

## **Influence of Supramolecular Complexes of Glycyrrhizic Acid with Phytohormones on the Germination of Wheat (*Triticum Aestivum* L.)**

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### **Abstract**

The research showed that Glycyrrhizic acid (GA): Supramolecular complexes of phytohormones (PG) (PG: indole-3-acetic acid (IAA); indole-3-butyric acid (IBA); naphthalene-1-acetic acid (NAA) and kinetin) (100  $\mu$ M ) in laboratory conditions significantly increased the intensity and amount of water-absorbing dynamics of «Do'stlik» wheat variety compared to the control, and the grain germination energy and germination level were optimized under experimental salinity conditions (NaCl=200 mM). The obtained results show the high prospects of using GA:PGsupramolecular complexes in agricultural practices to optimize the process of germination and development of plants under conditions of stress factors, including salinity.

**Keywords:**Glycyrrhizic acid, phytohormones, supramolecular complexes, «Do'stlik» wheat variety, germination rate, germination energy

## 1. INTRODUCTION

Globally, the salt level of cultivated land is increasing due to the intensive use of land resources, the increase in crop productivity and the excessive use of chemical preparations in the system of combating plant diseases, as well as the use of non-scientific agromelioration measures[1]. It has a negative impact on the growth and development of plants, as well as the productivity and quality of the harvest [2]. Therefore, the selection of salt-resistant varieties of agricultural plants or the increase of resistance indicators is stated as one of the urgent issues of science.

A wide variety of natural and artificial chemicals have been recommended according to the results of researches related to increasing salt tolerance properties of plants. Many of them have been put into practice until today. In this case, the prospects of using phytohormones, which perform the function of mediators of external signals in plant tissue cells, are highly appreciated [3,4]. Some results have been showed on increasing the level of resistance and the most important biological indicators at the stages of growth and development of agricultural crops with the help of bioregulators under the influence of salinity [5,6].

Clarifying the importance of phytohormones in the formation of resistance mechanisms in the plant organism under the influence of various stress factors, developing optimization technologies, methods and approaches is considered as a theoretical/practical issue of the research [7].

In this study, the research was conducted on the effect of supramolecular complexes of GA with PG (ISK; IMK; NSK and kinetin) on the parameters of grain germination of wheat variety "Do'stlik" (*Triticum aestivum* L.) under experimental salinity conditions.

## 2. EXPERIMENTAL SECTION

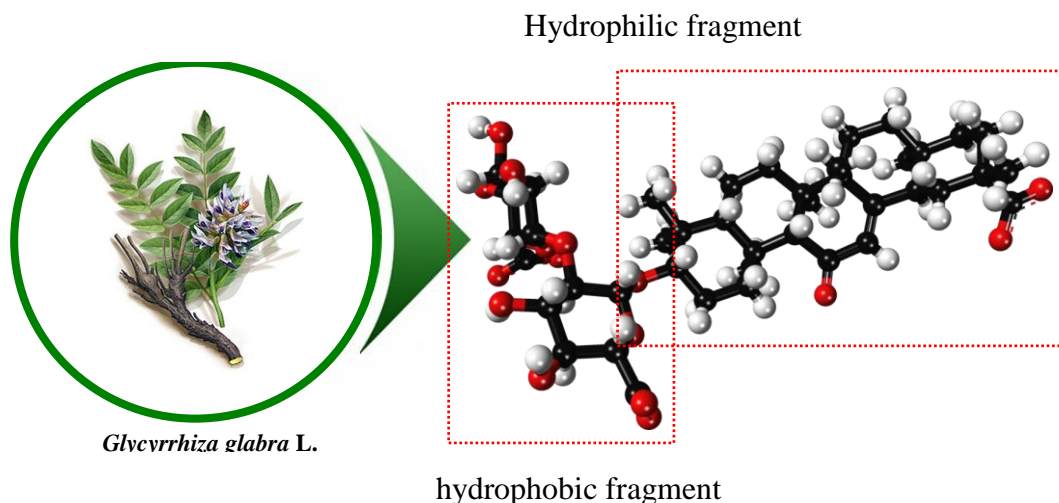
### 2.1. Research objects

Wheat variety in "Do'stlik"(*Triticum aestivum* L.) cultivated in large areas in Uzbekistan was selected in order to study the biological activity of auxins (ISK, IMK, NSK) and kinetin and GA:PGsupramolecular complexes, which are important in the growth and development of wheat in the experiments.

### 2.2. Isolation/chemical identification of GA from root plants of licorice (*Glycyrrhiza glabra* L.) and synthesis of supramolecular complexes GA:PG

The experiments was carried out using an IR-Fure spectrometer for the preparation of root extract of licorice plant (*Glycyrrhiza glabra* L.) and extraction of GA from its contents

(Fig. 1), chemical identification, synthesis of GA:PGsupramolecular complexes based on standard methods, "PerkinElmer Spectrum IR" (Germany; version 10.6.1) [8-10].



**Figure 1. Chemical structural formula of Glycyrrhizic acid**

(Empirical formula -  $C_{42}H_{62}O_{16}$ ; 20 $\beta$ -carboxy-11-oxo-30-norolean-12-en-3 $\beta$ -yl-2-O- $\beta$ -D-glucopyranuronosyl- $\alpha$ -D-glucopyranosiduronic acid)

[Schlotgauer, 2013; pp. 553–556; Yakovshin, 2018; p. 10–19].

### **2.3. Study of germination parameters of wheat (*Triticum aestivum* L.) grain in laboratory conditions**

Wheat grains were sterilized with NaClO (2%) solution [11] or KMnO<sub>4</sub> (1%) solution for 5 minutes or using ethanol solution (70%) for 2 minutes [12], in the next step they were washed in a stream of distilled water and levied in a Petri cup [13]. Petri cup was sterilized using ethanol solution (70% alcohol). Grains were cut to a size equal to the diameter of the Petri cup, 100 grains per cup, and placed on filter paper soaked in distilled water (10 mL) [6,14].

Filter papers «Whatman #1» («Sigma-Aldrich»; Germany) and D=110 («Chimreaktivkomplekt»; Russia) were used in the experiments.

Germination of wheat grains was carried out for 10 days (240 hours) in the dark, in a thermostat at a temperature of +22°C. In this case, after 24 hours, the process of germination began in grains [15].

In the germination process, the germination energy was calculated on the 3rd day (72 hours) and the germination level was recorded on the 10th day [14].

A root longer than half the length of the seed was considered as ripe [14].

*Germination energy*– represents the percentage (%) of grains that have matured at a normal level during the past time (3 days) in relation to the total number of grains [16].

*Germination rate* - represents the percentage (%) of grains ripened at the standard level compared to the number of grains used in the general experiment [16].

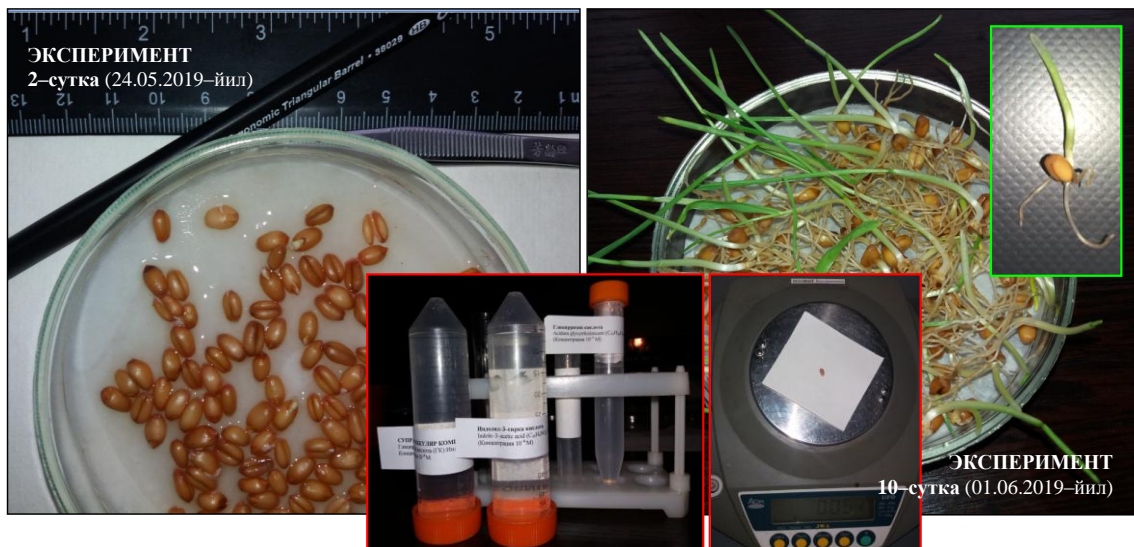
In the experiments, the germination rate (GR; Germination rate) was calculated using the following formula [17]:

$$GR = x_1/D_1 + (x_2 - x_1)/D_2 \dots (x_n - x_{n-1})/D_n$$

Here:*D*- represents the number of experimental days in which the calculation was made.

#### 2.4. Experimental salinity model

In the experiments, wheat (*Triticum aestivum* L.) germination parameters were analyzed using the universal method in the laboratory under experimental salinity conditions [18].



**Figure 2. Study of germination parameters of wheat (*Triticum aestivum* L.) grain in laboratory conditions**

Experimental salinity was created using NaCl (200 mM) incubation [2,19,20] and distilled water was used in the control group.

In the experiments, "Techniprot" (Poland; maximum 250 mg; accuracy level  $\pm 0.2$  mg); JW-1 (200 $\pm$ 0.2 g.; Acom; Korea) laboratory scales were used for preparing solutions of NaCl ("Sigma-Aldrich"; Germany), glycyrrhizic acid (GA), indolyl-3-acetic acid (IAA), indolyl butyric acid (IBA), kinetin and GA:PG supramolecular complex. 0.117 g of NaCl was dissolved in distilled water (10 mL) (200 mM) to create an experimental salinity model.

## 2.5. Mathematical-statistical processing of the obtained results

The results of the experiment were mathematically and statistically processed using the special software package OriginPro v.8.5 SR1 (EULA, USA) according to standard biometric methods [21].

## 3. RESULTS AND DISCUSSION

In the experiments, the average weight of 1 grain of wheat was equal to  $41.7 \pm 2.4$  mg, and after the soaking phase (72 hours) it increased by  $47.5 \pm 3.5\%$  compared to the control, and equal to  $61.5 \pm 2.4$  mg. In this case, it was noted that the intensity of dehydration is at a high level during the first 16-72 hours (1-3 days). Moreover, the intensity and amount of water absorption dynamics of wheat grain "Dostlik" increased compared to the control under the influence of GA:PG (PG: IAA, NAA, IBA and kinetin) supramolecular complexes ( $100 \mu\text{M}$ ) during the germination process in laboratory conditions, including the amount of water absorbed in 10 days respectively -  $28.4 \pm 2.5$ ;  $25.5 \pm 3.7$ ; and  $20.8 \pm 2.4$  and  $26.7 \pm 3.8$  mg (Table 1).

The obtained results are consistent with the existing literature data [6]. During the germination process, wheat grain enters the germination phase after water absorption of  $\sim 45\text{--}50\%$  than its dry weight [6]. In the researches, during the germination of wheat grain, the water absorption phase has a duration of  $\sim 2\text{--}6$  hours, and in the next phase ( $\sim 6\text{--}16$  hours) water reaches the endosperm, in this phase the activation of enzymatic reactions appears [22].

During seed germination, PGs perform the most important physiological function as important endogenous regulators [6].

**Table 1.** The effect of GA:PG (PG: IAA, NAA, IBA and kinetin) supramolecular complexes on the dynamics of water absorption of «Do'stlik» wheat grain in laboratory conditions ( $M \pm m$ )

Experience options	Dynamics of weight in the process of grain germination (mg)					Amount of water ingested (mg)
	Start time (hours)					
	6	16	24	72	240	
Control (distilled water)	$48.5 \pm 3.6$	$51.3 \pm 2.7$	$54.4 \pm 3.8$	$58.5 \pm 3.7$	$61.5 \pm 2.4$	$19.8 \pm 3.2$
GA:ISK (4:1) $100 \mu\text{M}$	$51.6 \pm 4.2$	$59.8 \pm 5.2^*$	$67.5 \pm 3.5^{**}$	$69.6 \pm 4.2^{**}$	$70.1 \pm 3.6^{**}$	$28.4 \pm 2.5$
GA: NSK (4:1)	$49.3 \pm 3.4$	$52.5 \pm 4.2^*$	$60.2 \pm 4.4^{**}$	$65.8 \pm 5.3^{**}$	$67.2 \pm 4.5^{**}$	$25.5 \pm 3.7$

100 μM						
GA:IMK (5:1)	48.7±5.2	59.4±5.7*	60.8±2.5**	61.4±3.1*	62.5±4.4*	20.8±2.4
100 μM						
GC:Kinetin (4:1)	50.6±2.6	55.4±4.1*	63.6±5.5**	67.6±4.8**	68.4±5.2**	26.7±3.8
100 μM						

**Note:**\* – difference of the values of the experimental group compared to the control with a statistical confidence level of  $r < 0.05$ , \*\* –  $r < 0.01$ .

Researches have reported the optimization of the permeability properties of the biological membrane under the influence of GA [23-25].

The obtained results can be explained by the optimization of permeability properties of biological membranes under the influence of GA:PG (PG: IAA, NAA, IBA and kinetin) supramolecular complexes (100 μM).

In the next series of experiments, the influence of GA:PG (PG:IAA, NAA, IBA and kinetin) supramolecular complexes on germination parameters of «Dostlik» wheat variety was analyzed under experimental salinity conditions.

In the experiments, after 72 hours in laboratory conditions, the energy of grain germination was equal to 38.9±4.3% in the control variant, and decreased to 16.4±3.5% in the experimental salinity (NaCl=200 mM) conditions, in turn, the value of this indicator was found to recover respectively 36.4±7.3%; 34.7±6.5%; 23.4±3.4% and 34.3±3.3% in this condition at a concentration of 100 μM under the influence of GA:IAA (4:1), GA:NAA (4:1), GA:IBA (5:1) and GA:Kinetin (4:1) incubation (Table 2).

Also, in experiments, the degree of grain germination in laboratory conditions was equal to 86.4±5.7% in the control variant, and decreased to 43.7±4.2% under experimental salinity conditions (NaCl=200 mM), in turn, under the influence of GA:IAA (4:1), GA:NAA (4:1), GA:IAA (5:1) and GA:Kinetin (4:1) during incubation under these conditions at a concentration of 100 μM the value of this indicator was 84.2±6.5%, respectively; 58.5±5.5%; Revealed 65.7±4.8% and 76.2±6.4% recovery (Table 2).

**Table 2.** Effects of GA:PG (PG: ISK, NSK, IMK and kinetin) supramolecular complexes on germination parameters of "Do'stlik" wheat variety in laboratory conditions (M±m)

Experience options	Grain germination energy(%)		Grain germination rate(%)	
	Control	NaCl (200 mM)	Control	NaCl (200 mM)
Control	38.9±4.3	16.4±3.5**	86.4±5.7	43.7±4.2**



(distilled water)				
GA (100 $\mu$ M)	42.4 $\pm$ 5.2*	25.9 $\pm$ 4.8*	87.5 $\pm$ 6.4*	51.5 $\pm$ 4.4*
ISK (100 $\mu$ M)	40.5 $\pm$ 6.4*	35.6 $\pm$ 5.5**	95.8 $\pm$ 6.3**	80.5 $\pm$ 6.6**
GA:ISK (4:1) 100 $\mu$ M	48.3 $\pm$ 5.3**	36.4 $\pm$ 7.3**	96.3 $\pm$ 6.5**	84.2 $\pm$ 6.5**
GA: NSK (4:1) 100 $\mu$ M	39.3 $\pm$ 4.5*	34.7 $\pm$ 6.5**	92.3 $\pm$ 3.7**	58.5 $\pm$ 5.5**
GA:IMK (5:1) 100 $\mu$ M	24.6 $\pm$ 4.2*	23.4 $\pm$ 3.4*	90.4 $\pm$ 3.5**	65.7 $\pm$ 4.8**
GC:Kinetin (4:1) 100 $\mu$ M	45.4 $\pm$ 6.6**	34.3 $\pm$ 3.3**	95.6 $\pm$ 4.4**	76.2 $\pm$ 6.4**

**Note:**\* – level of statistical reliability compared to the control  $r < 0.05$ , \*\* –  $r < 0.01$  ( $n = 3-4$ ).

Many researchers have noted that wheat grain germination indicators decrease under the influence of salinity, and this condition is caused by a decrease in the osmotic potential value, an increase in the concentration of  $\text{Na}^+$  ions, a cytotoxic effect, a slowing down of the transport process of reserve nutrients in the grain, a decrease in the degree of water absorption (absorption) of the seed [26-28], the disturbance of the  $\text{K}^+/\text{Na}^+$  balance in the cell membrane and, in turn, it is explained by the dysfunction of the embryonic development process [29], as well as the effects of genetic and other factors [30] together with dysfunctions in the biological membrane.

In the researches, it is explained by the decrease of morphometric/functional parameters of wheat grain during the germination of wheat grain under the influence of salinity [31], disruption of ion homeostasis in cells, cytotoxic effect due to the increase of  $[\text{Na}^+]$  in content [32].

Studies have shown that wheat grain germination energy increases under the influence of PG (auskin et al.) [33], and the obtained results are consistent with the data of this literature.

Also, the normalization of the morpho-functional indicators of the root was noted under the influence of PG in salinity conditions [32].

Considering that PG is important in the bioregulation of enzyme activity in plant seeds during germination [15], it is emphasized that the optimizing effect of PG in salinity conditions is related to the activation of functional enzymes [31].

The formation of a plant's resistance mechanism to stress-factors is considered a complex, multi-component process that includes specific/non-specific biochemical reactions [15], specific compensatory mechanisms are activated in the plant under the influence of stress-factors, and it is emphasized that phytohormones are considered one of the central functional components [4].

Thus, the positive effect of endogenous regulators on the germination parameters of wheat grain under salinity conditions and the increase of resistance to various abiotic stress factors, including salinity, have been noted by many researchers [34-42], and the use of PG's in optimizing the germination parameters of wheat grain under salinity conditions is noted to give effective results [31].

In the research carried out in the conditions of Uzbekistan, it was noted that GA and its derivatives isolated from the plant *Glycyrrhiza L.* and its roots optimize the fertility properties of the soil under salinity conditions, and have a positive effect on the growth and development and productivity indicators of agricultural crops [3].

In particular, studies have shown that GA reduces the level of salinity, increases the concentration of phenol compounds, increases the level of resistance of the plant to the effects of diseases, and has a positive effect on the germination-development and yield indicators of wheat due to the formation of a complex with soluble salts in the soil under salinity conditions [3].

Therefore, increasing the yield and quality of agricultural crops, ensuring food safety is considered one of the strategic priority issues [6], the solution of this issue are highly valued in the prospects of using environmentally safe endogenous phyto regulators, which have the property of optimizing the germination and development indicators of agricultural crops [6].

It has been proven that the use of endogenous phyto regulators in agricultural practice allows to increase the level of resistance to the effects of various phytopathogens and stress factors through the stimulation of complex biochemical/physiological processes in the plant organism [6].

In the conducted experiments, it was found that GA:PG (PG: IAA, NAA, IBA and kinetin) supramolecular complexes (100  $\mu\text{M}$ ) significantly increased the intensity and amount of water absorption dynamics of «Do'stlik» wheat grain during germination in laboratory conditions compared to the control. Also, during the germination process in the laboratory, significant optimization of grain germination energy and germination level was noted as the



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most important result after 72 hours in experimental salinity conditions (NaCl=200 mM). Under the influence of PG, the morpho-functional indicators of wheat grain in the initial phase of ontogenesis are higher than the control which can be explained on the basis of the fact that it is related to the metabolic-biochemical changes at the cellular level. The obtained results show that the high prospects of using GA:PG supramolecular complexes in agricultural practices to optimize the process of germination and development of plants under conditions of stress factors, including salinity.

## 5. CONCLUSIONS

It can be noted that the germination of wheat grains and the development of the root system in an environment with a salinity level is primarily related to the indicators of salinity resistance of seedlings. Based on the results of the conducted research, it can be noted that GA:PG (PG: indole-3-acetic acid; indole-3-butyric acid; naphthalene-1-acetic acid and kinetin) supramolecular complexes (100  $\mu$ M) significantly increase the intensity and quantity of the dynamics of water absorption of wheat grain compared to the control. Under the conditions of experimental salinity (NaCl=200 mM), the germination energy and germination level of wheat grain can be optimized under the effect of GA:PG. The obtained results show the high prospects of using GA:PG supramolecular complexes in agricultural practices to optimize the process of germination and development of plants under conditions of stress factors, including salinity.

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