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Research Article

Putrescine in Herbicide Stress Protection: Modulate the Genomic Instability and DNA Methylation Changes in Wheat

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Abstract

Wheat is one of the most consumed and important food in worldwide. During its growing season, weeds around the cultivated areas grow rapidly and inhibit the normal growth and development, stable yield and quality of wheat seriously. The prevention and removal of weeds are achieved by herbicide treatments. Dicamba is one of the herbicides that is used in agricultural areas which may represent a potential genotoxic risk to off-target crops. The present study was aimed to evaluate the effect of dicamba (0.2, 0.4 and 0.6 ppm) which caused to destabilize of genomic template stability (GTS) and DNA methylation changes in Triticum aestivum L. seedlings by RAPD (Randomly Amplified Polymorphic DNA) and CRED-RA (Coupled Restriction Enzyme Digestion-Random Amplification) techniques, respectively. Also, Full Methylation Ratio and Methylation Ratio were computed according to data of CRED-RA patterns. It was determined that the damage raised with an increasing dose of dicamba. To minimalize the genotoxic effects of dicamba, putrescine (0.01, 0.1 and 1 ppm), a kind of polyamine, were used. Especially, 1 ppm of putrescine was the best concentration to revert the stress-exposed wheat seedlings. Polyamines are positively charged organic cations and hence they interact with negatively charged macromolecules such as DNA and RNA and stabilize them. The results of this experiment have clearly shown that putrescine could be used effectively to protect wheat seedlings from the effects of dicamba on DNA damage and DNA methylation changes, also RAPD and CRED-RA could be used as ideal techniques to get reliable and accurate results.

Keywords: Dicamba, RAPD, CRED-RA, Polyamines.

Herbisit Stres Korumasında Putresin: Buğdayda Genomik Kararsızlığı Azaltma ve DNA Metilasyon Değişiklikleri

Öz

Buğday, dünyada en çok tüketilen önemli gıdalardan biridir. Büyüme mevsimi boyunca, ekili alanların etrafındaki yabani otlar hızla büyümekte ve buğdayın normal büyüme ve gelişmesini, verimini ve kalitesini ciddi şekilde engellemektedir. Yabancı otların önlenmesi ve giderilmesi, herbisit uygulamaları ile sağlanır. Dikamba, tarım alanlarında hedef olmayan ürünler için potansiyel genotoksik riskleri oluşturabilecek olan herbisitlerden biridir. Bu çalışmada Triticum aestivum L. fidelerinde genomik kalıp stabilitesinin (GTS) ve DNA metilasyonunun değişmesine neden olan dikambanın (0,2, 0,4 ve 0,6 ppm) etkisinin RAPD (Rastgele Çoğaltılmış Polimorfik DNA) ve CRED-RA (Çift Restriksiyon Enzimi Kesimi ve Rastgele Çoğaltım) teknikleri ile değerlendirilmesi amaçlanmıştır. Ayrıca Tam Metilasyon ve Metilasyon Oranları CRED-RA verilerine göre hesaplanmıştır. Dikamba dozu arttıkça oluşan hasarında arttığı belirlenmiştir. Dikambanın genotoksik etkilerini en aza indirmek için bir tür poliamin olan putresin (0.01, 0.1 ve 1 ppm) kullanılmıştır. Özellikle, 1 ppm putresin strese maruz kalan buğday fidelerini eski haline döndüren en iyi konsantrasyon olarak belirlenmiştir. Poliaminler pozitif yüklü organik katyonlardır ve bu nedenle DNA ve RNA gibi negatif yüklü makromoleküllerle etkileşir ve kararlılığını sağlarlar. Bu çalışmanın sonuçlarına göre buğday fidelerinde dikambanın sebep olduğu DNA hasarı ve DNA metilasyon değişikliklerinden korumak için putresin etkili bir şekilde kullanılabilirken, RAPD ve CRED-RA'nın güvenilir ve doğru sonuçlar elde etmek için ideal teknikler olarak kullanılabileceği önerilmektedir.

Anahtar Kelimeler: Dikamba, RAPD, CRED-RA, Poliaminler.

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1. Introduction

Dicamba (3,6-dichloro-2-methoxy benzoic acid) is a benzoic synthetic auxin herbicide which widely used to control broadleaf weeds in cereal crops cultivated areas all over the world. Dicamba is used as an important alternative herbicide especially in wheat cultivated areas, which is one of the most produced and consumed in people's diets (Kurt and Dizlek, 2020), for the control of weeds that are resistant to other herbicides such as 2,4-D, picloram, glyphosate, etc. Post-emergent applied dicamba acts as auxin and stimulates cell elongation and cellular differentiation, causing rapid growth of leaves, stems, and petioles. This abnormality damages the cellular transport system and leads to the death of the weeds (Heap, 2014). Despite the natural selectivity mechanisms to prevent damage to off-target crops, herbicides can cause several damages. In this case, off-target plants grown in the same area are exposed to dicamba at the same time and can experience some phytotoxic damages such as malformation, necrosis and also substantial injuries and yield loss (Egan and Mortensen, 2012). A previous research has documented that dicamba has caused to a reduction of yield, plant height and induction of visible injury in winter wheat. Also, wheats are influenced by this herbicide according to application timing and temperature (Robinson et al., 2015). As well as McCauley et al., (2006) reported that all of the pesticides have an impact on oxidative stress which stimulated by increasing reactive oxygen species (ROS), also DNA and RNA damages and gene expression changes. In our previous researches, we determined that picloram and deltamethrin have caused DNA damage by decreasing the genomic template stability (GTS) and also give rise to DNA methylation changes (Taspinar et al., 2017a; 2017b).

One possible remedy to reduce these negative effects of pesticides on off-target plants is to use externally applied alternative molecules. Polyamines (PAs) (putrescine, spermidine, spermine, and cadaverine) are linear polycations which were located in the nucleus of eukaryotic cells. Among them, putrescine is the first synthesized and most abundant in the cell. They were involved in a wide range of biological functions such as regulation of gene expressions and enzyme functions, activation of DNA synthesis, transcriptional mechanisms, cell proliferation, etc. (Iacomino et al., 2012). Additionally, PAs have important functions in cellular DNA protection against external impacts like abiotic stress because of their positively charged molecules causing the bond to the negatively charged DNA where they can stabilize and remodel the chromatin structure and modulate gene expression. It is known that changes in the cellular PA levels may affect the DNA methylation level. The increasing level of PAs during the plant development prevents DNA methylation by providing expression of some specific genes (Walters, 1997). The fact that moving from the major grooves of A-DNA to minor grooves of B-DNA where DNA methylation occurs less is evidence of this (Ruiz-Herrera et al., 1995).

Although the contribution of PAs to plant growth and the protective role on DNA is known, its response to during dicamba treatment is unknown. Thus, this study has been conducted on the aftereffect of dicamba on genomic instability and DNA methylation changes and whether exogenously applied putrescine has any protective effects on these parameters in bread wheat by using RAPD and CRED-RA techniques, respectively.

2. Material and Method

2.1. Plant material, growth and treatment conditions

The plant material (*Triticum aestivum* L. Bezostaja 1) was provided by Ataturk University, Faculty of Agriculture. The equal sizes of wheat seeds were immersed in 70% ethanol for 1 min and 1% sodium hypochlorite for 10 min, respectively and washed thrice with autoclaved water. 20 of sterile seeds were placed on 9-cm diameter glass Petri dishes have two sheets of filter paper (Whatman #1). Five-milliliter aliquots of dicamba solutions (0.2, 0.4 and 0.6 ppm) and putrescine solutions (0.01, 0.1 and 1 ppm) alone or combined with each other were applied to seeds. Also, a control group was created which only applied diluted water. Each treatment included three biological replicates. The Petri dishes were placed on a laboratory bench at room temperatures (18–20°C). After 8 days, the samples were harvested from three different plants for each replication of treatments. The strategy of bulk DNA sampling was applied.

2.2. DNA isolation

Total DNA isolation method used by Yildirim et al., (2014) was extended and modified. After DNA isolation, the concentrations and purities of DNA samples were checked in NanoDrop-1000 spectrophotometer (OD 260/280) and 1.2% agarose gel electrophoresis stained with ethidium bromide.

2.3. RAPD

The RAPD reaction was performed according to the method developed by Yildirim et al., (2014). 32 arbitrary RAPD primers (Operon Technologies Inc., USA) were tested. Only 10 of them (OPA-4, OPA-12, OPB-10, OPH-16, OPH-18, OPW-4, OPW-13, OPW-11, OPY-7, C-10) amplified polymorphic amplicons and used in RAPD-PCR reactions (Table 1). The RAPD-PCR reactions were carried out in a 20 µL volume in each reaction tube contained 30 ng template DNA, 300 µM of dNTP, 1.5 mM of MgCl2, 1 U Taq DNA polymerase, 1X PCR buffer (10X) and 25 pmol of primer. The amplification was performed in a thermocycler (Sensoquest GmbH, Labcycler Gradient, Germany) using the following steps: 95°C for 5 min; 36 cycles at 94°C for 1 min., 36°C for 1 min., 72°C for 2 min; final extension at 72°C for 15 min. PCR products were resolved on 1% agarose gel electrophoresis in 1X TBE buffer using 1 kb DNA ladder molecular mass marker and were stained with 0.5 mg/ml ethidium bromide, and were photographed under UV transilluminator.

Table 1. The primer sequences used for RAPD

Primer Name	Sequence (5'-3')	Primer Name	Sequence (5'-3')
OPA-4	AATCGGGCTG	OPW-4	CAGAAGCGGA
OPA-12	TCGGCGATAG	OPW-13	CACAGCGACA
OPB–10	CTGCTGGGAC	OPW-11	CTGATGCGTG
OPH-16	CAGGCCCTTC	OPY-7	AGAGCCGTCA
OPH-18	GAATCGGCCA	C-10	TGTCTGGGTC

2.3.1. RAPD analysis

The RAPD patterns were evaluated and confirmed according to the disappearance of a normal band and appearance of a new band compared with the control using the TotalLab TL120 computer software. Finally, genomic template stability (GTS, %) was calculated for each dose and each primer as per following formula: GTS = 100-(100x a/n), where a is the average number of polymorphic bands detected in each treated sample, and n is the number of total bands in the control.

2.4. CRED-RA

The CRED-RA technique was conducted according to Yildirim et al., (2014). The genomic DNA samples were digested with restriction enzymes (MspI and HpaII) according to manufacturers protocol (New England Biolabs, Beijing, China, #R0106 and #R0171). After checking the digestion of DNA samples on 1.2% agarose gel, the total digestions were randomly applied by random PCR using primers that used for RAPD analysis. Amplification and visualization methods are the same as described for RAPD techniques.

2.4.1. CRED-RA analysis

The CRED-RA patterns were analyzed with TotalLab TL120 program to determine the polymorphism value (%). After assigned the appeared and disappeared bands in all CRED-RA gel patterns, the polymorphism value (%) was computed with this formula: 100x a/n. Apart from this, methylation patterns were confidently confirmed into four classes (Table 2) according to the ability of MspI and HpaII enzymes digestions. Both enzymes recognize the same sequence (5'-CCGG-3') but digestion conditions are different from each other as shown in Table 2. Finally, Full methylation and Methylation ratios were determined (Shams et al., 2020).

Table 2. Methylation sensitivity and restriction pattern of enzymes (Zhang et al., 2016)

Types	Methylation status	Digestibility of enzymes	
		HpaII	MspI
Class I	CCGG C<u>C</u>GG GGCC GGCC	Active	Active
Class II	<u>C</u>CGG GGCC	Active	Inactive
Class III	C<u>C</u>GG GG<u>C</u>C	Inactive	Active
Class IV	<u>C</u>CGG GGC<u>C</u>	Inactive	Inactive

3. Results and Discussion

3.1. Genomic Template Stability Changes

Besides RAPD is rapid, reliable and nonradioactive technique compared to other techniques such as comet and micronucleus assay that determine genetic damages, it is best to determine the temporary DNA damages caused by lower concentrations of pollutants that may not finally be showing themselves as mutations (Rocco et al., 2014; Liu et al., 2005). We further investigated the changes in GTS by RAPD technique during the dicamba stress and putrescine treatments. Among the 32 arbitrary RAPD primers, only 10 of them amplified and gave specific band patterns (Table 3). While all of the primers generated totally 80 bands (ranged from 60 pb to 1050 pb) in control treatment, each of primer generated 3-8 new bands in all treatments. As shown in Table 3, both treatments (alone or combined with each other) obtained important differences. These were shown themselves as appearance and disappearance of bands. After dicamba treatments, totally 11 bands which existed in the control, disappeared and 18 bands appeared. Band changes in the RAPD-PCR profile prove that the genetic material has changed and there is existing damage to DNA. Values of GTS were 71.9%, 65.6% and 64.6% in 0.2, 0.4 and 0.6 ppm dicamba treatments, respectively (Table 3).

Table 3. Molecular sizes (bp) of bands (+: appearance / -: disappearance) and the average GTS values in RAPD profiles

Primers	Control	+/-	Dicamba	0 ppm putrescine			1 ppm putrescine				0.1 ppm putrescine				0.01 ppm putrescine			
				0.2 ppm	0.4ppm	0.6ppm	0 ppm	0.2 ppm	0.4 ppm	0.6 ppm	0 ppm	0.2 ppm	0.4 ppm	0.6 ppm	0 ppm	0.2 ppm	0.4 ppm	0.6 ppm
OPA-4	8	+		315; 562; 814	315; 562; 814	315; 562; 814	115	115	115	640	-	-	723	723	520	520	520	520; 312
		-		-	125	125	-	-	-	-	-	118	118	118	512	512	512	512
OPA-12	6	+		482	482	482	-	-	616	616	821	821	821	821	318	318	318	318
		-		216	216	216	-	-	-	-	216	-	-	-	-	-	618	618
OPB–10	9	+		465; 612	465; 612	465; 612	-	-	804	804	554	-	-	554; 380	719; 312	719; 312	719; 312	719; 312
		-		-	96	96	514	514	-	-	-	170	170	-	247	247	247	247; 319
OPH-16	10	+		-	615	615	330	330	330	330	661	-	661	661; 1025	186	186	186; 247	186; 247
		-		910	910; 554	910	-	-	-	-	432	-	-	-	-	-	319; 193	319; 193
OPH-18	8	+		248; 625	248; 625	248; 625	460	460	460	460	-	-	-	575	772	-	772	772
		-		122	122; 718	122; 718	-	215	-	215	-	184	184	-	-	-	-	614
OPW-4	6	+		184; 612	184; 612	184; 612	-	-	110	110	310	310	310	-	496; 513	496; 513	496; 513	496; 513
		-		-	-	-	-	-	-	-	-	-	-	147	-	-	-	-
OPW-13	9	+		-	517; 1015	517; 1015	314	314	314	-	-	-	-	-	358; 464	358; 464	358; 464	358; 464
		-		294	718	294; 718	-	-	-	152; 404	-	95	95	95; 406	-	-	-	298
OPW-11	8	+		182	182; 546	182	225	225	225	-	482	482; 715	482	482; 715	664	664	664	664
		-		305	305	305	90	90	-	-	-	-	-	-	-	-	712	712
OPY-7	6	+		818	818	-	715	715	715	715	954	-	954	-	256	-	256	256
		-		256; 590	256; 590	256; 590	-	-	-	-	-	-	-	156	-	-	882	882
C-10	10	+		118; 445	118; 445	118; 445	-	224	-	-	-	325	-	-	473; 562	473; 562	473; 562	473; 562
		-		-	-	-	-	-	-	519	-	-	-	610	-	-	-	-
GTS	8			**71.9**	**65.6**	**64.6**	**90**	**87.8**	**88**	**85.8**	**89**	**88.4**	**86.7**	**81.7**	**79.7**	**82.6**	**72.1**	**67.4**

These percentages proved that dicamba caused to DNA damage. Dicamba is a common herbicide worldwide to destroy broadleaf weeds. It is known that dicamba mixed with soil after application to the plant is very mobile and degraded by mineralization or biologic activation. Apart from this, its short half-life is considered an advantage instead of a threat to environment. However, the spread of the tolerant and resistant weeds in the planting areas spurred the farmers to use more frequent and higher concentrations application of herbicide using (Benbrook, 2016). So, it poses a danger to off-target plants and causes physiological, biochemical and molecular damages (Carla et al., 2018). In previous studies, it would be stated in any kind of plant and animal that dicamba caused to genotoxic effects such as point mutations and single/double-strand DNA breaks (González et al., 2006; Cenkci et al., 2010; González et al., 2011). Despite these effects is known, the mechanism of genotoxic damage caused by dicamba is not yet well characterized. However, it was assumed that dicamba had genotoxic damage by increasing the amount of ROS (Duchnowicz and Koter, 2003; González et al., 2006). In this study, we also evaluated the effects of putrescine on GTS in dicamba stressed wheat plants. Whereas putrescine had quite high percentages (90%, 89% and, 79.7%) in alone treatments, when applied putrescine and dicamba together, putrescine increased the rate of reduced GTS caused by dicamba alone (Table 3). Putrescine, a kind of PAs, is connected in a wide range of physiological and cellular processes, such as growth, development, and cell division in higher organisms (Miller Fleming et al., 2015). Since it has a positive charge, it creates a bond with the negatively charged DNA and RNA where they can stabilize and remodel the chromatin structure and modulate gene expression (Miller Fleming et al., 2015). Also, the amount of PAs in the cell are commonly accumulated in abiotic stress. Taie et al., (2019) stated that polyamines modulated growth, antioxidant activity and DNA in heavy metal stress treated wheat. According to Park et al., (2019), PAs could promote antioxidant capacity and osmotic adjustment ability of tobacco when plants were subjected to salt stress. Based on this, in our study, while the amount of endogenous putrescine increased with dicamba stress, when combined with exogenously applied putrescine it is thought they had a compact and stronger effect together.

3.2. DNA Methylation Changes

DNA methylation is one of the epigenetic mechanisms that change gene expression without altering DNA sequence (Deng et al., 2018). Recently, a growing number of studies have indicated that DNA methylation status changes when plants encounter with abiotic stresses such as drought, salt, UV radiation, pesticide stress, etc. (Colicchio et al., 2015; Brzezinka et al., 2016). DNA methylation may be a significant mechanism involved in the regulation of plant response and defense to contaminants like pesticides. Lu et al., (2016) reported that atrazine induced changes in DNA methylation by activating specific genes. In our study, we used CRED-RA technique to determine DNA methylation changes in dicamba treatments along with putrescine and we were able to get outstanding results. The average polymorphism values were detected for each concentration of each treatment (Table 4).

Table 4. CRED-RA band amounts and polymorphism %

Primers	Dicamba	0 ppm putrescine		1 ppm putrescine		0.1 ppm putrescine		0.01 ppm putrescine	
		H	M	H	M	H	M	H	M
OPA-4	0 ppm	-	-	11.1	0	0	0	0	16.6
	0.2 ppm	0	0	0	20	0	12.5	0	0
	0.4 ppm	14.2	0	0	0	0	0	14.2	0
	0.6 ppm	12.5	0	0	11.1	0	0	0	16.6
OPA-12	0 ppm	-	-	20	0	0	0	0	11.1
	0.2 ppm	25	0	0	0	0	0	0	11.1
	0.4 ppm	0	14.2	16.6	0	16.6	0	0	12.5
	0.6 ppm	0	20	0	11.1	0	16.6	0	0
OPB–10	0 ppm	-	-	0	14.2	0	16.6	0	0
	0.2 ppm	0	16.6	0	0	12.5	0	20	0
	0.4 ppm	0	0	11.1	0	0	0	20	11.1
	0.6 ppm	0	0	0	0	0	0	0	0
OPH-16	0 ppm	-	-	0	0	11.1	0	0	0
	0.2 ppm	0	14.2	20	0	0	0	16.6	0
	0.4 ppm	0	0	0	0	0	20	0	0
	0.6 ppm	0	0	0	0	20	0	11.1	20
OPH-18	0 ppm	-	-	0	0	12.5	0	14.2	14.2
	0.2 ppm	0	20	16.6	16.6	0	0	0	14.2
	0.4 ppm	40	16.6	0	25	0	0	11.1	0
	0.6 ppm	25	16.6	12.5	0	16.6	0	0	0
OPW-4	0 ppm	-	-	0	12.5	0	16.6	11.1	11.1
	0.2 ppm	33.3	16.6	0	0	14.2	0	0	11.1
	0.4 ppm	0	0	16.6	0	0	0	0	12.5
	0.6 ppm	20	33.3	0	0	0	0	20	0
OPW-13	0 ppm	-	-	0	12.5	20	0	11.1	0
	0.2 ppm	20	0	0	0	0	0	11.1	0
	0.4 ppm	0	16.6	0	0	0	11.1	0	0
	0.6 ppm	0	0	25	14.2	0	0	20	0
OPW-11	0 ppm	-	-	0	0	0	0	0	20
	0.2 ppm	14.2	20	0	0	0	20	0	20
	0.4 ppm	28.6	14.2	0	11.1	25	0	11.1	0
	0.6 ppm	0	0	0	0	0	14.2	0	0
OPY-7	0 ppm	-	-	12.5	0	0	14.2	11.1	0
	0.2 ppm	0	0	0	0	20	0	11.1	0
	0.4 ppm	0	0	0	0	0	16.6	0	20
	0.6 ppm	0	0	0	16.6	0	0	0	11.1
C 10	0 ppm	-	-	0	0	14.2	0	11.1	0
	0.2 ppm	0	0	0	0	0	11.1	11.1	0
	0.4 ppm	0	14.2	0	0	0	0	0	12.5
	0.6 ppm	0	0	0	0	0	11.1	0	0
Polymorphism value (%)	**0 ppm**	**-**	**-**	**4.3**	**3.9**	**5.8**	**4.7**	**5.8**	**7.3**
	0.2 ppm	**9.2**	**8.7**	**3.7**	**3.7**	**4.7**	**4.4**	**6.8**	**5.7**
	0.4 ppm	**8.2**	**7.5**	**4.4**	**3.6**	**4.2**	**3.1**	**5.7**	**6.1**

	0.6 ppm	5.7	6.9	3.8	3.6	3.7	4.2	5.1	5.0

While the averages of polymorphism were 8.7%, 7.5% and 6.9% in dicamba alone, these values were detected as 7.3%, 4.7% and 3.9% in putrescine alone. However, there was a reduction in this value when dicamba and putrescine used together. Therefore, we can say that dicamba reduces DNA methylation, but putrescine prevents this reduction depending on the concentrations.

On the other hand, we determined the classes of DNA methylation patterns according to the status of Hpa II and Msp I enzymes digestions. 10 primers were used for the differentiation of classes. According to the number of classes, Full Methylation Ratio and Methylation Ratio were computed for each treatment and concentrations (Table 5).

Table 5. Methylation levels of dicamba and/or putrescine treated wheat seedlings (Shams et al., 2020)

Classes	0 ppm putrescine			1 ppm putrescine				0.1 ppm putrescine				0.01 ppm putrescine			
	0.2 ppm	0.4 ppm	0.6 ppm	0 ppm	0.2 ppm	0.4 ppm	0.6 ppm	0 ppm	0.2 ppm	0.4 ppm	0.6 ppm	0 ppm	0.2 ppm	0.4 ppm	0.6 ppm
Class I %	67.5	64.44	63.83	65.96	74.5	70.83	70	69.57	72.55	67.44	67.92	65.96	65.91	65.85	64.44
Class II %	10	11.11	14.9	12.77	9.80	12.5	8	8.70	11.76	9.3	5.66	10.64	6.82	9.76	11.11
Class III %	2.5	4.44	8.51	4.26	3.92	6.25	6	4.35	1.96	6.98	9.43	8.51	6.82	4.88	8.89
Class IV %	20	20	12.77	17.02	11.76	10.42	16	17.39	13.73	16.28	16.98	14.89	20.45	19.51	15.56
Full Methylation Ratio[1]	22.5	24.44	21.28	21.28	15.68	16.67	22	21.74	16.69	23.26	26.41	23.4	27.27	24.39	24.45
Methylation Ratio[2]	32.50	35.61	36.18	34.05	25.48	29.17	30	30.44	28.45	32.56	32.07	34.04	34.09	34.15	35.56

1 Full Methylation Ratio: (III+IV / I+II+III+IV) X 100
2 Methylation Ratio: (II+III+IV / I+II+III+IV) X 100

All of the classes were confirmed in CRED-RA patterns. When the average rates of Classes were scanned, Class I (no methylation or only inner methylation in a single strand occurred) was the most prevalent one from them. It was followed by Class IV (both DNA strands are methylated at the outer cytosines), Class III (both DNA strands are methylated at the inner cytosines) and Class II (the outer cysteine of a single strand is methylated), respectively.

Full Methylation Ratios did not enable us to make a perfect distinction between treatments, however, it was not the same for Methylation Ratio. Dicamba had an important impact on ratios that Methylation Ratio gradually increased as the concentrations raised. The highest Methylation Ratio (36.18%) was detected in 0.6 ppm dicamba and the lowest (25.48%) was in 1 ppm putrescine combined with 0.2 ppm dicamba treatment. Therefore, there was a certain degree of difference between the dicamba and putrescine treatments. When we analyzed the putrescine concentrations combined with dicamba, we could say that 1 ppm putrescine was the most effective concentration that caused to decrease in the Methylation Ratio of 0.6 ppm dicamba (36.18%) to 30% (Table 5). This ameliorative effect of 1 ppm putrescine was positively correlated with its Polymorphism value (%) that were presented in Table 4.

As far as we know, there is no information available on the DNA methylation of treatment with different concentrations of putrescine together with dicamba in wheat seedlings. Previous studies found that polyamines affected selective inhibition of cytosine-DNA methylases (Ruiz Herrera et al., 1995; Valledor et al., 2007). Some studies revealed 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 cadaverine bind to both sugar-phosphate backbone and major and minor grooves. 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 (Minocha et al., 2004; Wada et al., 2004; Kuznetsov et al., 2006). So, polyamines could inhibit DNA methylation, which permits the expression of specific genes responsible for the synthesis of stress protein.

4. Conclusions and Recommendations

This study was clearly showed that dicamba caused to DNA damage and DNA methylation changes according to data of RAPD and CRED-RA. These effects of dicamba were gradually increased when the concentrations were raised. Also, we used putrescine to mitigate the genotoxic effects of dicamba and we got successful results so that putrescine was decreased these harmful effects effectively. RAPD and CRED-RA could be used as reliable techniques to determine the GTS, Polymorphism, Full Methylation and Methylation values. Even though there were some studies to understanding the relationship of herbicide stress on DNA damage and DNA methylation changes, the protective mechanism of polyamines against dicamba is not exactly known. Therefore, more detailed studies are needed for further understanding of this mechanism.

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