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Cytogenetic Effects of Maleic Hydrazide on *Helianthus annuus* L.

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Abstract: In the studies which are carried out for obtaining more productive, enduring and qualified yield several methods are used. One of these methods is fighting with the agricultural pests using pesticides. Herbicides are a group of pesticides. In the present study, the effects of different concentrations (10^{-2} M, 10^{-3} M, 10^{-4} M and 10^{-5} M) of Maleic Hydrazide (MH) which is an herbicide, on *Helianthus annuus* L. chromosomes undergoing mitotic and meiotic cell division were investigated. Preparates which were made for meiotic division and mitotic division were stained according to Feulgen squash procedure. With 10^{-2} M concentration of MH was not seen mitotic division stage. At the other MH concentrations various meiotic and mitotic division abnormalities were observed. MH concentrations caused a decrease in mitotic index. It is observed that all concentrations of MH caused important abnormalities according to control.

Key words: Maleic hydrazide, plant chromosome, chromosome abnormalities

INTRODUCTION

For nutrition of population of the world that is increasing it is necessary to obtain wide and high-grade yield. For this purpose, one of methods which used is fighting with the agricultural pests.

Environmental pollution is reached high dimensions which is caused by the pesticides with the other chemicals. It is known that environmental pollutants have effects on living things. One of these effects is the effect on genetic structure of living things. Many studies have been done for to indicate this effect[1,2].

Herbicides cause dying of these by effecting metabolism of plants. So they help agricultural fighting by preventing growing of unwanted plants. The mutagenic effect of herbicides also have been shown by investigators[3-5]. MH has been recognised as plant growth inhibitör. Darlington and McLeish[6] were first to report that it induced chromosome breaks. Singh[7] observed that MH inhibites mitotic division. It is known that MH causes chromosomal damage in root tip cells of plants and also different types of chromosomal aberrations[8-10]. Yuang and Zhang[11] have shown that MH induced SCE in root meristem cells of *Hordeum vulgare*. In contrast to the situation an order plants, MH is not mutagenic in *Arabidopsis thaliana*[12]. It is used in agriculture in despite its known effects as a mutagenic and clastogenic agent. Chromosome aberrations induced by mutagenic agents in plants is indicator of genetic damage. This cytogenetic events is considered with respect to mitotic and meiotic behaviour[13]. The abnormalities which were occured in meiosis are very important because they cause congenital abnormallities[14].

While effects of MH on mitotic behaviors have been investigated on several plants, there is no study about meiotic behavior of MH on plants. *Helianthus annuus* L. is the most important cultural plant of Turkish Trakya and cytogenetic effects of MH have not been investigated on *Helianthus annuus* L. This study was aimed to investigate the cytogenetic effects of different concentrations of MH on meiosis in anthers and mitosis in root tip cells of *Helianthus annuus* L.

MATERIALS AND METHODS

The seeds of *Helianthus annuus* L. used in this investigation were obtained from Agriculture Research Institute of Trakya. MH (SIGMA, CAS No. 123-33-1) was used as a test chemical.

Seeds pretreated with different concentrations of MH (10^{-5} M, 10^{-4} M, 10^{-3} M and 10^{-2} M) and controls were sown in fields of Agriculture Research Institute of Trakya for M_1 generation. Properties of soil of the fields is as follow; pH is 7.3, poor for organic substance (0.47) and phosphorus and medium but middle rich for Potassium.

Concentrations of MH were prepared with dissolving in tap water in 37°C water bath. To determine the effects of MH on meiotic cell division the seeds were kept in tap water for 6 h, then placed in different MH solutions for 2 h. Control group were kept in tap water for the same time period. Seeds obtained from M_1 generation were sown for M_2 generation without treatment. For meiotic division 100 seeds were used for each treated group and control.

The buds which had been taken in early developmental stage were fixed in Carnoy and hydrolyzed in 1 N HCl for 15 min in an oven in 60°C.

To determine the effects of MH on mitotic cell division seeds were soaked in tap water for 6 h and then they were germinated in petriplates. When the roots were about 2-3 cm long root tips were treated with different MH solutions for 2 hours. Control group were kept in tap water for the same time period. After the root tips were fixed in Carnoy, hydrolyzed in 1 N HCl for 15 min in an oven in 60°C. The buds and the root tips were squashed in feulgen stain[15].

To determine the effects of MH on mitotic index 3000 cells were scored in control and in each treated group. Obtained data were evaluated with the χ^2 test.

RESULTS

Effects of MH on *Helianthus annuus* L. chromosomes undergoing meiotic division were investigated in anthers of selected plants from M_1 and M_2 generations. An examination of meiotic division of M_2 generation was carried out in order to determine if chromosome aberrations were transmitted to the second generation.

While abnormality ratio of M_1 generation of control plants is % 1, abnormality ratio of M_2 generation is 1.1% (Table 1).

The higher frequency of meiotic aberrations in all the treatments was chromosome bridges. Total abnormality ratio in M_1 generation which obtained from seeds treated with 10^{-5} M concentration of MH was 5.6%. This ratio in M_2 generation was 3.7%. Abnormalities were observed in both I and II division stages. The most common abnormalities were chromosome bridges and laggard chromosomes at anaphase. The ratio of meiotic division abnormalities caused by 10^{-4} M concentration of MH was 8.6% in M_1 generation, 3.3% in M_2 generation. The most common abnormality was early separation of bivalents at metaphase I. In the M_1 generation the percentage of abnormalities caused by 10^{-3} M concentration of MH was 10.4%. This ratio in M_2 generation was found as 3.7%. Distribution of abnormalities according to generations were different. Because germinated plant ratio obtained from seeds treated with 10^{-2} M MH was very few couldn't evaluate them. Examples of abnormalities observed during meiotic cell division with MH are shown in Fig. 1.

Root tip cells of plant were used to determine the effects of different concentrations of MH on mitotic cell division. The mitotic index was seen to decrease with increasing MH concentrations when compared with the control. At 10^{-2} M concentration of MH root growth and mitotic division phases were not occured (Table 2).

Aberrant mitotic cell division were 4.4% in root tip cells of the control plants. The percentage of abnormalities in root tip cells was seen to increase with increasing MH concentrations. The results obtained from the control and treated plants are shown in Table 2. These abnormalities were anaphase and telophase bridges, fragment formation at metaphase, irregular metaphase. Mitotic division abnormalities mostly occured with different concentrations of MH are shown in Fig. 2.

All the results tested with the fishers' exact χ^2 test were significant according to control.

Table 1: Abnormalities of meiotic division produced by MH at M_1 and M_2 generations

Abnormalities	Observ. cell no.	Diakinesis Metaph. I	Metap, II Anaph. I Teloph. I	Anaph. II Teloph. II	Total Abnormality	Abnor-mality (%)	p-value
M_1							
Control	4680	12	24	12	48	1.0	
10^{-5} M MH	4908	60	132	84	276	5.6	<0.05
10^{-4} M MH	5264	348	60	48	456	8.6	<0.05
10^{-3} M MH	4144	204	96	132	432	10.4	<0.05
10^{-2} M MH	-	-	-	-	-	-	-
M_2							
Control	4772	20	24	12	56	1.1	
10^{-5} M MH	5360	52	100	48	200	3.7	<0.05
10^{-4} M MH	5200	80	52	44	176	3.3	<0.05
10^{-3} M MH	4448	56	80	32	168	3.7	<0.05
10^{-2} M MH	-	-	-	-	-	-	-

Table 2: Abnormalities of mitotic division observed in control and treated plants

Abnormalities	MI (%)	Observ. cell no.	Proph. Metaph.	Anaph. Teloph.	Total Abn.	Total Abn. (%)	p-value
Control	23	5690	240	15	255	4,4	
10^{-5} M MH	18	4965	195	210	405	8,1	<0.05
10^{-4} M MH	12	4250	270	340	610	14,3	<0.05
10^{-3} M MH	7	3865	315	435	750	19,4	<0.05
10^{-2} M MH	-	-	-	-	-	-	-

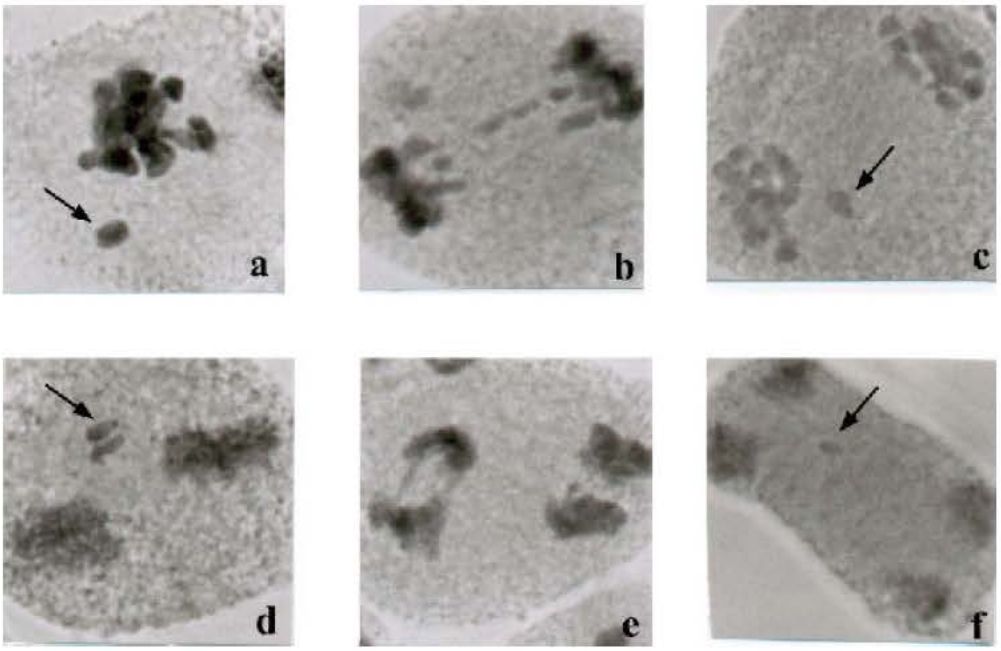

Fig. 1: Meiotic division abnormalities occurring most frequently with MH
- a: Univalents at metaphase I and chromosome stickiness (10^{-4} M)
- b: Bridges at anaphase I (10^{-5} M.)
- c: Laggard chromosome at telophase I (10^{-3} M)
- d: Early separated chromosome at metaphase II (10^{-4} M)
- e: Bridge at anaphase II (10^{-3} M)
- f: Fragment at telophase II (10^{-4} M)

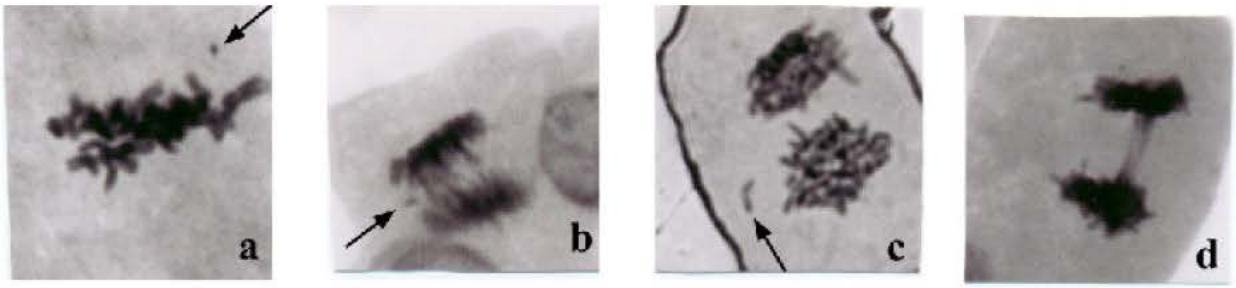

Fig. 2: Some mitotic division abnormalities occurring with the effect of MH.
- a: Fragment at metaphase (10^{-3} M).
- b: Chromosome bridge and fragment at anaphase (10^{-4} M).
- c: Laggard chromosome at telophase (10^{-3} M)
- d: Bridge at telophase (10^{-5} M).

DISCUSSION

In this study, percentage of chromosomal abnormalities observed from mitotic and meiotic cell divisions in the control series are generally the predicted values for *Helianthus annuus* L. Because also Georgieva-Todorova[16] have reported that in *Helianthus annuus* L. spontaneous chromosome abnormalities may occur up to 5% such as bridges and fragments or lagging chromosomes. Lewis and John[17] have suggested that such abnormalities are due to spontaneous breakage and exchange rather than the presence of a paracentric inversion.

According to Patil and Bhat[18] MH is a structural isomer of uracil, a pyrimidin of RNA. The mode of action of MH is possibly through its interference with synthesis

of uracil or becoming incorporated into RNA molecule replacing the uracil it reacts with sulphydril groups of nucleic acids. The final results in any case is presumably a weakness in the structure of the chromosome leading to chromosome breakage. According to Gil and Navarrate[19] this chemical is not an alkylating agent but, its mode of action seems to be like alkylating agent. Cortes *et al.*[20] are of opinion that cytogenetic action of MH in many respect resembles to the bifunctional alkylating agents. Patra *et al.*[21] indicated that the mechanism of genotoxic action of MH is not quite clear. However, it is generally considered that the effectiveness of MH lies in its ability to inhibit cell division[22].

Following studies have shown that MH is an effective clastogen in plant cells[10,23-26]. In contract, according to Gichner[12] MH is not mutagenic in *Arabidopsis thaliana*.

In the studies of Andersson and Kihlman[8] and Patra *et al.*[21] it is indicated that MH has induced chromosome aberrations in plants at natural pH. Cortes *et al.*[20] have demonstrated that MH at low pH produces high percentage of aberrant chromosome on root tips of *Allium cepa*.

Chromosome aberrations constitute a significant portion of genetic damage produced by most mutagenic agents. The results of the present study are more correlated with the results of Cortes *et al.*[20]. The present observations indicated that meiosis and mitosis of *Helianthus annuus* L. was affected by MH. This chemical induced significant chromosome abnormalities during meiotic division in both M_1 and M_2 generations when compared with control and genetic damage can be transmitted to M_2 generations, leading to congenital abnormalities. The highest concentration of MH used in this study has inhibited mitotic cell division. It has induced chromosomal aberrations at low concentrations. Also Patil and Bhat[18] have found that the 10^{-2} M MH concentration has inhibitory effect on mitotic activity and induced chromosome aberrations at low concentrations.

In both mitotic and meiotic cell divisions of *Helianthus annuus* L. stickiness, precocious movement of chromosome in metaphase, anaphase bridges, fragment formation, laggard chromosomes were found. Similar type of abnormalities and frequency have also been recorded by other investigators[10,27,28]. Chromosome stickiness may result from entanglement of chromatin fibers which fail to condense properly in preparation for mitosis[29]. It is suggested that some clastogen agents don't effect DNA directly, but indirectly because of stikiness of chromosomes[30]. Because of inducing chromosome stickiness, this chemical can be accepted as a clastogenic agent. Chromosome breakage, fragment formation and spindle inhibition possibly resulted due to the effect of MH on protein essential for spindle apparatus[27]. Precocious movement of chromosomes and laggards may be attributed to the failure of spindle apparatus to organize in a normal way[18].

It has been known that MH inhibites mitosis at higher concentration in plants[18,25]. Also in our investigation, the highest concentration of MH (10^{-2} M) inhibited mitotic cell division.

Findings of this study have demonstrated that MH is genotoxic. The main effect of MH in anthers and root tip cells of *Helianthus annuus* L. is clastogenicity. The plant meiosis is affected by MH and that potential genetic damage can be transmitted to the offspring via male gametes, leading to congenital abnormalities.

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