

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

Mahmut Sinan TAŞPINAR¹, Esra ARSLAN², Murat AYDIN¹, Burcu SİĞMAZ³, Güleray AĞAR²

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), respectively. 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) putresinin 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

¹ Mahmut Sinan TAŞPINAR (0000-0001-6399-2703), Murat AYDIN (0000-0003-1091-0609), Atatürk Üniversitesi Tarımsal Biyoteknoloji Bölümü, Erzurum, Türkiye
² Esra ARSLAN(0000-0002-9062-6896), Güleray AĞAR(0000-0002-8445-5082), Atatürk Üniversitesi Biyoloji Bölümü, Erzurum, Türkiye
³ Burcu SİĞMAZ(0000-0001-7411-9440), Atatürk Üniversitesi Tarla Bitkileri Bölümü, Erzurum, Türkiye
Sorumlu yazar/Corresponding Author: Murat AYDIN,maydin@atauni.edu.tr

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 (Lejzak-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 constituents. 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.

MATERIALS AND METHODS

Plant Growth and Treatment Conditions

Phaseolus vulgaris L. cv. Elkoca seeds were obtained from Department of Field Crops, Faculty of Agriculture, Atatürk 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. $25 \pm 1^\circ\text{C}$ 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 $25 \pm 1^\circ\text{C}$ with a 16 h photoperiod of 60 $\mu\text{mol photons/m}^2\text{s}$ 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-RA Procedures and PCR Methods

CTAB method (Taspınar 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	GTCCTGGGT
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 μM
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: $GTS = 100 - (100 \times a/n)$. 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 $100 \times a/n$ (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 412 (OPY-15) 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	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	-	2204	2204	2204	-	2204	2204	2204	2204	-	2204	2204	2204	-	2204	2204	2204	2204	2204
	+		2153; 1219	2108; 1187			2130					2204; 1485	2204; 1485	2204; 1485	2153; 1219				2204; 1485	2204
OPA-4	9	-	2255; 1321	2255; 1321	1947; 1321	654	2255	1947			654	1045	1947			1947; 437			1947	-
	+		2255; 1321	2255; 1321	1947; 1321	322	1045				2255; 1321		1947			1947; 437			1947	-
OPW-13	4	+	1790; 1083	1829; 1117	1133	1125	1731	1717	1801	1768	-	1768	-	1768	1801; 1117	1757	1768	1717	1757	1780
	-		913	1653	1653; 913	1000	1653	1653	1653	1653	1653	1653	1653	1653	1653	1653	1653	1653	1653	1653
OPA-1	3	-	2979	3173; 2979	3173; 2979	3173; 2979	-	2979	3173; 2979	3173; 2979	3173; 2979	-	2979	3173; 2979	3173; 2979	3173; 2979	-	3173; 2979	3173; 2979	2979
	+		955; 843	825	-	861	-	852	-	-	962; 834	-	852	-	852	-	869	-	-	-
OPH-18	5	-	658; 485	658; 485	658; 436	-	658	-	658	-	658	-	658	658; 485; 436	-	658; 485	658; 485	658	658	658
	+		1628	-	1609	2752; 1628	-	2752	-	2752	-	2790	-	1309	-	1609	1309	2752	-	1628
OPH-19	4	-	1507	1747	2645; 1747	2645; 1747	2645	1747	2645	-	2645	1747	2645	2645; 1747	1747	1747	1747	2645	2645	1747
	+		-	-	2065	-	1876	-	1876	-	-	1876	-	-	-	-	-	-	-	-
OPY-8	5	-	635	-	1056	-	1785	-	1785; 635	1785; 742	-	1785	-	1785	-	1785	635	1785	1785	1785
	+		1750; 1325	1325	1274	1307	1791	1811	1325	-	1883; 525; 488	525; 488	-	1750	1343	-	1527	-	1750	1967; 1830
OPW-17	4	-	1617	1617	1167	1617	1440	1617	1617	-	1617	-	1617	786; 705	786; 705	-	-	-	-	1440
	+		-	-	-	2065	-	1876	-	1876	-	1876	-	-	-	-	-	-	-	-
OPY-11	3	-	2900	2900; 846	2900; 846	2900; 846	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900
	+		1902; 1531; 1214	1550	1455	-	1621; 1275	2383; 1006	1568	1512	1621	-	1512	1621	1512; 1086	-	2040; 1512	2515; 1621	1586	1568
OPY-13	8	-	1125	2383; 2120	2383; 2120	2383; 1125	1318	1654	2383	-	2383; 2120	1125	-	2383	-	2120	2383	2383	2383	-
	+		1146; 573	1983	894	900	-	1113	1098	2000; 1121	-	2000; 1121	-	1098	2000; 1137	1092	2000; 1121	-	1098	1098
OPY-15	6	-	1004; 412	1891; 1550	1550; 1004	1004; 739	1550	1891; 739	1550	1891; 1004	452	1004	1550	1891; 1550	1550	1891; 1550	1891; 1550	1550	1550	1550
	+		1891; 1550	1891; 1550	1891; 1550	1891; 1550	1891; 1550	1891; 1550	1891; 1550	1891; 1550	452	1004	1550	1891; 1550	1550	1891; 1550	1891; 1550	1891; 1550	1550	1550
			412	739	739	739	739	739	739	739	739	739	739	739	739	739	739	739	739	739

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

+	1879	1905; 1879;	1905;	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	1473	1490	1486	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
OPW-4	1410;	-	2086;	1410;	1410	-	-	1410	2086	1448;	2086	1410	1410	1410	1500;	1448;	1410	
5	1192	-	1410	1192	-	-	-	1192	1192	1192	1192	-	-	-	-	-	-	
+	2861	-	2816	-	3000	-	2793	-	2816	2839	-	-	-	-	-	-	3261	
OPY-16	3000;	3000;	3205;	3205;	3205;	3205;	3205;	3205;	3205;	3205;	3205;	3205;	3205;	3205;	3205;	3205;	3205;	
4	859	859	859	859	859	859	859	859	859	859	859	859	859	859	859	859	859	
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
OPH-16	2079	2079	2079	2079	2079	2079	2079	2079	2079	2079	2079	2079	2079	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	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	12.5	0	0	0
	5 mg L ⁻¹	25	12.5	12.5	12.5	0	0	0	0	0	0
	10 mg L ⁻¹	12.5	25	0	12.5	0	0	0	0	0	0
	20 mg L ⁻¹	12.5	25	12.5	0	12.5	12.5	12.5	12.5	12.5	12.5
	40 mg L ⁻¹	12.5	0	0	12.5	0	12.5	25	25	0	0
OPY-13	0 mg L ⁻¹	-	-	0	0	0	0	0	0	0	14.2
	5 mg L ⁻¹	0	14.2	0	0	0	14.2	0	0	0	14.2
	10 mg L ⁻¹	0	14.2	0	0	0	0	0	0	0	14.2
	20 mg L ⁻¹	14.2	0	0	0	14.2	0	0	0	0	14.2
	40 mg L ⁻¹	28.5	28.5	0	0	0	0	14.2	14.2	0	0
OPW-17	0 mg L ⁻¹	-	-	16.6	0	14.2	0	0	0	0	0
	5 mg L ⁻¹	0	0	25	0	0	0	0	0	0	0
	10 mg L ⁻¹	0	0	14.2	0	0	0	0	0	0	0
	20 mg L ⁻¹	20	16.6	0	0	12.5	0	12.5	0	0	0
	40 mg L ⁻¹	12.5	0	0	16.6	14.2	0	14.2	0	0	0
OPA-1	0 mg L ⁻¹	-	-	0	0	0	0	0	0	12.5	33.3
	5 mg L ⁻¹	0	33.3	0	0	0	33.3	0	33.3	12.5	33.3
	10 mg L ⁻¹	0	0	0	33.3	20	33.3	0	33.3	0	33.3
	20 mg L ⁻¹	14.2	33.3	0	33.3	0	0	0	0	0	33.3
	40 mg L ⁻¹	0	33.3	16.6	0	16.6	0	0	0	0	33.3
OPH-18	0 mg L ⁻¹	-	-	0	0	14.2	20	14.2	20	16.6	20
	5 mg L ⁻¹	0	40	16.6	0	20	20	20	20	0	20
	10 mg L ⁻¹	16.6	40	12.5	0	20	0	20	16.6	16.6	20
	20 mg L ⁻¹	14.2	20	11.1	0	0	20	0	0	0	0
	40 mg L ⁻¹	0	20	0	0	16.6	20	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.

REFERENCES

- Aydin M, Arslan E, Taspinar MS, Karadayi G, Agar G, 2016. Analyses of somaclonal variation in endosperm-supported mature embryo culture of rye (*Secale cereale* L.). *Biotechnology and Biotechnological Equipment*, 30: 1082-1089.
- Balague C, Sturtz N, Duffard R, Evangelista de Duffard AM, 2001. Effect of 2,4-dichlorophenoxyacetic acid herbicide on *Escherichia coli* growth, chemical composition, and cellular envelope. *Environmental Toxicology*, 16: 43-53.
- Correia S, Lopes ML, Canhoto JM, 2011. Somatic embryogenesis induction system for cloning an adult *Cyphomandra betacea* (Cav.) Sendt. (tamarillo). *Trees*, 25: 1009-1020.
- D'Agostino L, Di Pietro M, Di Luccia A, 2005. Nuclear aggregates of polyamines are supramolecular structures that play a crucial role in genomic DNA protection and conformation. *FEBS Journal*, 272: 3777-3787.
- Duchnowicz P, Koter M, 2003. Damage to the erythrocyte membrane caused by chlorophenoxyacetic herbicides. *Cellular and Molecular Biology Letters*, 8: 25-30.
- Feng SJ, Liu XS, Tao H, Tan SK, Chu SS, Oono Y, Zhang XD, Chen J, Yang ZM, 2016. Variation of DNA methylation patterns associated with gene expression in rice (*Oryza sativa*) exposed to cadmium. *Plant Cell and Environment*, 39: 2629-2649.
- Grativol C, Hemery AS, Ferreira PCG, 2012. Genetic and epigenetic regulation of stress responses in natural plant populations. *Biochimica et Biophysica Acta*, 1819: 176-185.
- Khan AU, Di-Mascio P, Medeiros MHG, Wilson T, 1992. Spermine and spermidine protection of plasmid DNA against single-strand breaks induced by singlet oxygen. *Proceedings of the National Academy of Science of the United States of America*, 89: 11428-11430.
- Leljak-Levanic D, Bauer N, Mihaljevic S, Jelaska S, 2004. Changes in DNA methylation during somatic embryogenesis in *Cucurbita pepo* L. *Plant Cell Reports*, 23: 120-127.
- Miyamoto S, Kashiwagi K, Watanabe S, Igarashi K, 1993. Estimation of polyamine distribution and polyamine stimulation of protein synthesis in *Escherichia coli*. *Archives of Biochemistry and Biophysics*, 300: 63-68.
- Mohammed KB, Ma TH, 1999. Tradescantia-micronucleus and -stamen hair mutation assays on genotoxicity of the gaseous and liquid forms of pesticides. *Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis*, 426: 193-199.
- Oakes DJ, Webster WS, Brown Woodman PDC, Ritchie HE, 2002. Testicular changes induced by chronic exposure to the herbicide formulation, Tordon 75D® (2,4-dichlorophenoxyacetic acid and picloram) in rats. *Reproductive Toxicology*, 16: 281-289.
- Olea-Popelka F, Mclean DM, Horsman J, Almquist K, Bramble JE, Hall C, 2005. Increasing expression of an anti-picloram single-chain variable fragment (ScFv) antibody and resistance to picloram in transgenic tobacco (*Nicotiana tabacum*). *Journal of Agricultural and Food Chemistry*, 53: 6683-6690.
- Ruiz-Herrera J, Ruiz-Medrano R, Dominguez A, 1995. Selective inhibition of cytosine DNA methylases by polyamines. *FEBS Letters*, 357: 192-196.
- Salvi ND, George L, Eapen S, 2001. Plant regeneration from leaf base callus of turmeric and random amplified polymorphic DNA analysis of regenerated plants. *Plant Cell, Tissue and Organ Culture*, 66: 113-119.
- Sawamura S, Jackson WT, 1968. Cytological studies in vivo of picloram, pyriclor, trifluralin, 2,3,6-TBA, 2,3,5,6-TBA and nitratin. *Cytologia*, 33: 545-554.
- Sung S, Amasino RM, 2004. Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. *Nature*, 427: 159-164.
- Taspinar MS, Agar G, Yildirim N, Sunar S, Aksakal O, Bozari S, 2009. Evaluation of selenium effect on cadmium genotoxicity in *Vicia faba* using RAPD. *Journal of Food, Agriculture and Environment*, 7: 857-860.
- Temel A, Gozukirmizi N, 2013. Analysis of retrotransposition and DNA methylation in barley callus culture. *Acta Biologica Hungarica*, 64: 86-95.
- Wang W, Qiao Q, Sun F, Wang Y, Xu D, Li Z, Fu B, 2016. Genome-wide differences in DNA methylation changes in two contrasting rice genotypes in response to drought conditions. *Frontiers in Plant Science*, 7: 1675.
- Yildirim N, Agar G, Taspinar MS, Turan M, Aydin M, Arslan E, 2014. Protective role of humic acids against dicamba-induced genotoxicity and DNA methylation in *Phaseolus vulgaris* L. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, 64: 141-148.
- Zeid IM, Shedeed ZA, 2006. Response of alfalfa to putrescine treatment under drought stress. *Biologia Plantarum*, 50: 635-640.

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.