Bulgarian Journal of Agricultural Science, 26 (No 4) 2020, 772–776

Biological activity of the novel plant growth regulators: N-alkoxycarbonylaminoethyl-N'-arylureas

Antonida V. Kalistratova[1], Leonid V. Kovalenko[1], Maxim S. Oshchepkov[1,2*], Inna N. Solovieva[1], Anna G. Polivanova[1], Natalia A. Bystrova[3] and Konstantin A. Kochetkov[3]

[1]*D. Mendeleev University of Chemical Technology of Russia, 125047, Moscow, Russian Federation*
[2]*JSC "Fine Chemicals R&D Centre", 107258, Moscow, Russian Federation*
[3]*A. N. Nesmeyanov Institute of Organoelement Compounds, RAS, 119991, Moscow, Russian Federation*
[*]*Corresponding author:* maxim.os@mail.ru

Abstract

Kalistratova, A. V., Kovalenko, L. V., Oshchepkov, M. S., Solovieva, I. N., Polivanova, A. G., Bystrova, N. A. & Kochetkov, K. A. (2020). Biological activity of the novel plant growth regulators: N-alkoxycarbonylaminoethyl-N'-arylureas. *Bulg. J. Agric. Sci., 26 (4)*, 772–776

The synthetic cytokinins are the matter of the utmost importance and the subject of highly intensive studies due to the high activity in prevention of leaves fading, mobilization of nutrients, stem growth, etc. In a present study seven novel N-alkoxycarbonylaminoethyl-N'-arylureas are synthesized and tested for biological activity along with kartolin-2 and chlorocholine chloride taken as references. The results of the preliminary tests on tobacco cell culture (growth tests under controlled conditions) showed that tested compounds have an evident, although multidirectional effect on the metabolic processes.

Results of the wheat drought resistance tests demonstrated the superior positive effect of 1–2, 5 and 7 in water retardation as refereed to control compounds, kartolin-2 and chlorocholine chloride.

The results of frost resistance of winter wheat tested in the presence of 5 and 6 revealed their activity substantially exceeding the references. Thus the novel regulators seem to be very promising candidates as plant growth regulators and stress protectants.

Keywords: Anti-stress properties; N-alkoxycarbonylaminoethyl-N'-arylureas; Plant growth; Regulation activity; Synthetic cytokinins

Introduction

Plant growth regulators and stress protectants are the matter of increasing interest and intensive studies worldwide (Jiang &Asami 2018; Kawagishi, 2018; Rademacher, 2015; Wang et al., 2015; Ludwig-Mueller Luethen, 2015; Hambardzumyan et al., 2016; Zhang et al., 2015;Varejão et al., 2013; Mazid et al., 2011; Ricci & Bertolett, 2009). Although these chemicals are successively used in the agriculture, the number of publications in this field is constantly increasing. According to Chemical Abstracts (SciFinder) the number of annual publications for the key word combination "Plant growth regulators" has grown from 413 in 2000 to 660 in 2005, to 1851 in 2010 and to 2198 in 2017. Among these chemicals the natural cytokinins are known to be very effective ones (Mazid et al., 2011; Ricci & Bertolett, 2009; Zahajska et al., 2017; Savelieva et al., 2018; Oshchepkov et al., 2020), Figure 1.

Indeed, cytokinins reveal high activity in the regulation of numerous physiological processes of plants, such as prevention of leaves fading, stem growth, formation and activity of the apical shoot meristem, nutrient mobilization, etc.

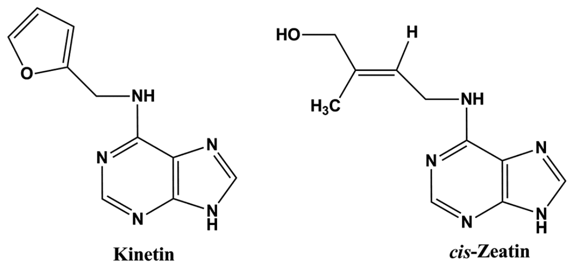


Fig. 1. The structures of natural cytokinins

(Mok&Mok, 2001; Rolli et al., 2012; Kumari et al., 2014; Ooiet et al., 2017). At the same time arylureas seem to become the most promising scaffold for elaboration of new synthetic analogues of cytokinins (Figure 2). The structure of the synthetic cytokinin analogues consists of two bioactive groups separated by an alkyl chain. Plant growth regulators include a number of arilcarbamates, such as 4PU-30, thidiazuron, EDU and kartolin-2, possessing cytokinin activity, which are effective in extremely small dosages (Brunonia et al., 2014; Stoilkova et al., 2014;Yonova, 2010). Comparing the structures of kartolin-2 and EDU, we proposed that compounds including two different groups − carbamate and urea, separated by the ethylene bridge, may also be active in regulating plant growth.

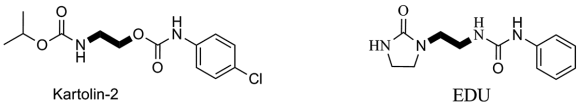


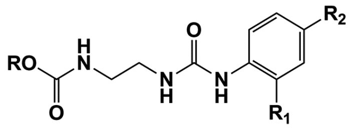
Fig. 2.The structures of synthetic analogues of cytokinins

Therefore, the target of the present study was to synthesize a series of bifunctional compounds having carbamate and urea bound by the ethylenediamine bridge, and to evaluate the phytoactivity of these compounds. It is expected that such synthetic analogues of cytokinins can become low-cost high-quality chemicals, which would be significantly meaningful for the reduction of the volume of the pesticide use and thereby can help to provide the environment preservation. Besides, the growth regulation activity of these synthetic analogues is supposed to be stably high in unfavorable environmental conditions such as drought and frost.

Materials and Methods

Experimental Details and Treatments

Reagents: Kartolin-2 was synthesized according to known procedures (Baskakov et al., 1991) (Figure 3). Compounds **1-7** were synthesized according to known procedures (Kovalenko et al., 2018). Chlorocholine chloride (BioReagent) was purchased from Sigma – Aldrich.



1: R= *2*-C_4H_9, R_1= H, R_2= CH_3
2: R= *2*-C_4H_9, R_1= CH_3, R_2= H
3: R= *i*-C_3H_7, R_1= CH_3, R_2= CH_3
4: R= *n*-C_4H_9, R_1= CH_3, R_2= H
5: R= *i*-C_3H_7, R_1= H, R_2= CH_3
6: R= *i*-C_3H_7, R_1= H, R_2= $COOC_2H_5$
7: R= *2*-C_4H_9, R_1= CH_3, R_2= CH_3

Fig. 3. Structures of N-alkoxycarbonylaminoethyl-N'-arylureas

Biological activity tests on tobacco cells culture

Plant growth regulation activity tests were carried out on a tobacco cell culture grown under strictly controlled conditions (modified Schenk and Hildebrandt environment, 26°C, growth regulator concentration $5 \cdot 10^{-6}$ mol·dm^{-3}, incubation time 120 hours, 10 replicates).

Wheat drought resistance test

The spring cultured wheat *Triticum aestivum L.,* varieties Moskovskaya 35 (Russian State Register of Protected Breeding Achievements, State catalog, № 7200935) was grown in water for 4 days and afterwards in solutions of the tested compound for 3 days. Wheat plants without roots and grains were slowly dried in thermostat at 24°C for 24 hours, then stored in a moist chamber on a wet filter paper and the number of survived plants was determined in 7 days. As a reference for comparing activity in various experiments chlorocholine chloride was used, as an agent that increases plant drought resistance. (Table 2).

Wheat frost resistance test

Winter cultured wheat *Triticum aestivum* L., varieties Konkurent (Russian State Register of Protected Breeding Achievements, State catalog, № 9908369) was germinated in a thermostat for 3 days and then grown in a solution of the test compound for 7 days. 10-Day-old wheat plants without roots, but with grains, were kept to stop growth at 2 °C for a week, and then they were frozen at −5 °C for 24 hours, and finally thawed at room temperature. The plants were placed in distilled water for 2 hours and the release of electrolytes from dead tissues was measured.

Statistical analysis

Statistical processing of the results was performed using Microsoft Excel software. To assess the statistical significance of various data sets, an acceptable value of significance was $p = 0.05$. The 95% confidence interval of true averages is shown in the tables 1, 2 and 3.

Results

Results of the biological activity tests on tobacco cells culture. The activity was measured by an inhibition of the gaseous CO_2 mass release due to the sells respiration (Kovalenko et al., 2017; Vorobev et al., 2017). The corresponding values are given in % of these indicators in control samples (free from the growth regulator). Kartolin-2 ($5 \cdot 10^{-6}$ mol·dm^{-3}) was taken as a reference compound шт this study (Table 1).

Table 1. Results of biological activity tests on tobacco cell culture

Compound	Specific respiration intensity (CO_2/d), % of control
1	109 ± 10
2	136 ± 10
3	30 ± 3
4	129 ± 15
5	128± 15
6	250 ± 20
7	55 ± 8
Control	100 ± 10
Kartolin-2	63 ± 5

Table 2. Results of the wheat drought resistance tests in comparison with chlorocholine chloride and Kartolin-2

Compound	C, mg/L	Water loss, %*	Number of survived plants upon dehydration, % to control*
Experiment 1			
1	10.0	59.0	106
2	10.0	61.0	122
7	10.0	59.0	89
Control	0	69.5	100
Chlorocholine chloride	10.0	65.8	85
Experiment 2			
3	10.0	70.0	155
Control	0	71.0	100
Chlorocholine chloride	10.0	74.0	82
Experiment 3			
5	10.0	61.5	146
Control	0	65.0	100
Chlorocholine chloride	10.0	68.0	84
Kartolin-2	10.0	68.0	84
The least significant difference ($HCP_{0.95}$): 2.3 % for water loss and 5% for the number of survived plants			

Wheat drought resistance tests results. In drought tolerance tests on spring wheat, the following parameters were evaluated: (i) ability to retain moisture in sprout tissues, which was defined as the loss of water during drying; and (ii) the number of surviving plants during dehydration (the same degree in all variants). The results of the biological activity study for the tested compounds compared to chlorocholine chloride are shown in Table 2.

The test results of wheat frost resistance test. The frost resistance was tested via the measurement of cell membranes damage degree by electroconductivity. Under the low temperatures the cell membranes get damaged and a leak of electrolytes from the plant tissues increases. The tests were run for compounds 5 and 6. The chlorocholine chloride and Kartolin-2 were used as reference compounds. The ratio of electrolyte yield in frost-treated tissues to electrolyte yield in dead tissues is a measure of the resistance of plant tissues to negative temperatures. Therefore, the lowest ratio corresponds to the highest resistance.

Discussion

The results of the preliminary tests on tobacco cell culture (growth tests under controlled conditions) showed that tested compounds have an evident (Table 1), although multidirectional effect on metabolic processes in plant cells. The compound 6 reveals significant cell growth stimulation activity, while compounds 3 and 6 demonstrate a remarkable inhibition of metabolic processes related to cell respiration. Moreover, the inhibition effect of the compound 3 is twice as high relative to that one for Kartolin-2. At the same time the compound 1 indicates no any noticeable effect relative to control within the experimental error.

As can be seen from Table 3 the tested compounds 1-3, 5 and 7 demonstrate the superior positive effect in water retardation as refereed to control, Kartolin-2 and chlorocholine chloride. The number of survived plants upon dehydration is also higher for the compounds 1 and 2, but not for the compound 6. The samples of 1 and 2 exhibit better results relative to control and chlorocholine chloride. The highest efficacy is observed for the compounds 3 and 5. The latter demonstrates much better results relative to both chlorocholine chloride and Kartolin-2. Thus the novel regulators look very promising candidates for the wheat drought resistance increasing agents.

The results of frost resistance testes performed for compounds 5 and 6 in comparison with chlorocholine chloride are given in Table 3. Both compounds 5 and 6 exhibit quite high activity relative to the blank (control) experiment. Moreover,

Table 3. Results of the wheat frost resistance tests in comparison with chlorocholine chloride and Kartolin-2

Compound	C, mg/L	Electrolyte leak, % of control
5	10.0	67
6	10.0	49
Control	0	100
Chlorocholine chloride	10.0	106
Kartolin-2	10.0	102
The least significant difference ($HCP_{0.95}$) 5%		

the frost resistance tests carried out for winter wheat revealed activity substantially exceeding that one registered for kartolin-2 and chlorocholine chloride. Particularly the sample 8 performs 39 and 35% effectively than chlorocholine chloride and Kartolin-2 respectively, while substance 9 demonstrated even better results: 57 and 51% higher activity.

Conclusions

The synthesized compounds exhibit quite high plant growth regulation activity, exceeding that one recorded for known structurally related analogues, such as Kartolin-2. According to the performed anti-stress tests, the highly efficient plant growth regulation activity for the obtained compounds was proved to be maintained at the condition of drought and frost.

Acknowledgements

This research was supported by the Russian Foundation for Basic Research (RFBR), Grants 19-03-00492 A (biological activity tests) and 18-33-01128 mol_a (synthesis of the novel N-alkoxycarbonylaminoethyl-N'-arylureas). The manuscript has no any potential competing interests or conflict of interest, including employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. For our data publication no Permission Note is required.

References

Baskakov, Yu. A., Shevelukha, V. S. & Simonov, V. D. (1991). Preparation of N-(hydroxyalkyl)- and N-[(aminocarbonyloxy)-alkyl]-carbamates as stress-reducing plant growth regulators PCT Int. Appl.1991,WO 9107381 A1 19910530.

Brunoni, F., Rolli, E., Dramis, L., Incerti, M., Abarca, D., Pizarro, A., Diaz-Sala, C. & Ricci, A. (2014). Adventitious rooting adjuvant activity of 1,3-di(benzo[d]oxazol-5-yl)urea and 1,3-di(benzo[d]oxazol-6-yl)urea: new insights and perspectives. *Plant Cell, Tissue and Organ Culture*, *18,* 111−124.

Hambardzumyan, E. N., Vorskanyan, A. S., Grigoryan, A. A. & Yengoyan, A. P. (2016). Synthesis and biological activity of novel 5-(alkylthio)-1,3,4-thiadiazol-2(3H)-thione derivatives. *American Chemical Science Journal,15,*1-9.

Jiang, K.&Asami, T. (2018). Chemical regulators of plant hormones and their applications in basic research and agriculture. *Bioscience, Biotechnology, and Biochemistry,82,*1265-1300.

Kawagishi, H. (2018). Fairy chemicals - a candidate for a new family of plant hormones and possibility of practical use in agriculture.*Bioscience, Biotechnology, and Biochemistry*, *82,*752-758.

Kovalenko, L. V., Kalistratova, A. V., Oshchepkov, M. S., Glukhoedova, E. A., Solovieva, I. N., Vorobev, M. M., Kochetkov, K. A. & Karakotov, S. D. (2017). Synthesis of new N-alkoxycarbonylaminoethyl-N'-arylureas showing plant growth regulation activity. *Butlerov Communications*, *50,*45−51.

Kovalenko, L. V., Kalistratova, A. V., Oshchepkov, M. S., Glukhoedova, E. A., Solovieva, I. N., Vorobev, M. M. & Kochetkov, K. A. (2018). N-alkoxycarbonylaminoethyl-N'-arylureas exhibiting antistress and plant growth regulation properties under unfavorable environmental conditions. *INEOS OPEN,2,* 98–102.

Ludwig-Mueller, J.&Luethen, H. (2015). From facts and false routes: How plant hormone research developed. *Journal of Plant Growth Regulation*, *34,* 697-701.

Mazid, M., Khan, T. A. & Mohammad, F. (2011). Role of secondary metabolites in defense mechanisms of plants. *Biology and Medicine*, *3(2),* 232−249.

Mok, D. W. & Mok, M. C. (2001). Cytokinin metabolism and action. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,52,* 89-118.

Naoki, K., Hiroshi, H. & Yutaka, N. (1993). Thermoplastic compositions containing polymers bearing carbamate groups and their manufacture. Patent Japan JP 05295017.

Oshchepkov, M. S., Kalistratova, A. V., Savelieva, E. M., Romanov, G. A., Bystrova, N. A., Kochetkov K. A., (2020). Natural and synthetic cytokinins and their applications in biotechnology, agrochemistry and medicine**.** *Russian Chemical Review*, *89 (8)* 787-810.

Ooi, S. E., Novák, O., Doležal, K., Ishak, Z. & Ong-Abdullah, M.(2013). Cytokinin differences *in vitro* cultures and inflorescences from normal and mantled oil palm (*Elaeisguineensis* Jacq.). *J. Plant Growth Regul.,32,* 865–874.

Pawar, N. S. & Patil, J. U. (2017). Studies as plant growth regulators of carvacryl ethers.*Int. J. Innovative Res. in Science, Engineering and Technology*, *6,* 18129–18133.

Rademacher, W. (2015). Plant growth regulators: Backgrounds and uses in plant production. *Journal Natural Product Research, 29,*149-157.

Ricci, A. & Bertoletti, C. (2009). Urea derivatives on the move: cytokinin-like activity and adventitious rooting enhancement depend on chemical structure. *Plant Biology*, *11,* 262–272.

Rolli, E., Incerti, M., Brunoni, F., Vicini, P. & Ricci, A. (2012). Structure–activity relationships of N-phenyl-N'-benzothiazol-6-ylurea synthetic derivatives: Cytokinin-like activity and adventitious rooting enhancement. *Phytochemistry*, *74,* 159–165.

Savelieva, E. M., Oslovsky, V. E., Karlov, D. S., Kurochkin, N.

N., Getman, I. A., Lomin, S. N., Sidorov, G. V., Mikhailov, S. N., Osolodkin, D. I. & Romanov, G. A. (2018). Cytokinin activity of N6-benzyladenine derivatives assayed by interaction with the receptors in planta, *in vitro*, and in silicon. *Phytochemistry (Elsevier)*, *149,* 161-177.

Stoilkova, G. M., Yonova, P. &Ananieva, K. (2014). Biological evaluation of newly synthesized aryl(thio)carbamoyl derivatives of 1- and 1-(2-aminoethyl)piperazines. *Plant Growth Regulation,72,* 303−312.

Sushma, K., Anil, S., Rohilla, A. K., Poonia, A. K. & Rana, J. S. (2014).Effect of some cyclic urea compounds on plant regeneration of *BacopaMonnieri* (L) Pennell. *International Journal of Pharma and Bio Sciences*, *5 (3),* 612−621.

Varejão, E. V., Demuner, A. J., Barbosa, L. C. &Barreto, R. W. (2013). The search for new natural herbicides. Strategic approaches for discovering fungal phytotoxins. *Crop Protection,48,* 41−50.

Vorobev, M. M., Kovalenko, L. V., Kalistratova, A. V., Oshchepkov, M. S., Filippova, V. S., Khodak, A. A. & Kochetkov, K. A. (2017). **β**-Oxalylamino-substituted *O*-ethyl *N*-arylcarbamates and *N*-ethyl-*N'*-arylureas. encapsulated into micelles of vinylimidazole–vinylcaprolactam copolymer. *Doklady Chemistry*, *473,* 84–87.

Yonova, P. (2010). Design, synthesis and properties of synthetic cytokinins.recent advances on their application.*Gen. Appl. Plant Physiol*., *36,* 124–147.

Zahajská, L., Nisler, J., Voller, J., Gucký, T., Pospíšil, T., Spíchal, L. &Strnad, M. (2017).Preparation, characterization and biologicalactivity of C8-substituted cytokinins.*Phytochemistry (Elsevier), 135,* 115-127.

Zhang, Q., Zhao, B., Song, Y., Hua, C., Gou, X., Chen, B. & Zhao, J. (2015). Synthesis and biological activity of **N**-Aroyl(aryloxyacetyl)-N'-ferrocenyl thiourea derivatives. *Heteroatom Chemistry*, *26,* 348-354.

Received: August, 26, 2019; *Accepted:* June, 25, 2020; *Published:* August, 31, 2020