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Hormonal Factors of Anti-Mutagenesis Regulation

Mekhtizadeh Emin RZA

National Academy of Sciences of Azerbaijan, Genetic Resources Institute, Baku - AZERBAIJAN

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Abstract: The genetic activity of phytohormones (Phs) was studied. The investigation was conducted with 4 plants (Allium fistulosum, Lycopersicon esculentum, Triticum aestivum, and Gossypium hirsutum). The action of Phs on plant genetic processes was evaluated by chromosome aberration testing. It was observed that the exogenic Phs exerted a stabilizing effect on spontaneous plant mutagenesis. The use of Phs before and after the action of mutagenic factors contributed to an increase in the anti-mutational or reparative function of the plants' genes. It was observed that the Phs exhibited genoprotective or reparative activity. The absence of clearly expressed dependence of these effects on Ph dose was recorded.

Key Words: Phytohormones, chromosome aberrations, antimutagenesis

Antimutasyonel Düzenlemenin Hormonel Faktörleri

Özet: Bitkisel hormonların (Ph) genetic aktivitesi çalışılmıştır. Araştırmalar değişik bitkisel objeler üzerine yoğunlaşmıştır. Bu hormonal preperatların genetik süreçler üzerine etkisi kromozom anarmallikleri testi ile değerlendirilmiştir. Ekzojenik bitkisel hormonların kendiliğinden mutasyon oluşumunda dengeleyici etkiye sahip olduğu tespit edilmiştir. Mutajenik faktörlerin etkisinden önce veya sonra Ph kullanımının genlerin antimutasyonel veya onarıcı fonksiyonunu artırıcı etki yarattığı bulunmuştur. Bitkisel hormonların, geni koruyucu veya tamir edici aktivite sergilediği gösterilmiştir. Ph dozunda bu etkilerin açık bağımlılığının olmadığı belirtilmiştir.

Anahtar Sözcükler: Bitkisel hormon, kromozom aberasyon, antimutagenesis

Introduction

Anti-mutagenesis is a vitally important manifestation of the protective-restorative function of biological systems on the genetic level. The importance of research of this phenomenon has increased due to the escalation of the pollution of habitats by technogenic factors occurring against a backdrop of unfavorable natural conditions. Many of these factors exhibit mutagenic activity. The effect of these factors on organisms enhances their risk of genetic vulnerability. The use of anti-mutagenic preparations is a promising way of improving the genetic stability of organisms in this situation. At present a large number of such preparations (1,2) are known and classified, but their use for anti-mutagenic protection in plants remains problematic.

Plants are the most important element in the structural makeup of ecosystems and provide primary biological productivity; no less important is their role in the bioremediation of habitats (3). Therefore, improving the genetic stability of a phytocomponent of anthropogenic ecosystems may be of paramount importance for ensuring the reliability of functioning of the noosphere, as a whole.

The search for protective agents has revealed a group of physiologically active compounds known as plant growth regulators. This group comprises phytohormones

(Phs) as well. Many positive effects of Phs have been well-studied. The participation of Phs in the processes of plant adaptation to unfavorable medium conditions is known (1) and suggests a high probability of their participation in the regulation of expression of the anti-mutative and reparative functions of the genes of plants.

Nonetheless, information on the genetic effects of Phs available in the scientific literature is contradictory (1). Thus, the present study was performed to investigate the regularities of manifestation of the genetic activity of Phs. The aim of the present study was to identify the genoprotective and reparative properties of Phs.

Materials and Methods

In this investigation *Allium fistulosum, Lycopersicon esculentum, Triticum aestivum,* and *Gossypium hirsutum* were used. On these plants the cytogenetic action of hormonal preparations produced by the company SERVA, namely an auxin (indole-3-acetic acid (IAA)), kinetin (6-furfurylaminopurine (K)), gibberellin (A_3-3-acetate-acetylgibberellic acid (GA_3)), and abscisic acid (ABA), was studied. The cytogenetic action of Phs was studied against a background of spontaneous and induced mutagenesis. Evaluation was performed on the basis of a chromosome aberration test in mitotic cells of root meristem of 2-3-day-old sprouts of the plants. To induce mutagenesis gamma-radiation (γ-R: 10-40 Gr), N-nitrose-N-methyl urea (NMU: 0.02% × 3-6 h), herbicide – 2,4-dichlorphenoxyacetic acid (2,4-D: 10 mg/l × 3-6 h), and saline, aqueous, and thermal stresses were employed. To create the saline stress, solutions of sodium chloride (NaCl: 0.05-0.2 M) were used. The aqueous stress was created with the use of polyethylene glycol (PEG) (MW: 3000) with osmotic pressure of 0.5 atm of the solutions. The thermal stresses was created by the action of high temperature (t° = 45 °C × 6 h). Phytohormones were used before or after the action of mutagens. For fixation, staining, and analysis of the preparations, well-known cytogenetic methods were applied (4,5).

Results and Discussion

First of all, the modifying action of phytohormones on the spontaneous mutagenesis of plants was studied. The more distinctive experimental data are given in Figure 1. They demonstrate that the group of phytohormones under study (IAA, GA_3, K, and ABA) does not exert any mutagenic action on the plants over the rather wide range of concentrations used. It is shown that the frequency of chromosome aberrations in experimental variants does not exceed the reference (spontaneous) level of mutability. Moreover, the presented experimental data show that under the influence of phytohormones stabilization of fluctuation of the spontaneous chromosome aberrations to their optimal level of mutability takes place in some experimental variants (Figure 1). To determine the genoprotective or reparative activity of hormonal factors on the basis of solely these facts is possible only with equal percent of probability. For obtaining an explicit answer the effect of phytohormones on the induced mutation process in the plants was investigated. Here, for the purpose of determining the possibility of participation of hormonal factors in the regulation of expression of the protective function of genes the use of phytohormones prior to the action of mutagenic factors on plants was supposed. According to this pattern, the action of individual phytohormones (IAA, GA_3, and K) on the mutation process induced by saline, aqueous, or thermal stresses in the plants was studied. The most characteristic results of the investigation are presented in Figures 2-4. These data show that the introduction of exogenic phytohormones to plants prior to the mutagenic effect caused by saline, aqueous, or thermal stresses contributes to the suppression of the induced processes of formation of chromosome aberrations.

The presented experimental data also show that under the influence of the hormonal factors complete protection of genetic structures from mutagenic effects is achieved in most experimental variants. The frequency of chromosome aberrations in these experimental variants is comparable to the reference (spontaneous) level of mutability (Figures 2-4). It is explicitly demonstrated that under the influence of the hormonal factors an increase in expression of the protective function of genes takes place.

Finally, a rise in the non-specific stability of genetic structures to various mutagenic factors occurs. Along with that, no clearly expressed dependence of the effect of phytohormones on their dose is observed. These results point to the manifestation of genoprotective activity of phytohormones when they participate in the regulation of genetic processes at the stage preceding the emergence of primary damage to DNA.

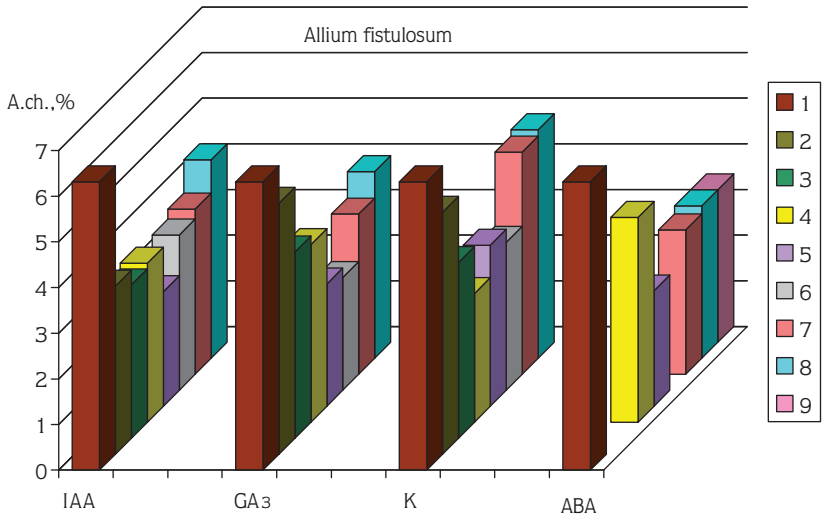

Figure 1. Spontaneous aberrations of chromosomes in the cells of *Allium fistulosum* root meristem in norm (1) and after the processing of seeds by different doses (2-9) of phytohormones (IAA, GA_3, K, ABA).

1. H_2O	1. H_2O	1. H_2O	1. H_2O
2. IAA (100 mg/l)	2. GA_3 (100 mg/l)	2. K (100 mg/l)	4. ABA (1 mg/l)
3. IAA (10 mg/l)	3. GA_3 (10 mg/l)	3. K (10 mg/l)	5. ABA (10^{-1} mg/l)
4. IAA (1 mg/l)	4. GA_3 (1 mg/l)	4. K (1 mg/l)	7. ABA (10^{-3} mg/l)
5. IAA (10^{-1} mg/l)	5. GA_3 (10^{-1} mg/l)	5. K (10^{-1} mg/l)	8. ABA (10^{-5} mg/l)
6. IAA (10^{-2} mg/l)	6. GA_3 (10^{-2} mg/l)	6. K (10^{-2} mg/l)	9. ABA (10^{-7} mg/l)
7. IAA (10^{-3} mg/l)	7. GA_3 (10^{-3} mg/l)	7. K (10^{-3} mg/l)	
8. IAA (10^{-5} mg/l)	8. GA_3 (10^{-5} mg/l)	8. K (10^{-5} mg/l)	

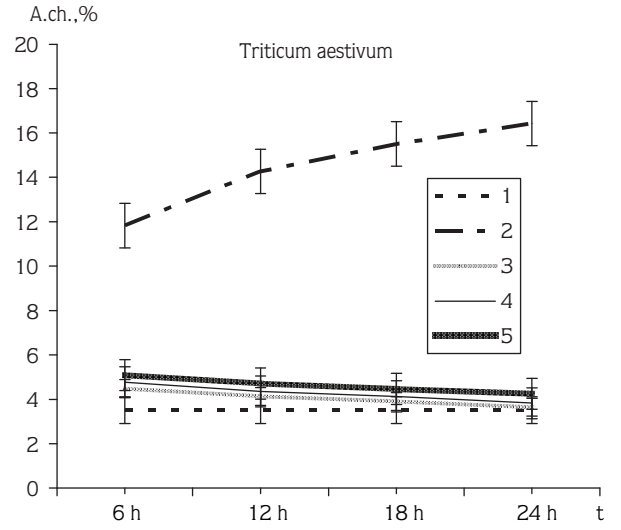

Figure 2. Influence of time of seeds' exposure in H_2O (1-2) and on the phytohormone (K) solutions of different concentrations (3-5) on the chromosomes' aberrations frequency, induced by salt stress.

(NaCl 0.2 M × 6 h)

1. H_2O
2. H_2O + NaCl (0.2 M)
3. K (1 mg/l) + NaCl (0.2 M)
4. K (10^{-1} mg/l) + NaCl (0.2 M)
5. K (10^{-2} mg/l) + NaCl (0.2 M)

It remained to elucidate how phytohormones can take part in the regulation of genetic processes after the emergence of the primary damage to DNA. For this aim Phs were applied after mutagenic factors. The influence of IAA, GA_3, K, and ABA on the mutation process induced by gamma-radiation or chemical mutagens was studied. Characteristic experimental data are given in Figures 5 and 6. These data show that the application of exogenic Phs to plants after they have been affected by gamma-radiation or chemical mutagens leads to a decrease in the frequency of the induced chromosome aberrations in the experimental variants to the reference (spontaneous) level of mutability. Under the influence of the hormonal factors complete recovery of the genetic consequences of the mutagenic effects takes place in the experimental variants. Under the influence of the hormonal factors the reparative function of genes is increased.

No clearly expressed dependence of this effect of phytohormones on their dose was observed. These results point to the manifestation of reparative activity of phytohormones when they participate in the regulation of

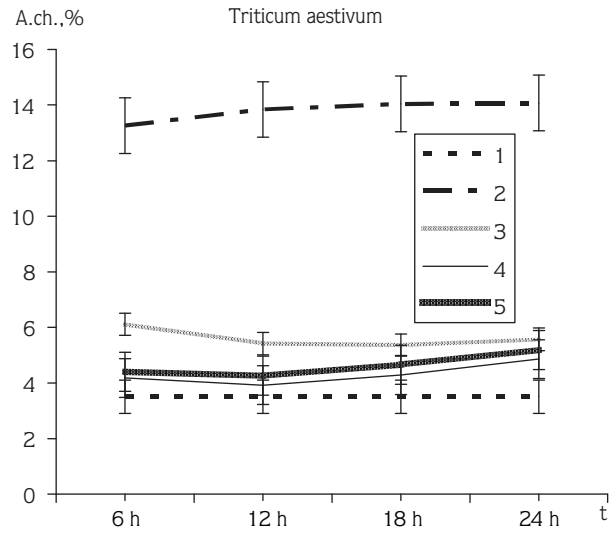

Figure 3. Influence of time of seeds' exposure in H_2O (1-2) and on the phytohormone (IAA) solutions of different concentrations (3-5) on the chromosomes' aberrations frequency, induced by water stress (PEG-0.5 atm × 6 h).

1. H_2O
2. H_2O + PEG (-0.5 atm)
3. IAA (1 mg/l) + PEG (-0.5 atm)
4. IAA (10^{-1} mg/l) + PEG (-0.5 atm)
5. IAA (10^{-2} mg/l) + PEG (-0.5 atm)

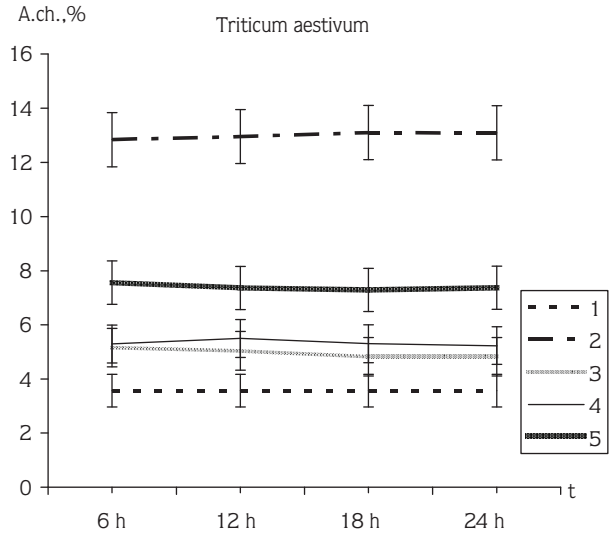

Figure 4. Influence of time of seeds' exposure in H_2O (2) and on the phytohormone (GA_3) solutions of different concentrations (3-5) on the chromosomes' aberrations frequency, induced by thermic stress.

(45 ºC × 6 h)

1. H_2O
2. H_2O + 45 °C
3. GA_3 (10^{-1} mg/l) + 45 °C
4. GA_3 (10^{-2} mg/l) + 45 °C
5. GA_3 (10^{-3} mg/l) + 45 °C

genetic processes at the stage following the emergence of damage to DNA.

Similar results were obtained in a number of other studies in which the author participated.

Manifestation of the reparative activity was observed for IAA and K through test of chlorophyll mutation and embryonic deaths induced by gamma-radiation in *Arabidopsis thaliana En.* (6). The same effect was seen for IAA and GA_3 through testing of chromosome aberrations induced by space flight factors in *Allium fistulosum* (7,8).

On the strength of the results obtained it is concluded that the phytohormones IAA, GA_3, K, and ABA do not exert any mutagenic action on the plants over a wide range of concentrations. Above all the ability of phytohormones to regulate genetic processes manifests itself in the stabilization of spontaneous mutations. No dependence of the effect of phytohormones on their dose is seen. The regular nature of this phenomenon suggests that exogenic phytohormones act as ignition keys for constitutional mechanisms of self-regulation of the balance between spontaneous mutation and antimutation processes in the genetic system of plants. In this case the necessary effect of stabilization of the spontaneous mutations is achieved even at a minimum dose of exogenic phytohormones.

The systemic nature of hormonal regulation of genetic processes is confirmed by data on the genoprotective and reparative activity of phytohormones. For manifestation of these properties and effectiveness of the action of exogenic phytohormones on induced processes no dependence on the dose of hormonal factor was seen. The regularity of this phenomenon suggests that exogenic phytohormones taking part in the regulation of genetic processes before or after the emergence of the primary damage to DNA serve as a factor of stimulation of endogenic hormonal system of regulation of expression of either antimutational or reparative function of genes. This may explain the fact that even a minimum dose of exogenic phytohormones is sufficient for achieving some effect. To sum up the discussion of the described results it is necessary to emphasize the following. The absence of dependence of the effect of phytohormones on their dose, which is found in this investigation, is not mentioned in scientific publications for other antimutagenic preparations known today (1,2). At the same time there

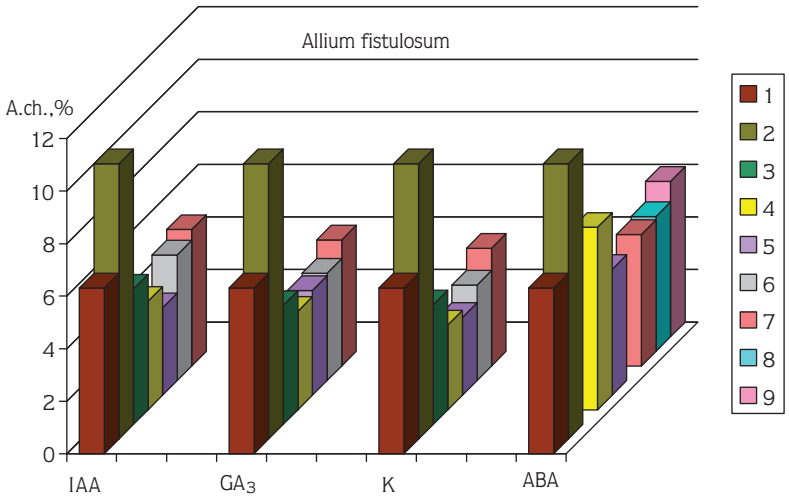

Figure 5. Aberrations of chromosomes in the cells of *Allium fistulosum* root meristem induced by γ-rays (2) and modified by different doses (3-9) of phytohormones (IAA, GA_3, K, ABA).

1. H_2O
2. γ-Ray (10 Gr) + H_2O
3. γ-Ray (10 Gr) + GA_3 (10 mg/l)
4. γ-Ray (10 Gr) + GA_3 (1 mg/l)
5. γ-Ray (10 Gr) + GA_3 (10^{-1} mg/l)
6. γ-Ray (10 Gr) + GA_3 (10^{-2} mg/l)
7. γ-Ray (10 Gr) + GA_3 (10^{-3} mg/l)

1. H_2O
2. γ-Ray (10 Gr) + H_2O
3. γ-Ray (10 Gr) + ABA (1 mg/l)
4. γ-Ray (10 Gr) + ABA (10^{-1} mg/l)
5. γ-Ray (10 Gr) + ABA (10^{-3} mg/l)
6. γ-Ray (10 Gr) + ABA (10^{-5} mg/l)
7. γ-Ray (10 Gr) + ABA (10^{-7} mg/l)

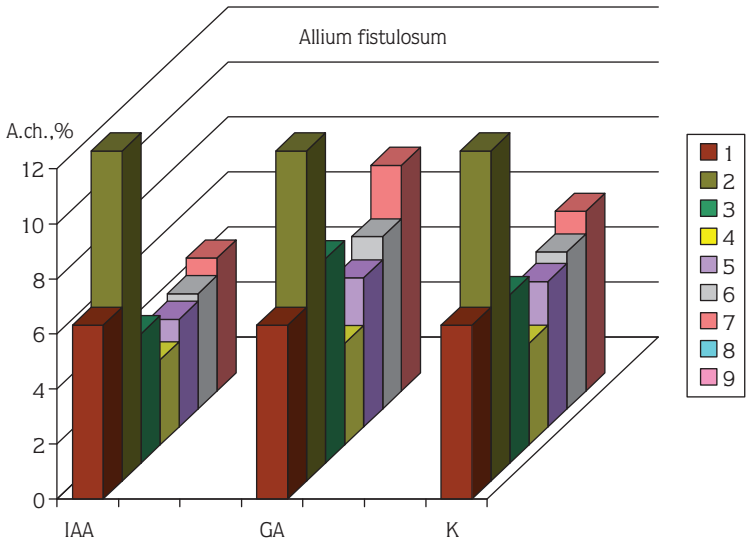

Figure 6. Aberrations of chromosomes in the cells of *Allium fistulosum* root meristem induced by NMU (2) and modified by different doses (3-7) of phytohormones (IAA, GA_3, K)

1. H_2O
2. NMU (0.02% × 3 h)
3. NMU (0.02% × 3 h) + IAA (10 mg/l)
4. NMU (0.02% × 3 h) + IAA (1 mg/l)
5. NMU (0.02% × 3 h) + IAA (10^{-1} mg/l)
6. NMU (0.02% × 3 h) + IAA (10^{-2} mg/l)
7. NMU (0.02% × 3 h) + IAA (10^{-3} mg/l)

1. H_2O
2. NMU (0.02% × 3 h)
3. NMU (0.02% × 3 h) + K (10 mg/l)
4. NMU (0.02% × 3 h) + K (1 mg/l)
5. NMU (0.02% × 3 h) + K (10^{-1} mg/l)
6. NMU (0.02% × 3 h) + K (10^{-2} mg/l)
7. NMU (0.02% × 3 h) + K (10^{-3} mg/l)

exist opinions that the phenomenon of imprinting (8) may be at the basis of regulation of gene expression by human and animal hormones. This hypothesis may also explain the regulatory effects of gene expression by phytohormones.

Corresponding Author:
Mekhtizadeh Emin RZA
National Academy of Sciences of Azerbaijan,
Genetic Resources Institute, Baku - AZERBAIJAN
emekhtizadeh@rambler.ru

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