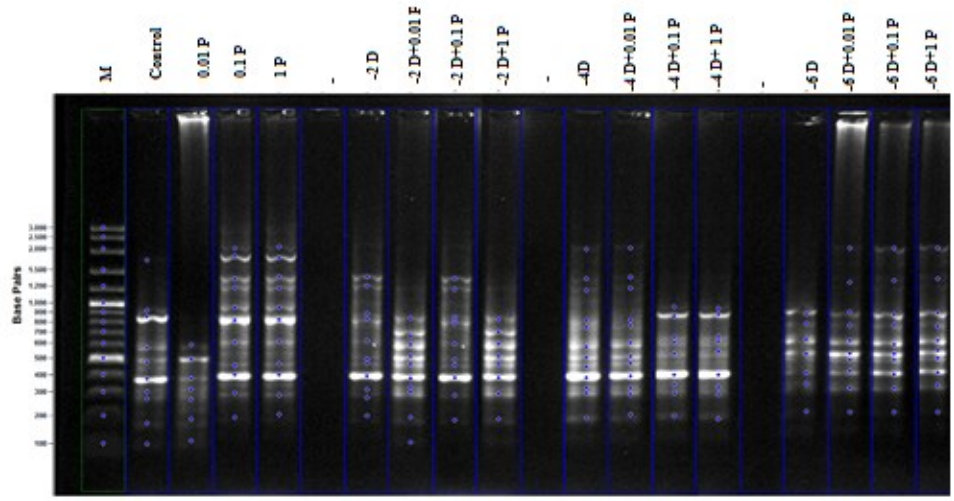

FIGURE 1. RAPD profiles of genomic DNA from *Triticum aestivum* Karasu 90 exposed to varying putrescine and/or PEG6000 concentrations with primer OPH-19 *M: marker, P: putrescine, D: drought.

3.1.2 Numerical analysis

PIC values of all primers varied between 0.284 and 0.360 and average became 0.321. While the primer OPW-18 was the highest PIC value, the primer OPH-19 was the lowest had (TABLE 2). D values of primers had been in 0.831-0.970 and average was 0.918. The primer OPW-18, which has both the discriminating power and the highest polymorphic band content, was determined as the most distinctive primer (TABLE 2). Similarity index of all treatments varied between 0.506 and 0.849. While the closest similarity coefficients to control was determined in -2 bar PEG6000 + 0.01 mM putrescine treatment as 0.645 ratio, the furthest similarity to control was in -6 bar PEG6000 + 1 mM putrescine treatment as 0.506 ratio (TABLE 3). The dendrogram (FIGURE 2) grouped all treatments into two main clusters. First cluster is untreated sample. Two cluster was divided into two main subclusters. The first subcluster was consisted of putrescine doses alone and combination with putrescine and -2 bar PEG6000 treatments while the second cluster was consisted of putrescine and -4 and -6 bar PEG6000 doses.

TABLE 1. Molecular sizes of bands (+: appearance / -: disappearance) and the average GTS values in RAPD profiles *P: primers, C: control

P	C	+/-	0 mM Putrescine / Drought (bar)			1 mM Putrescine / Drought (bar)				0.1 mM Putrescine / Drought (bar)				0.01 mM Putrescine / Drought (bar)			
			-2	-4	-6	0	-2	-4	-6	0	-2	-4	-6	0	-2	-4	-6
OPA-4	6	+	-	524	524	-	447 407	-	400	-	757	506 368	-	-	769	358	-
		-	980 537 443 388	980 537 443 388	980 537 443 388	980 740 443	-	-	556 378	980 740	800	1047 635	612 524 431	-	800	1047	556 431 378
OPA-12	6	+	579	392	500 362	613 423	763	-	-	-	-	-	987	-	-	1145 684	-
		-	745	1208 919	1208 566	1208 500 140	1500 1574	-	1075 362	1208 1102	1500 945 457 159	530 392 138	112	1208 1102 919 745 500	1500 945 457 159	530	745 112
OPH-16	5	+	1820 1470 1332 964 800 289	1322 892 509 292 207	1035 576 465 236	325	-	-	690	2303 1490 582	-	-	9135 09	2432 1720 311	-	473 176	-
		-	-	-	-	745 651	964 749 289	-	1085	631	964 749 800 611	728 509	6672 36	725	1132 800	738 207	964 236
OPH-18	7	+	6883 79	2202	836	1458	1237	836 465	993 600	628	1165	579	1526 991	1458 688	1200	400	15269 83 863
		-	1654 1237 723	1654 1373 1237	1654 1373 1237 723	1654 1373	1303	-	-	1654 1373 517	1303	2202 943	-	1654 1373 723	1303	2202 756 635	-
OPH-19	10	+	1352 881 463	1319 1315 746 503	1980 782 629	-	586 104	-	489	2000 1174	590 458	-	586 489	2062 1363 1166 465	-	649	500
		-	1684 918 97	918 97	1684 918 300 172 97	1684 918	1352 881	-	-	561 97	1200	1980 1319 382	629	97	1352 1200 881 392	746 503 382	-
OPB-10	6	+	-	619	-	-	-	-	-	-	-	-	633	-	-	-	633
		-	1017 836 727 491	563 491	1017 836 727 491	1017 836 727 491	593 370	984 823 504	1017 963 383	1017 836 727	593 370	504	581	1017 836 727 491	593 370	823	581

OPY-1	7	+	1123	446 191	955 500 191	500	506	245	430 322	-	452	274	446 311	-	204	-	12658 41
		-	1083 353 231 70	353; 231 70	888 353 231 70	353	452	1109 191	269	353	518	1109 191	269	1083 888 353	-	-	955 667
OPY-7	6	+	864	2046 1544 834	1940 1544 888	709	337 136	653 186	-	-	727 165	625 170	2454 993 181	-	-	1028 935	-
		-	917 681 337	1065 979 200	979 337 200	476 200	864 286	900	1940 1544 888 625	476 200	864 586	900	-	917 681 476 337	-	979	19401 544 888 625 315
OPY-13	6	+	496	676 541	844 473	1500	526	488 300 123	791	1691	213	187 127	-	823	890	1500 700	181 109
		-	507 200 115	754 115	904 507 115	115	496	1231 918 515	1030 844	115	-	1231 918	-	1152 754 115	942	541	10308 44
OPW-4	9	+	813	864	841	1526	930 605	520	583 492 209	1053	900 583	1354 684 503	-	1022	2000	2098 442	11215 68 500
		-	2439 1279 775 516 169	2439 1279 775 516 169	1761 1279 775 516 389 237 169	237	334 225	362 229	2023	237	225	362 229	-	237	-	362	20232 02
OPW-6	8	+	-	900 543	718 432	-	547	-	-	-	-	-	-	-	-	-	-
		-	1829 1555 500 400 218	1829 1555 500 400 218	1829 1555 757 500	1829 1555 400 218	-	1077 900 543	718 432 207	1829 1555 400 218	777	1077 900 543	718 432 207	1829 1555 400 218	777	770	-
OPW-13	9	+	2254 544	570 437	576	469	469 53	1458 770	63	469	50	1426	66	-	647 447 57	764 673 127	783 338 70
		-	1973 1567 500 63	1973 1567 920 714 138	1973 1567 714 507 138 63	1973 1567	2254 1275 770 155	570 330	-	1973 1567 714	2254 770 345 155	570 507 330	-	1973 1567 1280 920 714	2254 155	437	-
OPW-18	7	+	-	-	-	-	709	1448	1448	-	437 396	1225	416	-	-	1983 448	-
		-	1248 726 420 372 205	621 420 372 205	621 420 372 205	1479 1248 726 420	-	-	-	1479 1248 726 420	-	407	1448	1479 1248 726 420	1420 1329		-
GTS %	**100**		**33**	**28.6**	**19.1**	**75.4**	**60.3**	**57.4**	**56.6**	**68.6**	**59.8**	**45**	**43.9**	**64.6**	**50.3**	**44.3**	**43.5**

3.1.3 TOS and TAS

TOS and TAS values for treatments were presented in TABLE 4. TOS levels showed significant difference between the control and putrescine doses ($p<0.05$). The TOS value compared to the control decreased depending on the increase in putrescine doses (from 6.353 umol/L to 2.580 umol/L) whereas it increased due to increasing in drought stress doses (from 16.357 umol/L to 23.783 umol/L). When the effects of putrescine doses on the amount of TOS under drought stress were investigated, all putrescine doses applied under all doses of PEG6000 caused remarkable reductions in TOS value. A significant decrease in TAS value was occurred in drought stress and the difference between control and drought doses was significant ($p<0.05$). Furthermore, putrescine application caused a significant increase in TAS value compared to the control. On the other hand, putrescine applied in drought stress caused increase in TAS level compared to drought stress doses applied alone.

TABLE 2. Polymorphism Information Content (PIC) and Discriminating Power (D) of primers used in RAPD

Primers	PIC[a]	D[b]
OPA-4	0.327	0.933
OPA-12	0.346	0.956
OPH-16	0.324	0.927
OPH-18	0.314	0.912
OPH-19	0.284	0.831
OPB-10	0.298	0.879
OPY-1	0.321	0.924
OPY-7	0.319	0.920
OPY-13	0.314	0.912
OPW-4	0.340	0.949
OPW-6	0.304	0.893
OPW-13	0.328	0.933
OPW-18	0.360	0.970
Average	**0.321**	**0.918**

a: Botstein et al. (1980); b: Prevost and Wilkinson (1999)

TABLE 3. Jackard similarity index of treatments

	C	0.01 Put	0.1 Put	1 Put	-2 D**	-4 D	-6 D	-2 D + 0.01 Put	-2 D + 0.1 Put	-2 D + 1 Put	-4 D + 0.01 Put	-4 D + 0.1 Put	-4 D + 1 Put	-6 D + 0.01 Put	-6 D + 0.1 Put
0.01 Put	0.620														
0.1 Put	0.629	0.731													
1 Put	0.551	0.661	0.767												
-2 D	0.576	0.678	0.629	0.649											
-4 D	0.592	0.629	0.645	0.608	0.649										
-6 D	0.543	0.637	0.547	0.584	0.624	0.624									
-2 D + 0.01 Put	0.645	0.690	0.673	0.694	0.808	0.686	0.637								
-2 D + 0.1 Put	0.604	0.665	0.665	0.710	0.767	0.620	0.620	0.804							
-2 D + 1 Put	0.608	0.678	0.669	0.739	0.755	0.665	0.641	0.784	0.849						
-4 D + 0.01 Put	0.514	0.592	0.608	0.653	0.629	0.784	0.694	0.641	0.616	0.620					
-4 D + 0.1 Put	0.555	0.641	0.592	0.555	0.620	0.661	0.743	0.624	0.657	0.653	0.657				
-4 D + 1 Put	0.571	0.641	0.576	0.555	0.596	0.661	0.751	0.649	0.616	0.637	0.624	0.771			
-6 D + 0.01 Put	0.551	0.637	0.604	0.576	0.600	0.600	0.755	0.604	0.637	0.624	0.653	0.686	0.678		
-6 D + 0.1 Put	0.514	0.616	0.567	0.596	0.588	0.653	0.759	0.616	0.649	0.645	0.690	0.739	0.698	0.718	
-6 D + 1 Put	0.506	0.673	0.624	0.612	0.661	0.604	0.751	0.649	0.673	0.669	0.649	0.682	0.657	0.751	0.804

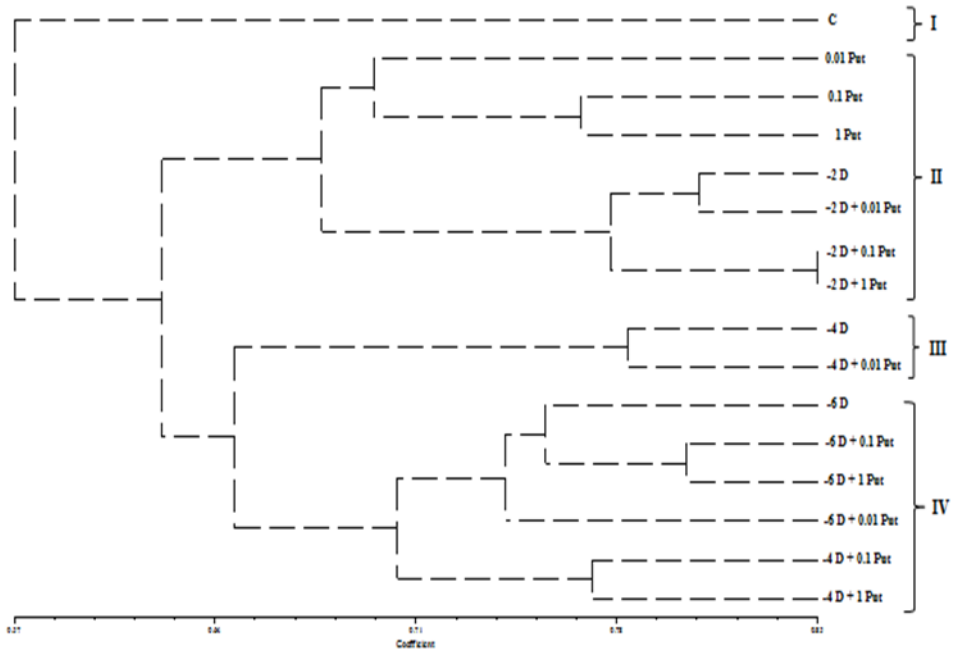

FIGURE 2. UGPMA dendrogram of the genetic similarity among putrescine and/or PEG6000 treatments inferred from a matrix of Jaccard coefficient

3.1.4 CRED-RA

Eight oligonucleotide primers which gave specific and sTABLE results used in RAPD analyzing were selected for CRED-RA analysis (TABLE 5). Compared to the PCR products obtained from the DNA of control treatment, putrescine and/or PEG6000 treatments resulted in certain changes in CRED-RA patterns. HpaII polymorphism values were higher than MspI polymorphism values for the most part of the whole treatments, since HpaII polymorphism ranged from 9.7% to 41.1% and MspI polymorphism ranged from 4.1% to 37% (TABLE 5). DNA methylation was emerged with all of doses of two treatments. The highest methylation value was 72.9% and the lowest was 47.3% in stress treatments. The highest methylation value was 18.5% and the lowest was 4.1% in putrescine treatments. The DNA methylation values changed in combined treatments according to dose variabilities. While MspI polymorphism was 20.7% in 1 mM put and -6 bar PEG6000, this value decreased as 16.3% in 1 mM put and -2 bar PEG6000 (TABLE 5).

TABLE 4. Comparison of TOS and TAS values based on the experimental treatments

Treatment	TOS (umol/L)	TAS (mmol/L)
Control	7.513^{k}	0.567^{f}
0.01 Put	6.353^{l}	0.664^{e}
0.1 Put	4.413^{m}	0.945^{b}
1 Put	2.580^{n}	1.567^{a}
-2 D	16.357^{c}	0.456^{gh}
-2 D + 0.01 Put	12.317^{h}	0.444g^{h}
-2 D + 0.1 Put	10.403^{i}	0.571^{f}
-2 D + 1 Put	8.447^{j}	0.888^{c}
-4 D	20.320^{b}	0.378^{i}
-4 D + 0.01 Put	18.713^{c}	0.436^{h}
-4 D + 0.1 Put	14.390^{f}	0.555^{f}
-4 D + 1 Put	10.767^{i}	0.738^{d}
-6 D	23.783^{a}	0.264^{j}
-6 D + 0.01 Put	20.737^{b}	0.435^{h}
-6 D + 0.1 Put	18.140^{d}	0.498^{g}
-6 D + 1 Put	13.340^{g}	0.661^{e}
Means	**13.036**	**0.629**
F value (Treatment)	1208.36**	255.71**
LSD$_{(0.05)}$ (Treatment)	0.520	0.055
Coefficient of variation (%)	2.40	5.30

TABLE 5. Percentage polymorphisms of studied CRED-RA amplicons

Primers	Drought (bar)	0 mM Putrescine		1 mM Putrescine		0.1 mM putrescine		0.01 mM putrescine	
		H	M	H	M	H	M	H	M
OPA-4	0	-	-	0	0	0	0	0	14.2
	-2	28.5	37.5	25	0	25	14.2	44.4	44.4
	-4	100	100	0	20	20	20	66.6	66.6
	-6	66.6	100	50	40	80	60	75	80
OPB-10	0	-	-	0	0	0	0	0	14.2
	-2	62.5	100	50	33.3	75	66.6	33.3	66.6
	-4	100	100	14.2	14.2	75	25	80	42.8
	-6	75	100	11.1	12.5	0	25	14.2	50
OPH-18	0	-	-	25	16.6	40	20	40	20
	-2	25	33.3	20	33.3	14.2	14.2	16.6	14.4
	-4	40	60	0	25	0	66.6	0	0
	-6	40	80	33.3	40	33.3	16.6	50	16.6
OPY-1	0	-	-	20	16.6	50	16.6	60	33.3
	-2	20	16.6	25	16.6	40	20	40	40
	-4	50	16.6	50	16.6	0	16.6	0	16.6
	-6	60	33.3	33.3	40	33.3	16.6	20	16.6
OPY-13	0	-	-	0	0	20	16.6	50	16.6
	-2	33.3	66.6	0	0	0	0	0	0
	-4	66.6	50	20	0	20	0	50	0
	-6	100	50	0	0	66.6	33.3	66.6	50
OPY-15	0	-	-	33.3	0	25	0	50	33.3
	-2	60	25	20	14.2	33.3	16.6	33.3	16.6
	-4	25	40	33.3	25	66.6	16.6	28.5	40
	-6	80	60	50	16.6	0	16.6	20	16.6
OPW-4	0	-	-	0	0	0	0	0	0
	-2	66.6	66.6	0	0	0	0	0	0
	-4	100	66.6	25	25	33.3	25	66.6	66.6
	-6	66.6	80	0	0	33.3	16.6	33.3	16.6
OPW-13	0	-	-	0	0	0	0	0	16.6
	-2	100	33.3	0	33.3	14.2	42.8	16.6	14.2
	-4	57.1	100	14.2	16.6	33.3	33.3	28.5	37.5
	-6	100	80	0	16.6	33.3	40	50	50
Average	**0**	**-**	**-**	**9.7**	**4.1**	**16.8**	**6.6**	**25**	**18.5**
	-2	**49.4**	**47.3**	**17.5**	**16.3**	**17.5**	**21.8**	**23**	**24.5**
	-4	**67.3**	**66.6**	**19.5**	**17.8**	**31**	**25.3**	**40**	**33.7**
	-6	**73.5**	**72.9**	**22.2**	**20.7**	**34.9**	**28**	**41.1**	**37**

4. DISCUSSION

In the current study, we investigated both genetic and DNA methylation changes in *Triticum aestivum* seedlings under drought stress conditions using RAPD and CRED-RA assays respectively, and effects of putrescine under these changes. The changes in the RAPD patterns generated by drought stress and putrescine included disappearance of normal bands and appearance of new bands when compared with control, as seen in TABLE 1. These changes differed from primer to primer among thirteen primers. According to PIC and D values the primer OPW-18 were the most distinctive primer in our study (TABLE 2). Also, we carried out the cluster analysis to determine the differences between all the treatments (TABLE 3, FIGURE 2). There was close relationship among putrescine and -2 bar PEG6000 treated groups. The other subcluster was shown that -4 and -6 bar PEG6000 treated groups were close to each other. It was thought that -2 bar PEG6000 had a separate effect in comparison with -4 and -6 bar PEG6000 groups.

As seen in TABLE 1, drought stress doses caused an enormous decrease on GTS value by comparison with other treatments (19.1 28.6 and 33%, respectively). These changes caused by drought were clearly dependent on extensive DNA damages [24-27]. Although many studies have proved that abiotic stresses induce DNA damage in different plants [2, 28], the molecular mechanism responsible for genotoxicity remains unclear even today. It was recommended that abiotic stress could stimulate the release of free radicals and ROS [29, 30]. In point of fact, we proved that TOS levels were gradually increased according to PEG600 doses (TABLE 4). Many ROS don't appear to interact with DNA but they are precursors for OH• radical. The reaction of OH• radical with DNA generates a multitude of products, since it assaults sugar, pyrimidines and purines, containing guanine residues to form 8-hydroxydeoxyguanosine (8-OHdG). In addition, 8-OHdG mostly produces transversion mutation (G to T). To limit ROS resulting damage, plants produce a wide range of antioxidants. After ROS has been occurs, detoxification mechanisms are effectively activated to minimize ROS-induced damage [4]. Antioxidant defense systems protect plant cells from oxidative damage by controlling the signaling pathways that lead to uncontrolled oxidations by scavenging ROS [2].

By the way, we determined that putrescine treatments caused an increase of GTS values against drought stress. According to results, especially 1 mM concentration of putrescine has increased GTS value and showed the most perfect effect in all stress treatments (TABLE 1). The defensive effects of polyamines contrary to DNA damage are related to its ability to bind to nucleic acid. Previous studies have shown the protective effect of polyamines against environmental stress in different plants [31-33]. It has been assumed that polyamines exhibit multiple functions by binding to negatively charged macromolecules due to basic net charge. Miyamoto et al. (1993) [17] have reported that total spermidine is bound to RNA, DNA and membrane lipids and protect DNA from enzymatic degradation, X-ray irradiation and mechanical shearing in Escherichia coli.

Therewithal, in this study we determined that TOS levels were quite low in dose-dependent of putrescine, while TAS levels were at the highest (TABLE 4). At this point, we are thinking of putrescine could be stimulate antioxidants and activate tolerance mechanisms in plant. These findings are consistent with Shi et al. (2013) [34] who reported that nucleoside diphosphate kinase (NDPK) and three antioxidant enzymes [2- Cys POD, ascorbate peroxidase (APX), Cu/Zn SOD] were generally regulated by polyamines (putrescine, spermidine and spermine) in bermuda grass. Similarly, Shi and Chan (2014) [35] found that the increased NDPK2 protein level by polyamine treatment is directly related with activities of antioxidant enzymes. Likewise, it was determined that overexpressing AtNDPK2 in Arabidopsis plants conferred enhanced tolerance to multiple environmental stresses that elicited ROS accumulation through interacting with oxidative stress-activated MPK3 and MPK6 and modulated the antioxidant enzyme activities such as APX, CAT and POD [36].

When plants are exposed to environmental stress, they activate mechanisms in biochemical, physiological and molecular levels induced DNA methylation and histon modification. DNA methylation is a well-characterized model to explain the epigenetically changes in gene expression. It is known that hypermethylation is associated with gene silencing while hypomethylation is linked with active transcription [37] and also known that hypermethylation and demethylation was periodic in nucleosomes. These status of methylation changes may be attributed to stress, kinds of plants and also tissue specificity. DNA demethylation was detected in salt stress in cotton [38], cold treated maize roots [39], heavy metal treated white clover [40], while hypermethylation was determined in chromium-exposed rapeseed [41], in pea exposed drought stress [9]. Our results well agreed with the outcomes of the earlier studies. We achieved the highest value of polymorphism (72.9%) in the -6 bar PEG6000 dose, so DNA methylation was showed quite a high rate of change (TABLE 5). Some researchers have emphasized that polyamines can inhibit direct DNA methylation by inhibition both the binding and activity of cytosine-DNA methylases [18, 42, 43]. Inhibition activity of cytosine-DNA methylases is non-competitive. It suggested that polyamines have an indirect effect on methylation as a mechanism for the antitrypanosomal effect of the ornithine decarboxylase inhibitor DFMO [44]. Other research provide that polyamines are capable of binding to A and B DNA, in A-DNA, binding occurs mainly to major groove, whereas in B-DNA putrescine and cadavarine bind to both sugar-phosphate backbone and major and minor grooves [33, 45, 46] Also experiment with B-DNA differing in the guanine to cytosine ratio showed that polyamines interacted mainly with phosphate groups and did not affect a native secondary structure DNA, thus providing for normal transcription of stress induced genes. So, polyamines could inhibit DNA methylation, which permits expression of specific genes responsible for the synthesis of stress protein. As would be expected, our results demonstrate that putrescine decrease cytosine DNA methylation (TABLE 5). Cleary, more information on molecular mechanism of the protective role of polyamines against DNA methylation in plants are needed.

5. CONCLUSION

As a conclusion we could state that putrescine is a protective material in drought stress conditions the points of DNA damage and DNA methylation alterations in wheat. RAPD and CRED-RA are used as accurate and reliable techniques as well as antioxidant and oxidant enzyme measurements confirm this opinion. In order to clarify the molecular mechanism of these applications it is necessary to measure the expression values of antioxidant enzyme genes in future studies.

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