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THE GENOTOXIC EFFECTS OF LOGRAN ON *HORDEUM VULGARE* L. AND *TRITICUM AESTIVUM* L.

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In the present study, the cytogenetic effects of the herbicide Logran on root tip cells of *Triticum aestivum* L. and *Hordeum vulgare* L. and changes of total protein content in root tip meristems were studied. The seeds of plants were treated with various concentrations of Logran (125, 250, 500 μg/ml) for 3 and 6 h. The percentages of abnormal cells were seen to increase with increasing treatment period and concentrations. The most dominant types of observed abnormalities were C-mitosis, distributed metaphase and anaphase, stickiness. All the used concentrations of Logran significantly induced a number of chromosomal aberrations in root tip cells of *Hordeum vulgare* L. and *Triticum aestivum* L. Logran also decreased mitotic index. The decrease of protein content in root tips of *Triticum aestivum* L. is significant at all the treated concentrations and treatment periods when compared with control.

Keywords: Logran – *Triticum aestivum* L. – *Hordeum vulgare* L. – genotoxic – chromosome abnormalities

INTRODUCTION

Due to increasing production and consumption of plant food it is necessary to control amounts of undesirable chemical compounds. The widespread use of these chemicals is usually connected with serious problems of pollution and health [24]. The chemicals come into agro-ecosystems especially from fertilisers, pesticides and industrial toxic products and other compounds. Effects of changes induced by these environmental pollutants may be from little significance to changing the hereditary constitution of organisms [33]. Herbicides are the pesticides used mostly against agricultural pests. It is widely known that herbicides are capable of inducing genetic

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effects on plants [34, 41]. Most sulfonylurea derivatives and hypoglycaemic drugs are widely used as herbicides [40, 51]. Sulfonlyurea herbicides are widely used to control broad-leaved weeds. The effects of some urea group herbicides on living organisms have been investigated [29, 32, 36, 45, 47]. Logran is one of sulfonylurea herbicides and its active substance is triasulfuron. Logran is used extensively, especially on crop grown in agricultural area. But there is no report about genotoxic effect of Logran on plants. Root tip systems of various plants have been widely used for determining the biological effects of chemicals [22, 23]. The main conclusion of all investigations was that plant assays are efficient and reliable test systems for rapid screening of chemicals for mutagenicity and clastogenicity [23, 41].

Protein quantity and quality are of increasing importance in modern plant breeding. In recent years protein assays were used for evaluating mutagenicity of environmental chemicals [27]. In the present study it is aimed to study the cytogenetic effects of Logran on root tip cells of *Hordeum vulgare* L. (barley) and *Triticum aestivum* L. (wheat), the changes of protein content in root tips and to determine whether there is a relationship between the changes on the mitotic cell division and protein content.

MATERIALS AND METHODS

Seeds of *Hordeum vulgare* L. and *Triticum aestivum* L. were used as test materials. The seeds were pre-soaked in water for 1 h at 25 °C to break the dormancy and then treated for 3 and 6 h with various concentrations of Logran (125, 250 and 500 μg/ml). Concentrations of Logran were prepared by dissolving in distilled water. Controls were placed in distilled water for the same period. After the treatment the seeds were thoroughly washed with distilled water and germinated in Petri dishes.

For cytological observations root tips of germling were fixed in Carnoy's solution for 24 h and squashes were prepared according to the method of Darlington and La Cour [12].

The Lowry method [31] was used for testing protein contents of seeds of treated and control groups of *Hordeum vulgare* L. and *Triticum aestivum* L. Root tips were homogenised and centrifuged 20′ at 3000 rpm in 4 °C. One ml Solution A (contains 100 ml 0.2% Na_2CO_3 dissolved in 0.1N NaOH, 1 ml 1% $CuSO_4$ dissolved in distilled water, 1 ml 2% sodium potassium tartrate dissolved in distilled water) and 0.2 ml distilled water were added to 0.1 ml homogenised root tip samples. After incubated in room temperature for 10′ in dark, mixed with 0.1 ml Solution B (1N Folin Reagent). The mixture incubated at room temperature for 30′ and the absorbance was read spectrophotometrically at 750 nm and the results were expressed in mg g^{-1} DW.

The mitotic index was calculated for each treatment as a number of dividing cells/100 cells. Obtained data were evaluated with the X^2 test. Total protein contents of treated and control groups were statistically evaluated by using ANOVA test.

RESULTS

In the present study, all the used concentrations of Logran induced a number of chromosomal aberrations in root tip cells of *Hordeum vulgare* L. and *Triticum aestivum* L. The percentages of abnormal cells were seen to increase with increasing treatment period and concentrations. These results were significant according to X^2 test (Table 1). The higher frequency of chromosomal aberrations in all treatments was observed at metaphase. The most dominant types of observed abnormalities at metaphase were C-mitosis, distributed metaphase and anaphase, stickiness. On the other hand, in this study the other mitotic abnormalities were observed such as

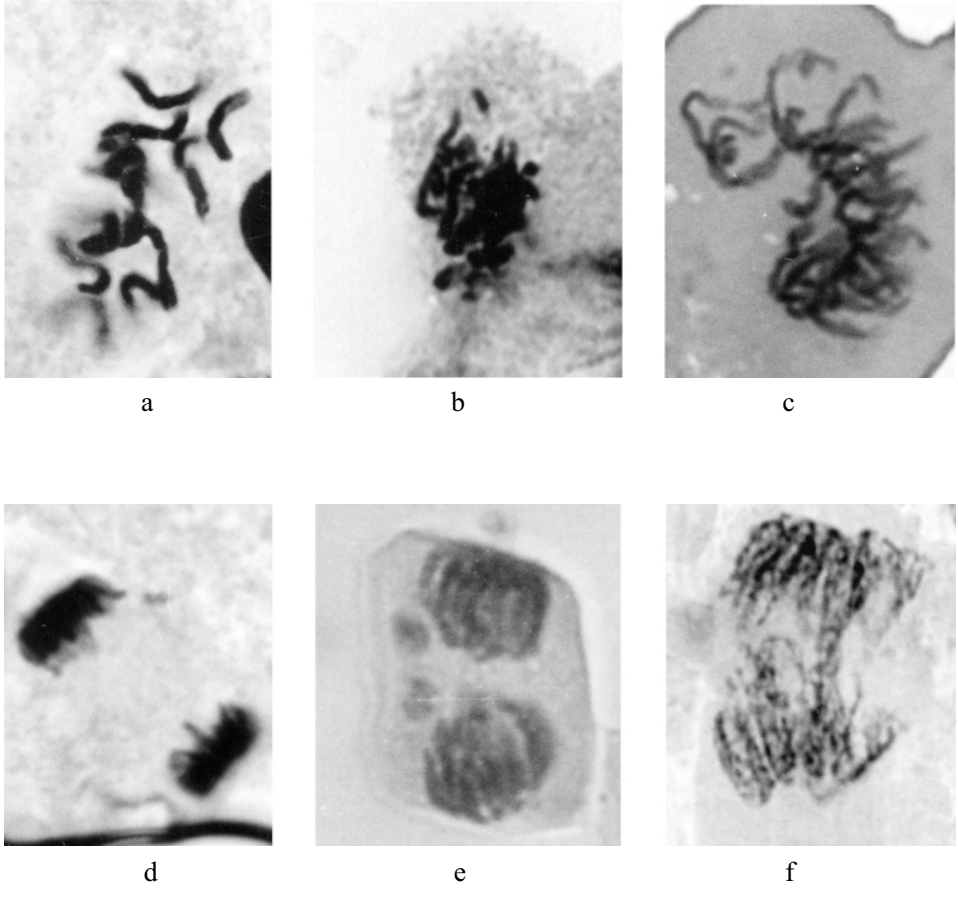

Fig. 1. Some chromosome abnormalities after treated with various concentrations of Logran. a: C-Mitosis (6 h – 500 µg/ml – *Hordeum vulgare* L.); b: Stickiness and fragment (6 h – 250 µg/ml – *Hordeum vulgare* L.); c: Distributed metaphase (3 h – 250 µg/ml – *Hordeum vulgare* L.); d: Fragment (3 h – 125 µg/ml – *Hordeum vulgare* L.); e: Micronuclei (3 h – 125 µg/ml – *Triticum aestivum* L.); f: Bridge (6 h – 500 µg/ml – *Triticum aestivum* L.)

Table 1
Chromosome abnormalities and mitotic index of barley and wheat after treated with different concentrations of Logran (125, 250, 500 µg/ml) for 3 and 6 hours

Treatment period	Concentration	Total number of cells	Abnormality %	Stickiness	C-mitosis	Irregular metaphase	Micronucleus	Bridge	Fragment	Total abnormality	MI
					Triticum aestivum L						
3 h	Control	1405	1	2	0	6	0	6	1	15	10.32
	125 µg/ml	1297	4.1	15**	0	18**	12***	4	5	54***	6.31
	250 µg/ml	1094	8.2	27***	12***	20**	8**	20**	3	90***	6.93
	500 µg/ml	1087	9.8	18***	24***	25***	5*	17**	8*	107***	5.90
6 h	Control	1269	1.9	8	8	0	2	3	4	25	12.19
	125 µg/ml	1262	5.1	10	12	19***	3	12*	9	65***	9.30
	250 µg/ml	1070	7.2	9	20*	10**	10*	18***	4	71***	8.62
	500 µg/ml	1328	10.3	43***	28**	21***	7	35***	6	140***	6.82
					Hordeum vulgare L.						
3 h	Control	1455	1	9	0	12	0	3	0	24	35.95
	125 µg/ml	1438	4	18	4	8	2	14*	8*	54***	22.93
	250 µg/ml	1358	6	30***	15***	28***	0	15**	6*	94***	12.19***
	500 µg/ml	1487	7.4	41***	12**	30***	0	19**	9**	111***	11.04***
6 h	Control	1338	1.8	14	4	4	0	2	0	24	19.94
	125 µg/ml	1250	7.6	28*	18**	28***	0	18***	4	96***	10.74
	250 µg/ml	1417	8.5	32*	15*	30***	0	22***	21***	120***	7.63*
	500 µg/ml	1473	13	57***	21**	60***	3	31***	20***	192***	6.2**

*P < 0.05; **P < 0.01; ***P < 0.001

Table 2
Protein content of barley and wheat after treated with different concentrations of Logran (125, 250, 500 µg/ml) for 3 and 6 hours

Treatment period	Concentrations	*Triticum aestivum* L.	*Hordeum vulgare* L.
		mean ± SE	mean ± SE
3 h	Control	324.6 ± 10.7	307.26 ± 25.1
	125 µg/ml	214.2 ± 19.2*	267.28 ± 24.1
	250 µg/ml	180.7 ± 33.3*	251.73 ± 29.7
	500 µg/ml	225.1 ± 24.7*	254.12 ± 32.0
6 h	Control	333.78 ± 7.13	325.5 ± 40.8
	125 µg/ml	386.25 ± 14.5*	279.3 ± 47.8
	250 µg/ml	173.25 ± 37.6*	241.7 ± 51.2
	500 µg/ml	200.63 ± 33.1*	281.6 ± 46.0

*P < 0.05; **P < 0.01; ***P < 0.001

micronuclei, lagging chromosomes and bridges at anaphase and telophase. Representative samples of the chromosomal aberration types are shown in Figure 1.

Logran also decreased mitotic index (MI). However, the decreasing of MI was not significant for all the treated tips in *Triticum aestivum* L. and 125 µg/ml concentrations of Logran in *Hordeum vulgare* L. when compared with control (Table 1).

The total protein contents of root tips of control and treated seeds with different concentrations of Logran are shown in Table 2.

Total protein content in treated plants with various concentrations of Logran decreased compared with control but total protein content of *Triticum aestivum* L. treated for 6 h with 125 concentration of Logran increased compared with control. The changes in protein content of *Triticum aestivum* L. were significant at all the treated concentrations and treatment periods when compared with control.

DISCUSSION

Triasulfuron is active substance of Logran that is used in this study. Triasulfuron is one of sulfonylurea group herbicides. Sulfonylurea herbicides are used to control a variety of broad-leafed weeds and grasses in cereals. Extra potential usage of these herbicides in agriculture areas can cause accumulation in soil and water and reach to organisms [1].

Although the effect on seed germination and root growth of plants of Triasulfuron group herbicides have been investigated [28], there is no report about cytogenetic effects of Triasulfuron on plants.

The plant tests have often been used for the determination of cytogenetic and genotoxic effects of environmental pollutants [19, 38]. Mutagenetic environmental effects may be analysed by macroscopic parameters, cytological parameters such as

the types and frequencies of chromosomal aberrations and abnormal cell division [16, 17, 19, 38), in addition, by the changes in the protein content [10, 26].

In this study, Logran induced chromosomal damage at all concentrations and treatment periods. The degree of chromosomal abnormalities was significant when compared with control. The most types of abnormalities were observed at metaphase. The frequently seen abnormalities were stickiness, C-mitosis and disturbed metaphase and anaphase.

Stickiness has been shown to be the result of entanglement of interchromosomal chromatin fibers and this leads to subchromatid connections between chromosomes [29, 35]. C-mitosis is one of the consequences of inactivation of spindle apparatus connected with the delay in the division of centromere [21]. The aneugenic substances inhibit the spindle formation and cause C-mitosis [15]. Disturbed metaphases and anaphases may be due to disturbance of spindle apparatus which allows that the chromosomes to spread irregularly over the cell [4, 5]. These abnormalities indicate that Logran has effects on spindle inhibition in both *Hordeum vulgare* L. and *Triticum aestivum* L.

Also Linuron is a substituted urea compound registered use as an herbicide for the control of broad leafed and grassy weeds. Papapaulou et al. [39] showed that Linuron has both clastogenic and aneugenic effects in human lymphocytes.

Logran also induced abnormalities such as bridge, fragment and micronuclei at anaphase and telophase. It is well known that fragment and bridges lead to structural changes in the chromosomes. Induction of subchromatid connections is the result of true exchanges between homologous or non-homologous chromosomes whereas the presence of heteromorphic chromosomes can be attributable to terminal deletion of one of the chromatids [44]. Micronuclei that are the product of acentric fragments [42], chromosomes with inactivated centromere and isochromosomes are not transfere to the daughter nuclei but remain as small nuclei [6]. Bridge, fragment and micronuclei formations are results of clastogenic events.

In study of Decision Document [13] it is reported that Triasulfuron did not induce point mutations in *Salmonella typhimurium*, *Saccharomyces cerevisiae* and mouse lymphoma cells. Also, in Chinese hamsters it is not observed chromosomal aberrations with studies of Triasulfuron. But Logran has induced significant chromosomal aberrations in human lymphocyte culture [37]. Chlorpropamide (sulfonylurea drug) used as hypoglycemic agent also significantly induced chromosomal aberrations in lymphocytes of diabetic patients [46]. Although it was found that chlorpropamide did not significantly induce chromatid aberrations and chromosome exchanges in chromosomal aberration test in Chinese hamster and mice [43].

The sulfonylurea herbicides exert toxic activity by interfering with the enzyme acetolactate synthase (ALS), which is specific for plants and microorganisms. The enzyme is involved in the synthesis of branched amino acids leucine, valine and isoleucine. Inhibition of ALS causes inhibition of cell division and cell elongation due to lack of essential amino acids [9].

The inhibition of mitotic activities is often used for tracing cytotoxic substances. Cytotoxicity is defined as a decrease in mitotic index [48] and as an increase in the

fraction of cells with C-mitosis, disturbed metaphase-anaphase, sticky and vagrant chromosomes [18].

In this study, all the used concentrations and treatment periods of Logran reduced mitotic activity in both *Triticum aestivum* L. and *Hordeum vulgare* L. But the decreasing of MI was not significant for all the treated tips in *Triticum aestivum* and 125 μg/ml concentrations in *Hordeum vulgare* when compared with control. The decrease in the mitotic index of root tip meristems was found to correlate with C-mitosis, chromosome stickiness and distributed metaphase and anaphase. It has been shown by many investigators that induction of stickiness, C-mitosis and distributed metaphase-anaphase observed as indicative of high toxicity [2, 18].

In the present study, mitotic index decreased as abnormalities due to increased inhibition spindle. Reduction of mitotic activity in root tip meristem of *Hordeum vulgare* L. was observed more than *Triticum aestivum* L. The differences observed in sensitivity to cytotoxicity between plants may be due to different genetic material of these plants.

The change in protein content or profile induced by a mutagen may be considered as a potential indicator of genotoxicity [10, 11]. Many investigators have studied the effects of chemical substances on total protein content [8, 10, 25, 45, 49]. Bilge [8] observed that streptomycin induced a decrease in protein amount in the root tips of *Vicia faba*. N-nitrosomethyl urea (urea herbicide) has induced changes in the biosynthesis of DNA and acidic nucleic changes in root meristem cells of *Vicia faba* L. [14]. Aluminium did not change total protein content in pineapple roots [30]. Endosulfan changed mitotic cell division and protein amounts and it caused chromosomal abnormalities on root tip cells of *Lentic*. It has been suggested that there is a relationship between the effects of endosulfan on mitotic cell division and protein content of root tips [3]. Cytotoxic and mutagenic effects of Olive Oil Mill Effluent (OOME) on the root tips of *Triticum aestivum* L. have been investigated and it was observed that OOMC causes chromosomal abnormalities, changed cell division frequency and decreased total protein amounts [7]. Genotoxic effects of EMS have been assessed in fish. EMS did not only cause chromosomal aberrations in somatic cells, nuclear anomalies in red blood cells, but also altered significantly both protein profiles and total protein contents in all tissues [26]. EMS has been extensively tested in various mammalian models and it has been reported to cause major chromosomal aberrations including chromosomal breakage in mammalian cells, by binding to DNA regions rich in G-C base pairs, causing those regions to become unstable or by disrupting the DNA backbone, perhaps in regions where protein is bound. Therefore, the change of gel-electrophoretic band profiles and total protein content observed in the EMS-treated fish might actually reflect damage in DNA or the protein synthesis system [27]. Effects of a mutagen on protein structure/synthesis can also give important clues although the appearance and disappearance of protein changes may or may not be directly related to cytogenetic changes that occur after exposure to chemical substances [10].

Phosphine gas in *Allium cepa L.* increased the rate of mitotic inhibition and chromosome aberration, but the protein content was not affected by phoshine treatment [50].

In our study, Logran reduced total protein content in both *Triticum aestivum* L. and *Hordeum vulgare* L. except for *Triticum aestivum* L. treated for 6 h with 125 µg/ml concentration of Logran. At this treatment group Logran increased protein content as compared with the control. As the chromosome abnormalities increased, total protein content decreased. The change in protein content of *Triticum aestivum* L. was significant when compared with the control.

Chromosome abnormalities may cause change in heredity material and affected DNA may alter protein synthesis leading to a decrease in protein content.

Formation and composition of seed proteins are dependent on both genetic and environmental factors [20]. In the present study the differences in total protein content may be due to different genetic structure of plants or variety of chromosomal abnormalities.

As a result, Logran significantly induced mitotic abnormalities and decreased total protein content. Due to its effect on hereditary material Logran is genotoxic. Logran also reduced mitotic activity of cells because of its cytotoxic activity. Therefore, this pesticide must be used under thorough control in agriculture areas.

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