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SHORT COMMUNICATION

Evaluation of potential and rate of the germination of wheat seeds (*Triticum aestivum* L) treated with bifunctional growth regulators under water stress

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ABSTRACT

The synthetic cytokinins belonging to the class of bifunctional carbamates and oxamates were tested for a biological activity in a wide concentration range of 10^{-2} M – 10^{-6} M along with chlorocholine chloride taken as reference. The compounds have a pronounced growth-regulatory activity for wheat seeds (*Triticum aestivum L*) at the optimal concentration of 10^{-5} M, at which the germination potential Gp and the germination rate Gr of seeds, as well as resistance to water stress, determined by RWC values reach their maximum values. It was found that the technique of spraying wheat seeds with solutions of the compounds used is more preferable than soaking.

Keywords: Wheat; Plant growth regulators; Carbamates; Oxamates; Water stress

INTRODUCTION

Abiotic stresses such as drought, extreme temperatures, salinity, flooding, lack of or high light levels, imbalances in inorganic nutrients, anthropogenic toxic compounds and oxidative stress result in crop losses annually (Ferreira et al., 2021; Seleem et al., 2021). Water stress (drought), being the second major factor in yield decline after diseases, alters the physiological, morphological, biochemical and molecular traits of plants (Rijala et al., 2021; Dhankher and Foyer, 2018; Dutta et al., 2016). In recent years, a large number of natural and synthetic plant growth regulators (PGR) with a wide range of biological, including cytokinin, activity have been tested, which increase the productivity of cultivated plants due to structural similarity with natural plant hormones and resistance to a complex of unfavorable environmental factors (Oshchepkov et al., 2020; Ishihara et al., 2019; Oh and Hoshi, 2019; Nakagawa et al., 2021). The data obtained as a result of biological tests (Kalistratova et al., 2021) indicate that the synthesized bifunctional compounds of a number of carbamates and oxamates have pronounced antistress and regulatory activity. The active ingredients soften the shell of wheat seeds, penetrate into the grain and activate physiological processes, promoting an increase in germination, growth of shoots, roots and improving resistance to water stress. The most effective carbamate (**I)** and oxamate (**II)** (Fig.1) were tested at a concentration of 10^{-3} M, since this value was used in most studies (Lone et al., 2021; Kovalenko et al., 2020; Alexopoulos et al., 2006). However, the question of the optimal concentration of compounds of this class for the treatment of wheat seeds remains open. The aim of this work is to determine the optimal concentrations of compounds **I** and **II**, as well as the treatment methods under which the germination potential, germination rate of wheat seeds (*Triticum aestivum* L), as well as resistance to water stress will be maximally possible.

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MATERIALS AND METHODS

Compounds

Synthesis of carbamate **I** and oxamate **II** was carried out at the Department of Chemistry and Technology of Biomedical Preparations of Mendeleev University of Chemical Technology of Russia (55°46'44°N; 37°35'43°E) and used as the objects of the current research study, Fig. 1.

O-*i*-Propyl-N-(2-hydroxyethylamino)carbamate **I** was synthesized according to procedure described before (Sheshenev et al., 2020) and O-*i*-Propyl-N-(2-hydroxyethyl) oxamate **II** obtained by analogy with reference (Kalistratova et al., 2021) and used at concentrations 10^{-3}, 10^{-4} and 10^{-5} M (T_1 - T_3 for **I** and T_4 - T_6 for **II**), respectively.

Preparation O-*i*-Propyl-N-(2-hydroxyethyl)oxamate **II**. 9,74 g (67 mM) of diisopropyloxalate in ethanol (3.4 ml) was placed in a round bottom flask equipped with a dropping funnel and magnetic stirrer. The solution was cooled on an ice water bath up to 0ºC. Then solution of 0.811 g (13.3 mmol) of the monoethanolamine in ethanol (7 ml) was added dropwise to a stirred cool mixture. After the addition the mixture was left to warm up to room temperature, filtered and concentrated under vacuum. The product of 95% purity was obtained after diisopropyloxalate excess distillation at 0.1 mm Hg. ^{1}H NMR (DMSO-d6, δ, ppm, *J*, Hz): 1.23 (d, 6 H, C$\underline{H}_3$, J^3 = 7.1); 3.15-3.23 (m, 2H, NH-C$\underline{H}_2$); 3.42 (t, 2H, C$\underline{H}_2$OH, J^3 = 5.6); 4.97 (sept, 1H, C$\underline{H}$). ^{13}C NMR (DMSO-d_6, δ, ppm): 21.52 ($\underline{C}H_3$); 42.01 (NH-$\underline{C}H_2$); 59.19 ($\underline{C}H_2$-OH); 70,39 ($\underline{C}H(CH_3)_2$); 157.71 (O-$\underline{C}$(O)); 160.45 ($\underline{C}$(O)-NH).

Compounds **I** and **II** are completely soluble in water. Structures of all synthesized compounds were confirmed by ^{1}H and ^{13}C NMR, mass-spectrometry and elemental analysis data. ^{1}H and ^{13}C NMR-spectra were recorded with «Bruker DRX-400» spectrometer operating at 400.13 MHz frequency, using DMSO-d_6 as solvent and TMS as an internal standard. Chemical shifts were measured with 0.01 ppm accuracy, coupling constants are reported in Hertz. Mass-spectra was recorded on an inductively coupled plasma mass spectrometer XSeries **II** ICP-MS (Thermo Scientific Inc., USA). The melting points are determined using the melting point apparatus Stuart SMP20 (UK).

Methods - soaking and spraying of seeds

The experiments on growing wheat were conducted at the laboratory of the Nesmeyanov Institute of Organoelement Compounds of Russian Academy Sciences, Moscow, Russia from March 2020 to December 2020. The geographic coordinates of the site are 55° 42'03" N; 37° 34'30" E.

Experiments on soaking and spraying were carried out simultaneously on seeds (*Triticum aestivum* L) in rectangular Petri dishes (85 x 75 mm) with three replications on an NLO-79-01-00 phyto-LED lighting setup (INEOS RAS) with a wavelength of Red 615/Blu 457 nm, providing illumination of the samples for 12/12 hours with an intensity of at least 250 lux at a temperature of 22.5 ± 1.5° C, relative humidity of 50 ± 1.5 %.

To study the growth-regulating activity of compounds **I - II**, wheat seeds (*Triticum aestivum* L.) named "selected wheat for germination" (Declaration EAEC NRUD-RU. AB97.V.0093/19), harvest 2019, provided by Biosphere LLC, Fedorovka village, Staroshaigovsky District, Mordovia, Russia, 54 ° 47'00" N, 46 ° 14'00" E were used. Wheat seeds were sterilized with a 0.2 % sodium hypochlorite solution for 10 min, washed three times with distilled water, and dried in an oven at 30° C for 48 hours. The dried seeds were stored at 5° C. As the first control group, seeds treated with distilled water (T0) were used as for soaking and spraying. A well-known preparation Chlormequat Chloride (CCC) recommended by BASF for the CIS countries, Asia and Africa, was used with the same concentrations from 10^{-3} to 10^{-5} M (T_7 - T_9) as the second control group for soaking and spraying.

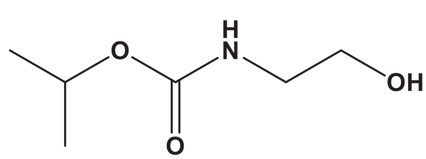
Determination germination index

Germination potential (Gp) and germination rate (Gr) were calculated using the following formulas (Eq. 1,2) (Guo et al., 2019):

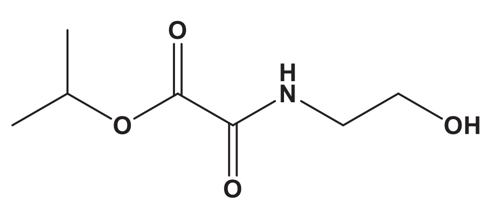
Germination potential (%) = [Number of germination seeds 1 d/Number of total seed] x 100 (1)

Germination rate (%) = [Number of germination seeds 4 d/Number of total seed] x 100 (2)

The relative water content (RWC) was determined by the formula (Eq. 3).(Mullan and Pietragalla, 2018):



I



II

Fig 1. Synthesis of carbamate I and oxamate II

RWC % = [(TW (FW - DW)/- DW)] × 100 (3)

where: FW = fresh weight; TW = obese weight; DW = dry weight.

Experiment on wheat growth

To soak the seeds, the technique described in reference (Semenov A.S., and Lukatkin, 2015) was used. Since compounds **I** and **II** are soluble in water at a concentration of 10^{-3} - 10^{-5} M, the formula (4) and analysis described in the reference (Golovatskaya, 2016) was used to determine the percentage of absorption:

$$\text{Water Uptake (\%)} = [\,(m_1 - m_0)/m_0] \times 100 \quad (4),$$

where m_o is the weight of dry seeds, m_1 is the weight of soaked seeds.

Spraying was carried out according to the methodology from (Kalistratova et al., 2021). After soaking and spraying, the seeds were covered with filter paper and filled with 10 ml of distilled water. Then all samples were moved to the shelves of the germination unit for 7 days. The experiment was carried out in the dark for the first 24 hours. The seeds were ventilated daily, opening the cups for 25 minutes, and 5 - 10 ml of distilled water were added as needed to prevent the seeds from drying out. On the third day of germination, the lids of the Petri dishes were removed so as not to interfere with the growth of seedlings. The last watering was 96 hours later. The germination potential Gp was determined 24 hours after the start of the experiment. The protrusion of the root by 1 mm was recorded as a criterion for germination. On the fourth day, the germination rate Gr of wheat seeds was determined. To measure the length of roots (RL) and the height shoots (Sh), fifteen seedlings were randomly taken from each Petri dish. The relative water content RWC was determined 72, 96 and 120 hours after the last watering, fifteen shoots taken at random were also selected according to (Mullan and Pietragalla, 2018). The table shows the data for the ninth day (120 hours after the last watering).

Statistical analysis

Statistical processing of the results was performed using Microsoft Excel software and STATISTICA 13.3 TRIAL (StatSoft Russia). Basic statistical parameters such as mean, standard deviation (SD) were computed along with one-way analysis of variance (ANOVA). To assess the statistical significance of various data sets, an acceptable value of significance was $p \leq 0.05$. The 95% confidence interval of true averages is shown in the Table 1.

RESULTS AND DISCUSSION

Preliminary studies of wheat seeds (*Triticum aestivum* L) treated with compounds **I - II** with a concentration of 10^{-2} M and 10^{-6} M showed the results below the data for T_0 by an average of 8 – 10 % at a concentration of 10^{-2} M and, accordingly, 3 – 5 % for 10^{-6} M, therefore, the values of these concentrations are not presented in further studies. The results of the experiment on the treatment of seeds (*Triticum aestivum* L) with substances **I - II** are shown in the Table 1. The first part of the table shows data on the percentage of water absorption (W_{Up}) by wheat seeds (*Triticum aestivum* L) during soaking, which range from 28.63 to 43.20 %. This means that the germination process has begun for most of the specimens. Solutions penetrate into more complex structural elements of the seed, such as organelles and the nucleus, protein synthesis on ribosomes and DNA transcription, hydrolysis of spare proteins and starch begins. In addition, respiration increases dramatically due to the completion of mitochondriogenesis by embedding cytochrome oxidase and succinate dehydrogenase subunits from the matrix into the inner membrane of mitochondria (Golovatskaya, 2016). The highest values > 40.0 % were observed when using compound **I** in samples T_2 and T_3, compound **II** in T_5, and CCC in T_8 treatments.

Plants constantly adapt to the environment, perceiving dawn and dusk as signals for organizing their growth, development and metabolism at the appropriate time of day (Seluzicki, 2017) so the first 24 hours of experiments were conducted in the dark. The germination potential Gp is the rate of root elongation; it is not a part of the germination process as such, but is an important component of pre-emergence seedling growth (Hatsig et al., 2015) Germination rate Gr is the main key element of vigorous seeds. In addition, it is very important to have rapid root and hypocotyl growth, as well as to know the number of shoots by the fourth day of the experiment (Finch-Savage et al., 2015). Seeds when soaked 24 hours after the start of the experiment showed a higher germination potential Gp than when irrigated, since soaking seeds in the dark promoted the activation of their metabolism, which led to a rapid lengthening of their hypocotyl. However, by the fourth day of the experiment, the germination rate Gr of the soaked seeds slowed down compared to spraying. This is due to the fact that light stops the etiolation program and activates the transformation of etioplasts into chloroplasts (Zhong et.al., 2014). This entails stopping hypocotyl elongation, opening and growth of petioles and cotyledons, differentiation of proplastids into chloroplasts and elongation of roots (Sinclair et.al., 2017).

Despite the fact that the germination potential Gp of the sprayed seeds was less than that of the soaked seeds, the germination rate Gr when the seeds were sprayed with compound **II** with a concentration of 10^{-5} M was 16 % higher compared to the seeds treated with compound **II**

Table 1: Wheat (*Triticum aestivum L*) seed treatment results soaking

	Soaking									
	Carbamate I				Oxxamate II			CCC		
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
WUp,%	38.7^{ns}	40.3^{ns}	42.22**	42.51*	32.89**	41.11*	28.63^{ns}	39.27**	43.20*	35.93^{ns}
Gp,%	76*	62^{ns}	78*	78^{ns}	70*	72^{ns}	72*	72*	68**	64^{ns}
Gr, %	78^{ns}	72^{ns}	80**	80*	72**	74*	72^{ns}	82*	70^{ns}	72*
R_L,cm	6.3*	7.6*	7.4^{ns}	7.3*	8.5**	7.6*	6.8^{ns}	7.3*	8.7^{ns}	7.1*
Sh_h, cm	10.2*	13.5**	11.4**	11.8*	12.8*	12.1**	12.3^{ns}	10.9^{ns}	10.8**	12.6**
R_L/Sh_h,%	100	91.80*	104.9^{ns}	100**	108.1^{ns}	101.63**	90.16*	108.19**	129.5^{ns}	91.80*
RWC,%	16.8^{ns}	17.64*	11.76^{ns}	14.26**	19.47**	15.03^{ns}	17.79**	19.24**	4.79^{ns}	21.74*
	Spraying									
	Carbamate I				Oxamate II			CCC		
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
Gp,%	51**	51^{ns}	50*	54**	49*	51^{ns}	55*	46**	54*	70^{ns}
Gr, %	79**	86^{ns}	85*	82^{ns}	82^{ns}	76**	88*	84*	75^{ns}	86*
R_L,cm	3.7^{ns}	4.7*	5.6**	$8,8^{ns}$	6.2*	5.3**	8.8**	3.8^{ns}	3.5*	7.1*
Sh_h, cm	5.3*	7.4**	9.0^{ns}	11,7*	8.1**	8.8^{ns}	14.2*	5.5*	5.3**	12.9^{ns}
R_L/Sh_h,%	100	91.30**	89.85*	107.73^{ns}	110.0^{ns}	86.95*	88.4**	100^{ns}	95.65**	78.84*
RWC,%	20.96^{ns}	27.89**	27.65^{ns}	30.34*	29.7**	27.89^{ns}	35.71*	24.98**	22.86^{ns}	30.25*

T_0: Without treatment, T_1 -10^{-3}M, T_2 -10-4M, T_3 -10^{-5}M - carbamate I;
T_4 -10^{-3}M, T_5 -10^{-4}M, T_6 -10^{-5}M - oxamate II;
T_7 -10^{-3}M, T_8 -10^{-4}M, T_9 -10^{-5}M - CCC; R_L, cm - root, Sh_h, cm - shoot
*significant at 5 % and ** significant at 1 %; ns - not significant according to the F test.

with a concentration 10^{-5} M after soaking. In addition, the germination rate Gr of seeds treated with compound **II** was 9 % higher than the first control group T_0 and 2 % higher than the second control group treated by spray of 10^{-5} M T_9.

Seed size does not affect germination, but affects growth, development and yield. Larger seeds have advantages over smaller seeds, they have faster seedling growth and a higher grain yield. In addition, the advantage of larger seeds is manifested when the crop is grown under environmental stresses, especially drought (White and Edwards, 2007) Shoot height in some plants was higher when soaked than when spraying. This is a consequence of the lengthening of the hypocotyl during etiolation. However, when seeds were sprayed with compound **II** with a concentration of 10^{-5} M (T_6 treatment), the shoot length was on average 13.39 % longer than the length of the shoot treated with compound **II** with a concentration of 10^{-5} M after soaking. In addition, 2.67 times higher than the shoot of the first control group T_0 and 9.16 % higher than the shoot length of the second control group T_9, treated with spraying.

It is known that ratio the root length and shoot height (R/S) is one of the most important indicators for assessing plant health, it serves as an indicator of the hormonal response to various types of stress caused by environmental changes, chemical or physical agents (Agathokleous et al., 2018). Studies have shown that the response of plants to carbomate **I** and oxomate **II** of various concentrations upon soaking was higher than upon spraying. The lowest value was in the T_6 samples both when soaking (90.16 %) and when spraying (88.40 %) compared to the results of the first control group TO (100 %). Thus, compound **II** with a concentration of 10^{-5} M (T_6) in this experiment has the most minimal effect on wheat seeds (*Triticum aestivum* L).

Measuring the relative water content RWC in leaves is a reliable and simple method for assessing the water status of a leaf, usually used to describe the state of water in a plant at a given point in time (Tanentzap et al., 2015). Typical RWC values for turgid leaves reach 98 %; about 60–70 % is during wilting of leaves and up to about 40 % in strongly drying and aging leaves (Alexopoulos et al., 2006). The relative water content in the RWC was determined 72, 96 and 120 hours after the last watering, as we wanted to understand how the RWC in the leaves changes when watering stops.

Through 72 hours after the last watering, all plants retain their turgor, and after 96 hours, the beginning of the wilting process is observed. Therefore, it is impossible to conclude which growth regulators are able to minimize water stress.

Only through 120 hours, after soaking, RWC dropped sharply and amounted to 4 - 21 %, that is, the plants were on the verge of drying out. After spraying, the RWC was 20 - 35 %. The highest value of 35.71 % (T_6) was during spraying and this value is almost 2 times higher than T_6 (17.79 %) after soaking. In addition, plants (T_6) after spraying had a lower degree of wilting compared to the first group T_0 (20.96 % after spraying, 16.8 % after soaking) and the second control group T_9 (30.25 % after spraying and 21.74 % after soaking).

CONCLUSION

A study of the growth of wheat seeds (*Triticum aestivum* L) treated with compounds belonging to the class of carbamates **I** and oxamates **II** in a wide concentration range of 10^{-2} M - 10^{-6} M was carried out. As a result, it was found that bifunctional compounds **I**, **II** have a pronounced growth-regulatory activity for wheat seeds at a concentration of 10^{-5} M, at which the germination potential Gp and the germination rate Gr of seeds, as well as resistance to water stress, determined by RWC values reach their maximum values. It was found that the technology of spraying wheat seeds is more preferable than soaking them.

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Declarations of interest

None.

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