Albanian j. agric. sci. 2015;14 (4):338-343 *Agricultural University of Tirana*

RESEARCH ARTICLE **(Open Access)**

Studying the Intensity of Physiological and Genetic Processes in Plants Treated with Maleic Hydrazide

AFET MAMMADOVA[1], RAMIZ ALIYEV[1], SEVDA BABAYEVA[1], MEHRAJ ABBASOV[1,2*]

[1]Genetic Resources Institute of National Academy of Sciences of Azerbaijan, AZ1106, Baku, Azerbaijan
[2]Baku State University, AZ 1148, Baku, Azerbaijan

Abstract

Different plant species were analysed for the changes in the physiological and genetic processes in response to the maleic hydrazide treatment. Treatments at very low doses increased the germination of seeds as compared to the control in all tested plants. Stimulation of growth processes caused by the influence of 0,001% MH, accompanied by a decrease in the activity of DNA synthesis, increase of the amounts of RNA, the transcriptional activity of the DNA, fraction of euchromatin DNA in the nucleus and the ratio of labile DNA fraction to stable. This event approves the fact that maleic hydrazide in small doses significantly activates metabolism and significantly intensifies the life of the plant.

Keywords: maleic hydrazide, germination, nucleic acids, wheat, rye, triticale

1. Introduction

In recent years, the use of biologically active substances in agriculture has increased [4, 5, 7, 9, 10, 23].

The application of growth regulators seems a promising way to increase yields, the resistance of plants to unfavorable factors such as drought and frost [6, 8, 11, 12, 21, 22].

Plant growth regulators are natural and synthetic organic substances which may stimulate or inhibit the growth and development of plants, depending on complex factors - the dosage, the age and physiological state of the plant, environmental conditions etc.

One of the biologically active substances which are an effective mean for regulating the growth, development and other vital processes of plants is maleic hydrazide (MH). Publications to date on the effect of MH (3-oksipiridazon-6) to plant growth suggest that it acts as an inhibitor of plant growth and development [19]. However these suggestions are still quite tentative and need to be revised as more data are secured. It is also reported that in small doses can be used to stimulate growth and development of plants, and in large doses - as a herbicide with continuous action [4, 9, 13].

During the use of MH in stimulating doses usually occur only minor metabolic disorders.
Therefore the present study was taken up to observe the effect of low concentration of MH on the physiological and genetic processes in different plants.

2. Materials and methods

Experiments were performed with Triticale (*Triticosecale*), wheat (*Triticum* L.) and rye (*Secale cereale* L.).

The seeds of control plants (100 seeds per plant) were rinsed in sterilized distilled water and placed in petri dishes, while the seeds of experimental plants were rinsed in 0.001% solution of MH. The experiments were conducted in three replications in thermostate (at 22 ^{O}C). The number of germinated seeds was monitored during 5 days.

Total cell DNA and RNA were extracted by the gradual fractionation method according to Alekseev [1]. To study the fractional composition of DNA stepwise impact on chromatin with solutions of different ionic strength and its deproteinization were implemented which allowed to split the cellular DNA to free or weakly bound, functionally active (DNA labile); completely blocked with histones (stable DNA) and firmly linked (residual DNA) DNA.

*Corresponding author: Mehraj Abbasov; E-mail: mehraj_genetic@yahoo.com
(Accepted for publication on December 15, 2015)
***ISSN**: 2218-2020, © Agricultural University of Tirana*

Stabile, labile and residual chromatin and RNA content was measured by UV absorbency at 270 and 290 nm wave-length as mg mL^{-1}.

Cell counting was performed according to the method of Brown with modification of Ali-Zade [2].

The results were processed statistically [3].

3. Results and Discussion

The data on germination percentage of different plant species showed increase at low doses of chemical mutagen - 0.001% maleic hydrazide in all tested plants.

Table 1. Effect of MH on seed germination percent at different days in triticale, wheat and rye.

Varieties	Seed germination, % On the third day	Seed germination, % On the fifth day
Triticosecale		
control	92	96
experiment (0.001% concentration of MH)	96	100
***Triticum* L.**		
control	74	78
experiment (0.001% concentration of MH)	78	82
***Secale cereale* L.**		
control	96	100
experiment (0.001% concentration of MH)	100	100

As shown in Table 1 the germination percentages recorded at 3 and 5 days in untreated control seeds of triticale were higher (92 and 96% respectively). Treatment 0.001% of MH showed 5,4% and 5.1% increase of germination as compared to the control. So, germination percentage of triticale seeds increased up to 96 and 100% with mutagen exposure (Figure 1).

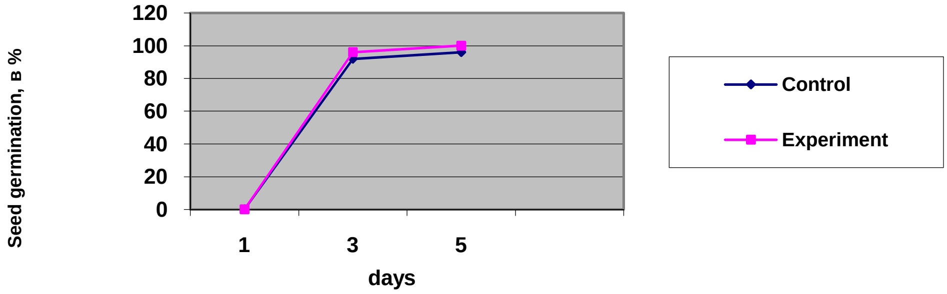

Figure 1. Effect of MH on seed germination percent in triticale.

Similar results were obtained for wheat (Figure 2) and rye (Figure 3).

The experimental seeds of rye showed 100% germination already at 3 days, while in control plants this value was noted only at 5 days.

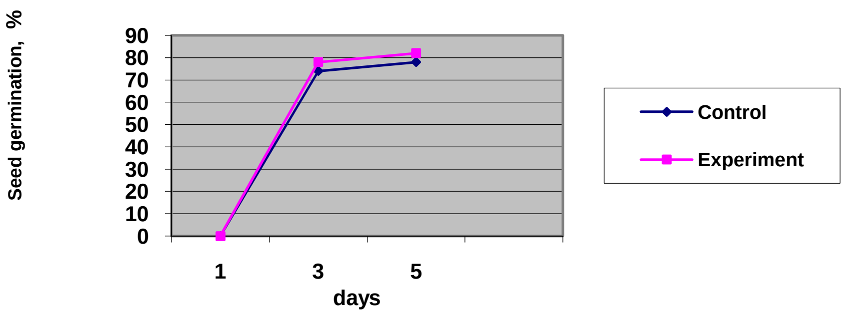

Figure 2. Effect of MH on seed germination percent in wheat.

The lower germination percent was observed in wheat seeds. However, the application of low dose of MH led to the increase of germination (up to 82%) in wheat as well. So, in the present work influence of MH at a concentration of 0.001% had a stimulating effect on seed germination. Numerous studies suggest that maleic hydrazide is a strong mutagen that induces mitotic inhibition and brings heritable alterations in the genes [16]. It possesses growth regulating properties, so stem, root growth and seed germination can be regulated by its treatment. However it is also reported that the germination of seeds is largely dependent on the timing of treatment and the concentration of MH. The stimulating effect obtained in our experiments on one hand can be explained by the fact that only small doses of maleic hydrazide were applied, on the other hand the plant material was exposed only to two days treatment of MH.

The results obtained were in accordance with the earlier reports of Sukul et al. [20] who carried out experiments on pigeon pea, Lady's finger, cow pea and rice to see if plant growth inhibitors serve as growth promoters at their ultra low doses. In all the cases significant increase in morphometric as well as biochemical parameters were observed. In Lady's finger plant growth significantly improved and water, chlorophyll and protein content in leaves significantly increased. Similarly, in experiments on sugar beet genotypes to detect the reaction of plants on maleic hydrazide it was shown that genotypes processed with 0.001-0.005% solution of MH, continued to grow vigorously. There was a small stimulation of growth in comparison with the control plants [24].

Growth stimulants can cause numerous structural and functional changes in plants. Among these changes, the reaction of genetic system plays a significant role, on which largely depend which proteins, with which intensity and the sequence will be synthesized by the cell in the current situation.

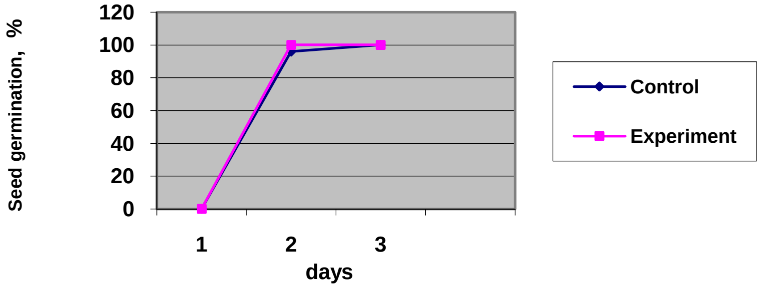

Figure 3. Effect of MH on seed germination percent in rye.

Table 2 shows the changes in nucleic acid content per cell after the stimulation of growth processes with MH. Analysis of data obtained per cell showed activation of RNA synthesis.

Table 2. Changes of RNA and DNA content in cells of rye and wheat during stimulation and inhibition of growth processes caused by MH.

Varieties	RNA, g. 10^{-12}	Fractions of DNA, g. 10^{-12}: labile	stabile	residual	total	% content of labile DNA in the total DNA	labile / stabile	RNA / DNA
Rye (after 24 h)								
Control	18,390	4,203	4,693	0,458	9,540	45	0.90	1,93
Experiment	17,752	4,018	2,984	0,713	7,715	52,1	1,35	2,30
Wheat (after 24 h)								
Control	31,340	2,058	6,658	1,384	10,100	20,5	0,31	3,10
Experiment	48,198	2,160	5,942	1,632	9,734	22,2	0,36	4,95
Rye (after 48 h)								
Control	13,498	2,788	3,653	0.425	6,866	40,6	0.76	1,97
Experiment	22,587	4,994	6,339	0,320	11,653	42,9	0,79	1,94
Wheat (after 48 h)								
Control	22,048	2,544	3,614	1,506	7,664	33,2	0,70	2,88
Experiment	22,616	2,104	1,495	0,465	4,064	51,8	1,40	5,57

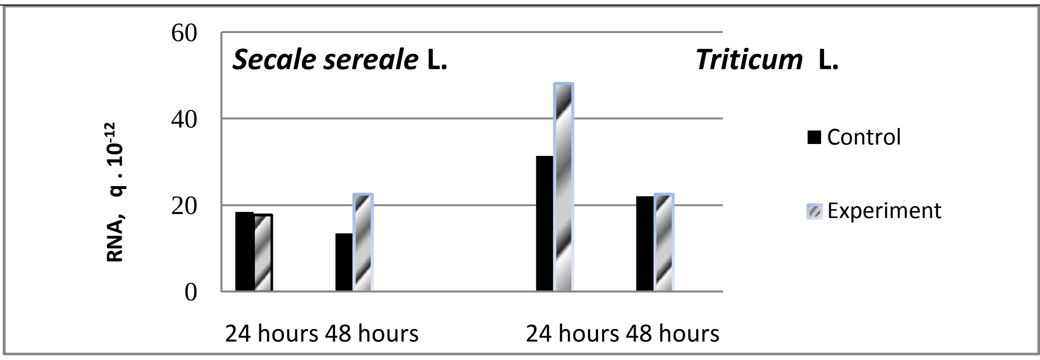

Figure 4. Changes of RNA content per cell after the influence with MH.

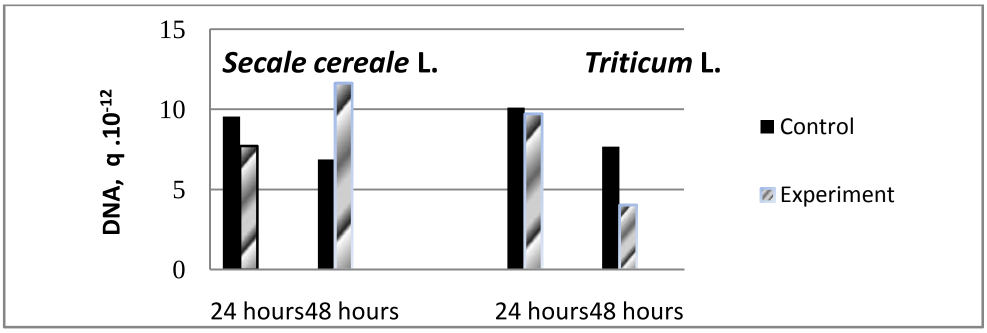

Figure 5. Changes of DNA content per cell after the influence with MH.

So, for example, after 24 hours in the experimental wheat plants treated with MH, increase in RNA content per cell, in comparison with the control plants, was 53.8%, in rye after 48 hours - 67.3% (Figure 4).

Studying the intensity of DNA synthesis in cells of rye and wheat seedlings exposed to MH demonstrated that the experimental plants, mostly inferior to the control in the rate of DNA accumulation. The only exceptions were rye plants after 48 hours of treatment, for which was noted enhancement of DNA synthesis in the cell, in comparison with the control options (Figure 5).

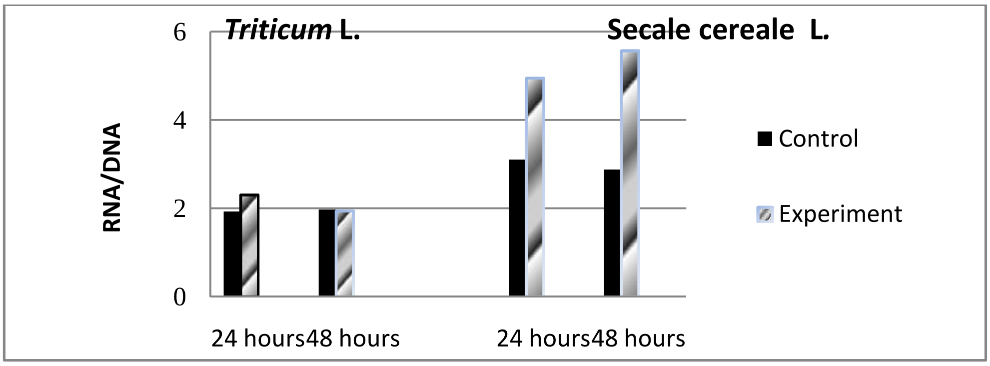

Figure 6. Changes in transcriptional activity of DNA after the influence with MH.

Other experiments carried out in corn and pea seedlings showed that maleic hydrazide inhibited nucleic acid synthesis. Lag time for inhibition of DNA synthesis in corn roots was 4 hr [15].

Although during the stimulation of growth processes decrease of DNA synthesis was noted, however, attention was drawn to the fact of enhance of the transcriptional activity of the DNA in the test plants (Figure 6). Study of fractional composition of DNA in rye and wheat seedlings showed (Table 2), that during the stimulation of growth processes in plants were not observed certain patterns of change in the content of labile fraction of DNA: in some cases was noted the activation of labile DNA synthesis (rye, 48 hours), in the other - a slight increase in the labile fractions (wheat, 24 h) and in the third - the labile DNA fraction decreased. Increase of labile DNA can be explained by the accumulation of loci with identical function, or gene copies [18]. This event is qualified as partial genome reorganization and leads to the intensification of transcription, translation, gene expression and other physiologic-biochemical and biophysical processes [14].

With regard to the stabile DNA was mainly observed attenuation of the synthesis of this fraction. The exception was only the option - rye, 48 hours. However, in all the experimental variants (both rye and wheat), an increase of the percentage content of labile DNA within the total DNA was detected. Thus, in rye after 24 hours of stimulation of growth processes increase in this index was 52.1%, after 48 hours - 42.9%. In wheat these indices were 22.2% and 51.8%, respectively, in comparison with the control plants. As it is known MH in large doses acts as an inhibitor of the synthesis of nucleic acids and proteins. While small doses produced the opposite effect and enhanced nucleic acid synthesis and protein synthesis [20]. In our experiments at the specified time of exposure with MH in all experimental options was noted the increase in the ratio of labile DNA to stable, which may indicate a high rate of biosynthetic processes in these experimental options (Figure 7).

Increase of biosynthetic processes might be explained by increase in the pool of enzymatic antioxidants that help plants in overcoming the oxidative stress [17]. Cells contain important non-enzymatic antioxidants for mitigating the toxic effects of free radicals and active oxygen species under oxidative stress. Initial increase of the both RNA content and the ratio of labile DNA to stable in our experiments induced by low doses of MH might be due to the increased activities of stress relief genes and their gene products.

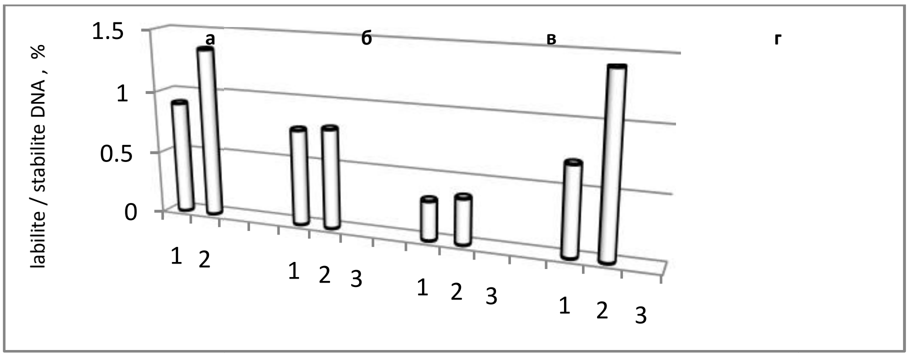

Figure 7. Dynamics of changes of ratio of labile DNA to stabile in rye and wheat cells after the influence with MH.

1. Control — a. rye 24 h.
2. Experiment — b. rye 48 h.

c. wheat 24 h.
d. wheat 48 h.

4. Conclusions

Thus, in our studies, stimulation of growth processes caused by the influence of 0,001% MH, accompanied by a decrease in the activity of DNA synthesis, increase of the amounts of RNA, the transcriptional activity of the DNA, fraction of euchromatin DNA in the nucleus and the ratio of labile DNA fraction to stable. This can be explained by the fact that when using the MH in stimulating doses usually occurred only minor metabolic disorders, but it significantly activates metabolism and significantly intensifies the life of the plant.

5. References

1. Alekseev VG: **The heterogeneity of the DNA of wheat seedlings and activity of the genome**. *Works of applied botany, genetics and breeding* 1973, **52**(1):46-56.
2. Alizade MA, EM Akhundova, Aliyev RT, Hajiyeva SI: **The method of conversion of relative content of substances per single cell of plant leaf**. *Proceedings of the Academy of Sciences of the Azerbaijan SSR* 1979, **6**:29-33.
3. Dospekhov BA: **Methods of field experience (with the basics of statistical of experimental results**). M .:Agropromizdat. 1985. 351p.
4. Dvorechkiy SA: **Influence of growth regulators to reduce the impact of xenobiotics in winter wheat crops in the Republic of Moldova.** PhD dissertation. Penza. 2012.
5. Efimenko VV, Grekhova IV, Matveeva NV: **The response of spring wheat on the use of regulators and micronutrient in seed dressing.** *Agrarian Herald Urals* 2014, **1**:6-8.
6. Irfan A, Shahzad MA., Amir I: **The effects of seed soaking with plant growth regulators on**

seedling vigor of wheat under salinity stress. *Journal of stress physiology-biochemistry* 2005, **1**(1):6-14.

7. Jachin OI, Lubyanov AA, Jachin IA, Kalimullina ZF, Gareeva GB, Markelov EM, Batraev RA, Kabirov RR, Fazlutdinova AI, Ishtimirov EI, Khalilova DI, Shaydullina GF, Suleymanova RV, Kadyrov FF: **Comprehensive assessment of the biological activity of natural plant growth regulators.** *Proceedings of the annual meeting of the Society of Plant Physiologists Russia* 2014, 537-539.
8. Kaspirovsky, AV: **Influence of growth regulators on plant productivity of spring wheat in the conditions of forest-steppe of the Volga**. PhD thesis. Samara. 2013.
9. Kirdar E and Allahverdiyev SR: **Regulators of plant growth and development and their action**. In: *Standardization of seedlings*: University named after Suleyman Demirel, faculty of Forestry, Sparta, Turkey; 2007: 243 – 259.
10. Kirilov NA: **Use of biostimulators in agriculture**. **Recommendations for specialists of agriculture**: Chebocsary, ChNTI; 2001. 32 p.
11. Kostin VI and Yerofeyev EN: **Adaptation of the population of winter wheat to abiotic environmental factors in the autumn, winter and spring under the effect of natural growth regulators**. *Bulletin of the Altai State Agrarian University* 2010, **6**(68):9 -13.
12. Kukushkin AV and Radjabov AK: **Impact of new growth regulators on yield and quality of grape harvest in terms of Novorossiysk district**. *Materials Intern. Distant scien. prac. confer. "Sustainable production of grapes in vine industry based on modern science* 2010, 49-53.
13. Mammadova AD: **Nuclear-mitochondrial interaction of cells genetic systems, under the stimulation of the growth processes by maleic acid hydrazide**. *Scientific enquiry in the contemporary world: theoretical basics and innovative approach.* Publishing Titusville, FL, USA 2012, 99-100.
14. Mammadova A, Aliyev R, Babayeva S, Abbasov M: **Adaptive reaction of cotton accessions of *G. hirsitum* L. and *G. barbadense* L. species to drought stress**. *Genetika* 2015, **47**(2):617-626.
15. Nooden LD: **Inhibition of nucleic acid synthesis by maleic hydrazide.** *Plant Cell Physiol* 1972, **13**(4): 609-621.
16. Nooden LD: **The mode of action of maleic hydrazide: inhibition of growth.** *Physiologia plantarum* 1969, 22: 260-270.
17. Nwani ChD, Lakra WS, Nagpure NS, Kumar R, Kushwaha B, Srivastava SK: **Toxicity of the herbicide atrazine: Effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish *Channa punctatus* (Bloch).** *Int J Environ Res Public Health* 2010, 7(8): 3298–3312.
18. Rahimli VR, Abbasov MA, Shiri M: **Effect of drought and phytohormones on nucleic acids content in drought tolerant and susceptible barley varieties**. *Trends in Applied sciences research* 2011, **6**:96-101.
19. Singh AK, Singh SK, Verma SK, Singh HV, Mishra AK, Agrawal PK, Mathur A, Siddiqui MA: **Accumulation of natural antioxidants in ferns exposed to mutagenic stress**. *International Journal of Chemical, Environmental and Pharmaceutical Research* 2011, **1**(2):52-55.
20. Sukul S, Sukul NCh, Mondal S, Sukul A: **New homeopathic potencies promote plant growth and development**. *Int J High Dilution Res* 2013, **12**(44):98-99.
21. Tosun, YK: **Increase productivity and quality of tomato under the influence of growth regulators**. PhD thesis. Krasnodar. 2008.
22. Troyazykov DD, Gavrilov EK, Korzinnikov YS: **Crop yields of spring wheat under drought conditions influenced by growth regulators.** *Agricultural Biology* 2004, **3**:106-108.
23. Vakulenko VV and Shapoval OV: **Regulators of plant growth**. *Plant protection* 2000, **11**:36-40.
24. http://www.activestudy.info/primenenie-regulyatorov-rosta-v-semenovodstve-saxarnoj-svekly/.