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

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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 thermostat (at 22 °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.
Stable, 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].

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

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Seed germination, %</th>
<th>On the third day</th>
<th>On the fifth day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Triticosecale</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>92</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>experiment (0.001% concentration of MH)</td>
<td>96</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><strong>Triticum L.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>74</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>experiment (0.001% concentration of MH)</td>
<td>78</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td><strong>Secale cereale L.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>96</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>experiment (0.001% concentration of MH)</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

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](image1.png)

**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](image2.png)

**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...
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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.

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.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>RNA, g. 10^{-12}</th>
<th>Fractions of DNA, g. 10^{-12}</th>
<th>% content of labile DNA in the total DNA</th>
<th>RNA / DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>labile</td>
<td>stabile</td>
<td>residual</td>
<td>total</td>
</tr>
<tr>
<td><strong>Rye (after 24 h)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>18,390</td>
<td>4,203</td>
<td>4,693</td>
<td>0,458</td>
</tr>
<tr>
<td>Experiment</td>
<td>17,752</td>
<td>4,018</td>
<td>2,984</td>
<td>0,713</td>
</tr>
<tr>
<td><strong>Wheat (after 24 h)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>31,340</td>
<td>2,058</td>
<td>6,658</td>
<td>1,384</td>
</tr>
<tr>
<td>Experiment</td>
<td>48,198</td>
<td>2,160</td>
<td>5,942</td>
<td>1,632</td>
</tr>
<tr>
<td><strong>Rye (after 48 h)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>13,498</td>
<td>2,788</td>
<td>3,653</td>
<td>0,425</td>
</tr>
<tr>
<td>Experiment</td>
<td>22,587</td>
<td>4,994</td>
<td>6,339</td>
<td>0,320</td>
</tr>
<tr>
<td><strong>Wheat (after 48 h)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>22,048</td>
<td>2,544</td>
<td>3,614</td>
<td>1,506</td>
</tr>
<tr>
<td>Experiment</td>
<td>22,616</td>
<td>2,104</td>
<td>1,495</td>
<td>0,465</td>
</tr>
</tbody>
</table>
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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.

Figure 6. Changes in transcriptional activity of DNA 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).

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.](image)

1. Control . 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

6. Irfan A, Shahzad MA., Amir I: The effects of seed soaking with plant growth regulators on...


