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Protective Role of Putrescine against Picloram Induced Genomic Instability and DNA Methylation in *Phaseolus vulgaris*

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ABSTRACT: Picloram (4-amino-3-5-6-trichloropicolinic acid) is an important synthetic auxin and it is one of the most widely used herbicides in agriculture. The use of picloram is representing a potential hazardous to ecosystems and the human health. Putrescine is a kind of polyamine which has a role in plant metabolism such as protecting membrane stability, removing free radicals and nucleic acid and protein synthesis, etc. In this study, DNA damage levels in *Phaseolus vulgaris* treated with picloram, DNA methylation changes and whether putrescine was any alleviative impact on these alterations were investigated. DNA methylation pattern changes and DNA damage levels were determined by using Randomly Amplified Polymorphic DNA (RAPDs) and Coupled Restriction Enzyme Digestion-Random Amplification (CRED-RAs). The obtained results indicated that all doses of picloram (5, 10, 20 and 40 mg L^{-1}) had a negative effect on RAPDs profile changes (increased DNA damage levels) and decreased of Genomic Template Stability (GTS) and also DNA hypomethylation was seen. However, all concentrations of putrescine (0.01, 0.1 and 1 ppm) reduced these harmful effects of picloram. Consequently, putrescine can be an alternative for reducing genotoxic damage against chemical mutagens in plants.

Keywords: DNA methylation, genomic instability, picloram, putrescine

Phaseolus vulgaris'te Genomik Kararsızlık ve DNA Metilasyonunu İndükleyen Piklorama karşı Putresinin Koruyucu Rolü

ÖZET: Pikloram (4-amino-3-5-6-trikloropikolinik asit) önemli bir sentetik oksin olup, tarımda en çok kullanılan herbisitlerden biridir. Pikloram kullanımı, ekosistem ve insan sağlığı için potansiyel bir tehlike oluşturmaktadır. Bir poliamin çeşidi olan putresinin bitki metabolizmasında; membran stabilitesinin korunması, serbest radikallerin uzaklaştırılması ve nükleik asit ve protein sentezi gibi rolü vardır. Bu çalışma, pikloram uygulanan *Phaseolus vulgaris'* teki DNA hasar düzeyleri ve DNA metilasyon değişiklikleri ile putresinin bu değişimler üzerinde koruyucu etkisinin olup olmadığının belirlenmesini amaçlamıştır. DNA hasar düzeylerini ve DNA metilasyon değişimlerini belirlemek için Rastgele Çoğaltılmış Polimorfik DNA (RAPD) ve Çift Restriksiyon Enzim Kesimi-Rastgele Çoğaltım (CRED-RA) kullanılmıştır. Sonuçlar, tüm pikloram dozlarının (5, 10, 20 ve 40 mg L^{-1}) RAPD profil değişikliklerini (DNA hasarının) artırdığını ve Genomik Kararlılık Stabilitesini (GTS) azalttığını ve ayrıca DNA hipometilasyonunun oluştuğunu göstermiştir. Bununla birlikte, kullanılan tüm putresin konsantrasyonları (0.01, 0.1 ve 1 ppm) pikloramın bu zararlı etkilerini azaltmıştır. Sonuç olarak, putresin, bitkilerdeki kimyasal mutajenlere karşı genotoksik hasarı azaltmak için bir alternatif olabilir.

Anahtar Kelimeler: DNA metilasyonu, genomik kararsızlık, pikloram, putresin

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INTRODUCTION

Picloram is an important synthetic auxin that was used often as a supplement in plant cell culture media such as Murashige and Skoog's (MS) in the plant research laboratories. It was also used to prevent unwanted and invasive broadleaf weeds present in fields. However, picloram is highly toxic to many non-target plants that were grown nearby plants through the roots in soil. Moreover, irrigation water is polluted with picloram application into rangeland, pastures and forests and may be hazardous to endangered plants. Studies indicated that picloram caused increasing viscosity of the cytoplasm in leaf tissue and in stamen hairs, necrosis, destroying of nuclear structure, mitosis decreasing, micronucleus increasing, pink mutation and chromosomal aberration in higher plants (Mohammed and Ma, 1999; Olea-Popelka et al., 2005). It was determined to be a teratogen, carcinogen, potent mutagen and also reproductive effects in several species of experimental animals were identified (Oakes et al., 2002). Recently, several studies have demonstrated that the some herbicides such as 2,4-dichlorophenoxyacetic acid lead to alteration of gene expression levels of some genes through DNA methylation (Leljak-Levanic et al., 2004). Temel and Gozukirmizi (2013) reported that dicamba caused increasing in cytosine methylation and changed in retro element movements in barley callus culture. Plants may be followed different ways in order to protect from stress; resistance, tolerance and avoidance. These strategies effect somatic growth (Sung and Amasino, 2004), heritable, modifications of gene expression known as epigenetic and abscisic acid (ABA), salicylic acid and polyamine levels (Grativol et al., 2012). Especially, polyamines such as putrescine, spermidine, spermine may establish a correlation with proteins, nucleic acids, membrane phospholipids and cell wall constitutents. Numerous studies claim that polyamines bound to guard DNA from enzymatic degradation, mechanical shearing and X-ray irradiation and also stabilize RNA to counteract of ribosomal dispersion (Miyamoto et al., 1993; Ruiz-Herrera et al., 1995). Additionally, some researchers also suggested that polyamines have ability to protect replicating DNA from oxidative damages (Khan et al., 1992; Miyamoto et al., 1993; D'Agustino et al., 2005). It was first reported that polyamines had the role of selective inhibition cytosine- DNA methylases by prevent both the binding and activity of these enzymes (Ruiz-Herrera et al., 1995). Although we have a lot of knowledge about the effects of picloram on non-target organisms, there is no report shown the effect of picloram on DNA methylation and DNA damage (by using the RAPD technique). Thus, the goal of this study was to explain DNA methylation changes and genotoxic potential of the picloram on bean seedlings and discuss if putrescine has any effect on these parameters.

MATERIAL AND METHOD

Plant Growth and Treatment Conditions

Phaseolus vulgaris L. cv. Elkoca seeds were obtained from Department of Field Crops, Faculty of Agriculture, Ataturk University, Erzurum, Turkey. Firstly, sterilization was made with in 0.5% sodium hypochlorite (NaOCl) solution for 10 min, after sterile water was used for rinse the seeds. 25 seeds were put in each plastic box which included two layers of filter paper moistened with 25 ml distilled water. 25 ± 1°C and dark conditions were provided until primary roots were grown at 0.5-1 cm length. Then, different concentrations of picloram (5, 10, 20 and 40 mg L^{-1}) and putrescine (0.01, 0.1 and 1 ppm) solutions were exposed to bean seedlings for 8 h (Zeid and Shedeed, 2006). Treated seedlings were grown in pots of a peat/soil mix (5 plants/pot) at 25±1°C with a 16 h photoperiod of 60 μmol photons/m^2s provided by white fluorescent lamp, at a relative humidity of 70–75% in a growth chamber (Sanyo Model, MIR 253, Sanyo Electric Biomedical Co. Ltd., Japan). 3 replicates were made. Bulk leaves were randomly collected from ten plants for each treatment after emergence of leaf 3 (leaves numbered from base) and immediately taken in -80°C.

Genomic DNA Isolation, RAPDs and CRED-RAs Procedures and PCR Methods

CTAB method (Taspinar et al., 2009) was used for genomic DNA (gDNA) isolation. RAPD-PCR was used for determining the genetic damage. 32 RAPD primers (Operon Technologies Inc., Alameda, CA, USA) were tested with bulked DNA of control treatment (0 mg L^{-1} picloram + 0 ppm putrescine). Only 14 primers were used in RAPD-PCR reactions due to amplified polymorphic amplicons and 8 primers were selected for CRED-RA PCR reactions according to have most polymorphic bands from RAPD primers (Table 1).

Table 1. The primer sequences used for RAPD and CRED-RA analysis

Primer Name	Sequence (5'-3')
OPW-20	TGTGGCAGCA
OPA-4	AATCGGGCTG
OPW-13	CACAGCGACA
OPA-1	CAGGCCCTTC
OPH-18	GAATCGGCCA
OPH-19	CTGACCAGCC
OPY-8	AGGCAGAGCA
OPW-17	GTCCTGGGTT
OPY-11	AGACGATGGG
OPY-13	GGGTCTCGGT
OPY-15	AGTCGCCCTT
OPW-4	CAGAAGCGGA
OPY-16	GGGCCAATGT
OPH-16	TCTCAGCTGG

RAPD-PCR reaction mix contents and conditions were given in Table 2.

Table 2. PCR reaction mix contents and conditions

PCR Reaction Mix Contents	
PCR buffer	1X
$MgCl_2$	2.5 mM
dNTP	400 μM
gDNA	50 ng
Primer	10 pmol
Taq DNA polymerase	1 *U*
Total volume of each sample	20 μL
PCR Conditions	
RAPD:1 cycle at 95°C for 5 min, 42 cycles of (94°C for 1 min, 36°C for 1 min and 72°C for 2 min), 1 cycle at 72°C for 15 min **CRED-RA:**1 cycle at 95°C for 5 min, 38 cycles of (94°C for 1 min, 36°C for 1 min and 72°C for 2 min), 1 cycle at 72°C for 15 min.	

There is a significant difference in CRED-RA-PCR reaction mix that gDNA samples from each treatment were separately digested with *HpaII* (New England Biolabs, Beijing, China, #R0171) and *MspI* (New England Biolabs, Beijing, China, #R0106) endonucleases according to manufacturers's instructions (which cut the sequence 5´-C/CGG-3´with different sensitivity to cytosine methylation; *MspI* cuts if the inner C is methylated, whereas *HpaII* cannot cleave in the presence of methyl groups). So, 50 ng digested

DNA (for each endonuclease) samples were added in PCR mix instead of non-digested gDNA.

Electrophoresis

RAPDs and CRED-RAs amplification products were resolved on 1% agarose gel (0.5X TBE buffer, 80 V for 150 min). Gels dyed with ethidium bromide displayed under UV light and photographed by gel visualization system (DNR MiniBis 16mm pro). DNA ladder (50-10 000 bp, Sigma-D7058) was used for estimation of the sizes of fragments.

Analysis

Total Lab TL120 computer software was used for evaluating of RAPD patterns. Genomic Template Stability (GTS, %) was calculated as follows: GTS = 100-(100x *a*/*n*). 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* (Yildirim et al., 2014).

RESULTS AND DISCUSSION

Picloram is an important synthetic auxin and is used in agriculture as a herbicide to control a wide range of agricultural and horticultural crops with many annual weeds and broad-leaved weeds. Picloram is highly toxic to many non-target plants. Furthermore, irrigation water contaminated with picloram can damage or kill crop plants. In fact, picloram applied to pastures, rangeland, or forests may be hazardous to endangered plants. This study evaluated the genotoxic effects of picloram by using RAPD technique. In RAPD analysis, a set of 32 different synthetic oligonucleotids were tested with control DNA sample and 14 out of these were resulted specific and stable DNA profiles in *Phaseolus vulgaris* genome. The treated plant samples (picloram and/or putrescine) showed significant differences according to the untreated samples. These differences were showed as variation in band intensity, loss of normal bands or appearance of new polymorphic bands. Each primer produced a band gap of 3-9 with an average of 4.85 bands per primer. According to the obtained results; amplified bands were ranged from 322 (OPA-4) to 3205 (OPY-16). The molecular sizes of bands that gained or lost among the control and treatment groups were given in Table 3. Changes in RAPD profiles were measured as GTS (a qualitative measurement reflects changes in RAPD patterns) as regards the pattern showed in the control treatment. Increased picloram concentration caused decreasing GTS value. The highest GTS value (60.9%) was observed in 5 mg L^{-1} picloram treatment, while the lowest GTS value that shown harmful effects of picloram (46.6%) was observed in 40 mg L^{-1} picloram treatment. However, increased putrescine concentration caused increasing GTS value in picloram exposed plants.

The high level putrescine dose (1 ppm) was found to be the best dose for GTS (87.5%) in both picloram treated and putrescine untreated plants. Our conclusions are consistent with the previously demonstrated genotoxic effect of picloram with various mutagenens tests in plants (Mohammed and Ma, 1999; Salvi et al., 2001; Correia et al., 2011). Sawamura and Jackson, (1968) have reported that picloram decreased the rate of cytoplasm streaming in stamen hairs of *Tradescantia*, while Brownian movement increased and numerous and size of chloroplasts affected in *Vicia* leaf.

Although the DNA damage mechanism of picloram is not illuminated yet, previous researches indicated that acidic herbicides such as 2,4-D can stimulate the release of free radicals, including reactive oxygen species with oxidative DNA damage (Balague et al., 2001; Duchnowicz and Koter, 2003). Moreover, there is no study on the role of picloram on DNA methylation changes in putrescine-exposed plants. In our previous study, we determined the effect of picloram on DNA methylation in somaclonal variation conditions (Aydin et al., 2016). This paper aims indicating DNA methylation changes that emerged picloram and putrescine interactions in bean. The obtained results demonsrated that picloram caused DNA hypomethylation.

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

Primers	C	+/-	0 ppm putrescine				1 ppm putrescine					0.1 ppm putrescine					0.01 ppm putrescine				
			5mg L^{-1} pic	10mg L^{-1} pic	20mg L^{-1} pic	40mg L^{-1} pic	0mg L^{-1} pic	5mg L^{-1} pic	10mg L^{-1} pic	20mg L^{-1} pic	40mg L^{-1} pic	0mg L^{-1} pic	5mg L^{-1} pic	10mg L^{-1} pic	20mg L^{-1} pic	40mg L^{-1} pic	0mg L^{-1} pic	5mg L^{-1} pic	10mg L^{-1} pic	20mg L^{-1} pic	40mg L^{-1} pic
OPW-20	**5**	+	-	2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		-	2204	2204	2204	2204	-	2204	-	2204	2204	-	-	2204; 1485	2204; 1485	-	-	2204	2204	2204; 1485	2204
OPA-4	**9**	+	2153; 1219	2108; 1187	-	-	-	-	2130	-	-	-	-	-	-	2153; 1219	-	-	-	-	-
		-	2255; 1321	2255; 1321	2255; 1947; 1321; 322	2255; 1947 1321; 1045	654	654	2255	1947	-	654	1045	1947	-	2255; 1321	-	1947; 437	-	1947	-
OPW-13	**4**	+	1790; 1083	1829; 1117	1133	1125	1731	1717	1790	1801	1768	-	1768	-	1768	1801; 1117	1757	1768	1717	1757	1780
		-	913	1653	1653; 913	1653; 1000	1653	1653	1653	1653	1653	-	1653	-	1653	1653	1653	1653	1653	1653	1653
OPA-1	**3**	+	--	-	-	-	-	-	-	-	-	-	-	2098	-	-	-	-	-	-	-
		-	--	2979	3173; 2979	3173; 2979	-	-	2979	3173; 2979	3173; 2979	2979	-	-	3173; 2979	3173; 2979	2979	-	3173; 2979	3173; 2979	2979
OPH-18	**5**	+	955; 843	825	-	861	-	-	852	-	-	-	962; 834	-	-	852	-	869	-	-	-
		-	-	-	658; 485	658; 436	-	-	-	658	-	658	-	658; 485; 436	658	-	-	658; 485	658; 436	658	-
OPH-19	**4**	+	1628	-	1609	1609	2752; 1628	-	-	2752	-	2790	-	-	1309	-	1609	1309	2752	-	1628
		-	1507	1747	2645; 1747	2645; 1747	2645	2645	1747	2645	-	2645	1747	2645; 1747	-	1747	1747	1747	2645	2645	1747
OPY-8	**5**	+	-	-	-	2065	-	1876	-	-	-	-	1876	-	-	-	-	-	-		-
		-	635	-	1056		-	1785	-	1785; 635	1785; 742	-	1785	-		-	1785	635	1785	1785; 635	1785
OPW-17	**4**	+	1750; 1325	1325	1274	1307	1791	1811	1325	-	1883; 1728	525; 488	-	-	1750	1343	-	1527	-	1750	1967; 1830
		-	1617	1617	1167	1617	1440	1617; 1440	1617	-	1617	-	-	786; 705	-	786; 705	-	-	-	-	1440
OPY-11	**3**	+	-	-	-	-	-	-	-	593	-	-	-	850; 593	-	-	-	-	-	-	-
		-	2900	2900; 846	2900; 846	2900; 846	-	2900	2900; 846	-	2900	846	2900; 846	2900	2900	2900	2900; 846	2900	2900	2900	-
OPY-13	**8**	+	1902; 1531; 1214	1550	1455	-	1621; 1275	2383;	2257; 1006	1568	1512	1621	-	1512	1621	1512; 1086	-	2040; 1512	2515; 1621	1586	1568
		-	-	1125	2383; 2120	2383; 2120; 1125	1318	1654	2383	-	2383; 2120	1125	-	-	2383	-	-	2120	2383	2383	-
OPY-15	**6**	+	1146; 573	1983	894	900	-	1113		1098	2000; 1121	-	2000; 1121	-	1098	2000; 1137	1092	2000; 1121	-	1098	1098
		-	1004; 412	1891; 1550 1004; 412	1891; 1550; 1004; 739	1891; 1550; 1004; 739	1550	1550 1004	1891; 739	1550	1891; 1550; 1004	452	1891; 1550; 1004	1891; 1550	1550	1891; 1550; 1004	1550	1891; 1550; 1004	1891; 1550; 1004; 452	1550	1550; 452

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

OPW-4	**5**	+	1879	1879; 1473	1905; 1490	1905; 1486	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		-	1410; 1192	-	-	-	-	2086; 1410	-	1410	1410; 1192	-	-	-	1410	2086	1448: 1192	2086	1410	1410	1500; 1448; 1410
OPY-16	**4**	+	2861	-	-	-	2816	-	3000	-	2793	-	2816	2839	-	-	-	-	-	-	3261
		-	-	3000; 859	3000; 859	3205; 3000; 859	-	-	859	3205; 3000; 859	859	-	-	-	3205; 3000; 859	3205; 3000; 859	-	-	3205; 3000; 859	3205; 3000; 859	3205
OPH-16	**3**	+	-	-	-	-	-	-	-	-	3137	-	3102	-	-	-	-	-	-	-	-
		-	-	2079	2079	2079	-	-	-	2079	3000	-	3000	2079	2079	-	-	-	-	2079	2079
GTS %			**60.9**	**58.1**	**49.8**	**46.6**	**87.5**	**73.6**	**69.8**	**65.3**	**63.1**	**82.8**	**70.8**	**67.9**	**65.1**	**61.2**	**79.0**	**69.8**	**65.7**	**64.3**	**61.1**

C: Control, pic: picloram

Table 4. CRED-RA band amounts and polymorphism %

Primers	Picloram	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 mg L^{-1}**	-	-	0	0	12.5	0	0	0
	5 mg L^{-1}	25	12.5	12.5	12.5	0	0	0	0
	10 mg L^{-1}	12.5	25	0	12.5	0	0	0	0
	20 mg L^{-1}	12.5	25	12.5	0	12.5	12.5	12.5	12.5
	40 mg L^{-1}	12.5	0	0	12.5	0	12.5	25	0
OPY-13	**0 mg L^{-1}**	-	-	0	0	0	0	0	14.2
	5 mg L^{-1}	0	14.2	0	0	0	14.2	0	14.2
	10 mg L^{-1}	0	14.2	0	0	0	0	0	14.2
	20 mg L^{-1}	14.2	0	0	0	14.2	0	0	14.2
	40 mg L^{-1}	28.5	28.5	0	0	0	0	14.2	0
OPW-17	**0 mg L^{-1}**	-	-	16.6	0	14.2	0	0	0
	5 mg L^{-1}	0	0	25	0	0	0	0	0
	10 mg L^{-1}	0	0	14.2	0	0	0	0	0
	20 mg L^{-1}	20	16.6	0	0	12.5	0	0	0
	40 mg L^{-1}	12.5	0	0	16.6	14.2	0	0	0
OPA-1	**0 mg L^{-1}**	-	-	0	0	0	0	12.5	33.3
	5 mg L^{-1}	0	33.3	0	0	0	33.3	12.5	33.3
	10 mg L^{-1}	0	0	0	33.3	20	33.3	0	33.3
	20 mg L^{-1}	14.2	33.3	0	33.3	0	0	0	33.3
	40 mg L^{-1}	0	33.3	16.6	0	0	0	0	33.3
OPH-18	**0 mg L^{-1}**	-	-	0	0	14.2	20	16.6	20
	5 mg L^{-1}	0	40	16.6	0	20	20	0	20
	10 mg L^{-1}	16.6	40	12.5	0	20	0	16.6	20
	20 mg L^{-1}	14.2	20	11.1	0	0	20	0	0
	40 mg L^{-1}	0	20	0	0	16.6	20	20	0

Table 4 (Continue). CRED-RA band amounts and polymorphism %

OPY-16	**0 mg L^{-1}**	-	-	12.5	16.6	20	16.6	0	16.6
	5 mg L^{-1}	0	0	0	16.6	0	16.6	14.2	16.6
	10 mg L^{-1}	0	33.3	0	0	0	16.6	0	16.6
	20 mg L^{-1}	16,6	16.6	0	0	33.3	16.6	25	16.6
	40 mg L^{-1}	0	16.6	0	0	14.2	16.6	0	16.6
OPW-6	**0 mg L^{-1}**	-	-	0	0	0	0	0	0
	5 mg L^{-1}	20	20	0	0	20	0	0	0
	10 mg L^{-1}	25	20	0	0	0	0	0	0
	20 mg L^{-1}	0	0	16.6	0	16.6	0	0	20
	40 mg L^{-1}	16.6	0	0	0	0	0	0	20
Average	**0 mg L^{-1}**	**-**	**-**	**4.1**	**2.3**	**8.7**	**5.2**	**6.1**	**12.0**
	5 mg L^{-1}	**6.4**	**17.1**	**7.7**	**4.1**	**5.7**	**12.0**	**3.8**	**12.0**
	10 mg L^{-1}	**7.7**	**14.7**	**3.8**	**6.5**	**5.7**	**7.1**	**2.3**	**11.7**
	20 mg L^{-1}	**13.1**	**15.9**	**3.3**	**4.7**	**12.7**	**7.0**	**5.3**	**10.9**
	40 mg L^{-1}	**10.0**	**14.0**	**2.3**	**4.1**	**6.4**	**7.0**	**8.4**	**9.9**

H:Hpa II, M: Msp I

Seven primers used for RAPD-PCR amplification produced specific and stable amplicons in CRED-RAs analysis. Compared with the PCR products obtained from the control DNA, picloram and/or putrescine treatments showed changes in CRED-RA patterns (Table 4). DNA hypomethylation occurred and its value increased depending on the decrease in picloram concentration. However, putrescine led to a reduction in picloram-induced DNA methylation. DNA methylation values were 14.0% in 40 mg L^{-1} picloram exposed plant whereas this value was determined as 4.1% in 40 mg L^{-1} picloram and 1 mM putrescine treated plant (Table 4).

With an increase in the dose of putrescine, DNA methylation values of picloram treated plants were decreased. The results indicated that putrescine had the antagonistic effect against picloram. Recently, several studies have indicated that stress positively contributed to tolerance as alter genes expression by DNA methylation and histone modifications (Wang et al., 2016; Feng et al., 2016). In additional, the present study evaluated the protective role of putrescine against picloram genotoxicity. Our results showed that putrescine treatment caused amelioration effect against DNA hypomethylation and DNA damage that was caused by picloram.

Previous research have revealed that polyamines guard DNA from enzymatic degradation, X-ray irradiation and also stabilize RNA, to counteract of ribosomal dispersion (Miyamoto et al., 1993; Ruiz-Herrera et al., 1995). Additionally, some researchers also suggested that polyamines have ability to protect replicating DNA from oxidative damages as well as DNA and RNA biosynthesis stimulate (Khan et al., 1992; Miyamoto et al., 1993; D'Agustino et al., 2005). The protective effects of putrescine may relate to positive charged properties. It has ability to binding minor groove of B-DNA, moving to the

major groove of A-DNA. Also, these studies have been proposed that positive charged polyamines bind to the minor groove of B-DNA, moving to the major groove in the A form. This paper is the first report that showed the effect of treatment with different concentrations of picloram and putrescine on DNA methylation. DNA methylation changes induced by putrescine may affect some specific genes activation to increase plant resistance under stress conditions.

CONCLUSION

The random application of these pesticides has already created a public health hazard. This study suggests that polyamines could be an alternative for reducing genotoxic damage against chemical mutagens in plant. However, more research should be done for understanding the mechanism of the protective effect of polyamines on plants as shown in these experiments.

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