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Healing Effect of Ascorbic Acid against Genetic and Epigenetic Changes Caused by Pendimethalin in Wheat

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Abstract
Because of the increasing need for agricultural products in the world, the use of pesticides, which are used to increase yield, is increasing day by day. Herbicides constitute a large part of the total amount of pesticides used, such as 20%. It is known that herbicides have toxic effects and irreversibly disrupt DNA and gene expression. Pendimethalin is a widely used herbicide against weeds in the production of grains, legumes, and vegetables. Ascorbic acid has an antioxidant effect. Molecular markers are frequently used to determine genotoxic and mutagenic effects at the DNA level. It was aimed to determine the curative effect of ascorbic acid on the negative effects of pendimethalin. IRAP and ISSR molecular markers were used. It was found that the Genomic Template Stability (GTS) ratio decreased as a result of increasing the dose of pendimethalin applied in wheat, resulting in DNA damage and the positive effect of applied ascorbic acid on DNA damage.

1. Introduction

Because of the rapid increase in the world population, the gradual decrease in agricultural lands, urbanization, unconscious resource use, climate changes and similar factors, it has become more and more difficult to meet the increasing demand for food. Physical and chemical applications are carried out to increase the yield in agricultural production and to obtain products equivalent to the need. Some of these are the use of quality seeds, tillage, irrigation, fertilization. Besides these to these applications, pesticides are used to combat harmful factors [1]. Unconscious application of pesticides used in chemical control produces negative effects in non-target organisms. Mutagenic, carcinogenic, teratogenic effects are the most well-known effects of pesticides [2], [3], [4]. When exposed to pesticides for a certain period, disorders in the liver, reproductive and nervous systems cause toxicity that causes allergic reactions [5].

Pendimethalin is a selective herbicide belonging to the dinitroaniline group, targeting single-year-old grasses and broad-leaved wild shrubs. In grain, legume, vegetable cultivation it is also used to remove unwanted weeds from decorative ornamental plants used for landscaping. [6], [7], [8]. Pendimethaline affects the mitotic division of root tips in the *Vigna mungo* plant by disrupting microtubule formation, preventing chromosome separation and cell wall formation [9]. Pendimethaline, which has high efficiency and long duration of action, medium and high soil permanence and low leakage property, increase the risk of polluting the environment [10]. There are studies in the literature on the cytotoxic and genotoxic effects of pendimethaline in different animal organisms [11], [12], [13] and studies in plant organisms are very limited [14], [2], [15].

Ascorbic acid, a water-soluble glucose derivative, has significant antioxidant activity *in*

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vitro, in part because of its ease of oxidation and the low reactivity of the semide-hydroascorbate radical derived from it [16]. Ascorbic acid has shown antioxidant effects in many test, systems such as human lymphocyte cells, eukaryotic tissues, *Drosophila* [17], [18], [19], [20].

This study, it was aimed to determine the genetic and epigenetic changes caused by pendimethalin in wheat with ISSR and IRAP techniques and the curative effects of ascorbic acid after pendimethalin treatment.

2. Material and Method

2.1. Chemicals Example

Pendimethalin (CAS 40487-42-1. Molecular Weight 281.31) and ascorbic acid (CAS Number: 50-81-7; Molecular Weight: 176.12) are commercially available from Sigma-Aldrich.

2.2. Treatment of Triticum aestivum L. seeds with pendimethalin and ascorbic acid

Triticum aestivum L. seeds were selected and kept in 5% sodium hypochlorite (NaOCl) solution for 10 minutes. The sterilized seeds were placed in petri and germinated with distilled water at 25 ^{0}C. Increased doses (0, 0.033, 0.044, 0.055 and 0.066 g kg−1) applied to germinated wheat germs and taken to pots and left to grow. Pendimethalin concentrations used in the study were determined according to Verna et al. [21]. Wheat leaves that reached sufficient maturity were treated with ascorbic acid in 2 different doses (50 and 100 ppm) by spraying and watered with pure water for 7 days. Ascorbic acid concentrations used in the study were determined according to Barakat [22]. The control group was treated only with distilled water. For molecular examination, samples were stored at -80^o C.

Table 1. Pendimethalin, ascorbic acid, control groups and administered doses

No	Group	Pendimethalin	Ascorbic Acid
1	Control	-	-
2	Control	-	50 ppm
3	Control	-	100 ppm
4	1.Group	0.033 g kg^{-1}	-
5	1.Group	0.033 g kg^{-1}	50ppm
6	1.Group	0.033 g kg^{-1}	100 ppm
7	2.Group	0.044 g kg^{-1}	-
8	2.Group	0.044 g kg^{-1}	50ppm
9	2.Group	0.044 g kg^{-1}	100 ppm
10	3.Group	0.055 g kg^{-1}	-
11	3.Group	0.055 g kg^{-1}	50ppm
12	3.Group	0.055 g kg^{-1}	100 ppm
13	4.Group	0.066 g kg^{-1}	-
14	4.Group	0.066 g kg^{-1}	50ppm
15	4.Group	0.066 g kg^{-1}	100 ppm

2.3. DNA isolation

It implemented the isolation of genomic DNA from plant samples with minor changes to the protocol of Shagai-Maroof et al. [23]. It carried the quantity determination of DNA samples out using the Epoch Microplate Spectrophotometer instrument.

2.4. ISSR analysis

We used 8 primers for ISSR analysis. The protocol followed for the ISSR PCR process; 3µL 10x PCR buffer, (10 mg mL^{-1}), 0.3 µL dNTP (10 mM), 1.15 µL MgCl2 (25 mM), 1µL DNA (100 ngm L -1), 1 µL primary (25 pmol), 0.5 µL 5 Unit mL L -1 Taq DNA polymerase was placed in the 0.2 ml PCR tube. By adding pure water, the volume is completed to 20 µL. Samples denatured for 4 minutes at 94 ºC are then processed for each cycle for 34 cycles; It is arranged so that there are 40 seconds at 94 ºC, different annealing temperatures for each primer for 45 seconds and 2 minutes at 72 ºC. Then, at the end of 1 cycle lasting 6 minutes at 72 ºC, the samples were removed to +4 ºC. The PCR procedure, the samples were loaded into the pre-prepared 0.8% agarose gel and executed in 1 X TBE (Tris Borat Edta) buffer [24].

2.5. IRAP analysis

I used 5 primers for IRAP analysis. Protocol followed for IRAP PCR processing; 3 µL 10xPCR buffer, (10 mg ml-1), 0.3 µL dNTP (10 mM), 1.15 µL MgCl2 (25 mM), 1 µL DNA (100 ng ml -1), 0.5 µl 5 Unit mL L -1 Taq DNA polymerase was placed in the 0.2 ml PCR tube. By adding pure water the volume is completed to 20µl. Samples denatured at 95 ºC for 2 minutes are then 41 cycles for each cycle; It is arranged so that it is 30 seconds at 95 ºC, different annealing temperatures for each primer for 60 seconds and, 2 minutes at 72 ºC. Then, at the end of 1 cycle lasting 5 minutes at 72 ºC, the samples were removed to +4 ºC [24].

2.6. Determination of genomic pattern stability (GTS*)*

The presence and absence of amplified DNA bands in all samples for each primer, decreases and increases in band densities according to negative control ISSR AND IRAP profiles were determined by agarose gel imaging device and Total LAB TL 120 (Nonlinear Dynamics) software. Genomic pattern stability (%) for all primary products; It was calculated using the formula 100 x 1-a/b. Formula; a; the ISSR and IRAP polymorphic profiles detected for each application sample, n; DNA obtained in the relevant primary and negative control group was selected as the total band number. The polymorphism observed in the ISSR and IRAP profiles of the application groups included the emergence of a new band or the loss of an existing band according to the negative control group [25].

3. Results and Discussion

Triticum aestivum L. in the samples, 8 primers were used in the ISSR analysis and 129 polymorphic bands were detected. Some observe that the size of these bands varies between 1630 bp and 224 bp. Polymorphism was detected in all samples where pendimethalin was applied. The rate of polymorphism is between 6.06% and 24.24%. In the evaluation of the samples with regarding to the control group**,** we observed an increase parallel with the dose increase. It was found that the samples treated with pendimethalin showed an increase inversely proportional to the dose increase where the GTS values were between 77.27% and 93.93%. Some observe that ascorbic acid applied in two different doses to pendimethalin groups reduces the polymorphism occurring and increases the GTS value (Table 1.)

Triticum aestivum L. in the samples, 5 primers were used in the IRAP analysis and 75 polymorphic bands were detected. Some observed that the size of these bands varies between 1189 bp and 27 bp. Polymorphism was detected in all samples where pendimethalin was applied. The polymorphism rate is between 31.57% and 73.68%. In the evaluation of the samples with reference to the control group, an increase in polymorphism was observed in parallel with the dose increase. We found the samples treated with pendimethalin showed an increase inversely proportional to the dose increase in GTS values between 26.32% and 63.16%. It was determined that ascorbic acid applied in two different doses to pendimethalin groups reduced the polymorphism and increased the GTS value (Table 2).

the environment and acute or chronic toxic effects in organisms. In addition, the inducing of DNA damage by herbicides leads to potentially adverse reproductive outcomes in humans, such as cancer and many other acute or chronic diseases. The negative impact of genetically damaged crops, potentially on both natural ecosystems and human health, has been reported. The genotoxic effects of most herbicides on agricultural crops are unknown [7], [11], [12].

There are many studies on the harmful effects of pendimethalin on non-target organisms. There are studies showing that pendimethalin has a significant toxic effect, especially on aquatic life [26], [13]. In studies conducted in animal organisms, it has been determined that pendimethalin causes toxicological effects, oxidative stress and DNA damage [27], [12], [11]. Alavanja et al. [28], it was determined that the herbicide pendimethalin showed a statistically significant exposure-response relationship with pancreatic cancer. Arici et al. [29], examined the effect of pendimethalin on inflammation caused by pancreatic cancer and drew attention to the use and toxic effect of pendimethalin because of oxidative damage in the results obtained.

In the literature, there are the limited number of studies investigating the cytotoxic and genotoxic effects of pendimethalin in plant organisms [15], [8]. In their of growth and DNA damage at the root ends of the Allium cepa, they determined that pendimethalin caused DNA damage at all concentrations compared to the control group [30]. Akbulut [15], determined that gene expression levels decreased depending on pendimethalin concentration and revealed that salicylic acid application had an effect on reducing the toxic effect caused by pendimethalin. Promkaew et al. [31]; found a significant increase in chromosome aberrations and mitotic index because of the increase in the amount of pendimethalin administered in Allium cepa and three Zea mays varieties. Anghel et al. [32]; we found that the increased dose of pendimethalin in the Allium test system induced a mitodepiric effect and caused an abnormal cell increase.

Table 2. ISSR analysis results of the wheat samples

Primer name	K	A1	A2	P1	P1/ A1	P1/A2	P2	P2/ A1	P2/ A2	P3	P3/ A1	P3/A2	P4	P4/ A1	P4/ A2
UBC 816	6	-	-	+1093 -330	-330	-330	+1152 -330	+1137 -330	+1137 -330	+1079 -330	+1107	+1112	+1093 -330	+1065	+1093
UBC 817	12	-803	-	+1269	+1269	-	- +1269	+1269	+1269	+1269 +552	+1269 +418	+1269	+1269 +413 -650	+1269	+1269
UBC 824	5	-	-	-	-	-	-	-	-	+1162 +698	+1142	+1159	+1154 +947	+1165 +987	+1148
UBC 825	9	-	-440	+775 +242	+812	-	-	-	-	+1348 -317	-394 -317	-394 -317	-394 -317	-394 -317	-394 -317
UBC 840	8	-	+256	-655 -485 -351	-485 -351	-	+1230 -485 -351	-351	+1230 -351	+1230 -485 -351 -310	+1630 +1214 -351 -310	+1290 -351 -310	+1306 -485 -440 -351 -310	-485 -440 -351 -310	-485 -440 -351 -310
UBC 841	11	-	-	-	+950 -230	-	-230	-230	-	+638	+605 -230	-	-	-733 -465 -415	-733
UBC 856	15	-	-	-367 -224	-1330 -1198	-1330 -1198 -790	-367 -224	-367 -224	-367 -224	-367 -224	-367 -224	-367 -224	-367 -224	-	-367 -224
Total Band	66	1	2	10	9	4	9	7	7	15	14	10	16	13	12
% Pol.		1,51	3,03	15,15	13,63	6,06	13,63	10,60	10,60	22,72	21,21	15,15	24,24	19,69	18,18
GTS		98,4	96,9	84,84	86,36	93,93	86,36	89,39	89,39	77,27	78,78	84,84	75,75	80,30	81,81

Table 3. IRAP analysis results of the wheat samples

Primer name	K	A1	A2	P1	P1/ A1	P1/ A2	P2	P2/ A1	P2/ A2	P3	P3/ A1	P3/ A2	P4	P4/ A1	P4/ A2
Nikita	4	-	-	+734 +613 -143	+718 +605 -361 -143	-361 -143	+753 +627 -143	-491 -143	+618 -143	+1163 +637 -143	+1169 +640	+613	+1189 +783 +670 -143	+812 +685 -143	+670 -143
LTR 6150	5	-	-	+300	+308	-	+500 +443	-	-	+1070 +982	-	-	+1062 +450 +291	+449 +294	+498 +392
5LTR1	3	+164	+351	-	-	-	-	-	-	+447 +389	+362	+383	+527 +457 +394	+517 +451 +398	+531
3LTR5	2	-	-	+358	-	-	+354	-	-	+428 +351	+432	+437	+435 +390	+387	+411
Sukkula	4	-	+27	+202 +38	-	+47	-	-	-	-	-	-	+584 +486	+393	+396
Total Band	19	1	2	7	5	3	6	2	2	9	4	3	14	10	7
% Pol.		5,26	10,52	36,84	26,31	15,78	31,57	10,52	10,52	47,36	21,05	15,78	73,68	52,63	36,84
GTS		94,74	89,48	63,16	73,69	84,22	68,43	89,48	89,48	52,64	78,95	84,22	26,32	47,43	63,16

Using high-dose herbicides causing contamination in

4. Conclusion and Suggestions

One of our most basic needs is nutrition. Sales of agricultural products are increasing at a rapid pace worldwide and are shrinking. In this case, methods such as breeding methods and removing pests are used to increase productivity. Although the use of pesticides is aimed at the target organism, these studies have negative effects on nontarget organisms. Today, chemicals used unconsciously cause environmental pollution and negatively effect the vital activities of organisms in many ecosystems. It is stated in the literature that the widespread use of herbicides causes genotoxic effects on plant and animal organisms, it was also detected in our study. Here, it would be appropriate to restrict the use of herbicides and raise awareness among users.

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Contributions of the authors

Muhammed Semih Dartar carried out the molecular genetic studies and drafted the manuscript. Nalan Yıldırım Doğan participated in the design of the study and helped to draft the manuscript.

Conflict of Interest Statement

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics.

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