Fagopyrum 41 (1): 19 -25 (2024)

**Research Paper**

# Adaptability of Buckwheat *in vitro*

**Svetlana A. BOROVAYA, Alexey G. KLYKOV, and Natalia G. BOGINSKAYA**

*Federal Scientific Center of Agricultural Biotechnology of the Far East named after A.K. Chaiki, 30 Volozhenina st., Timiryazevsky stl., Ussuriysk, Russia 692539*

**E-mail addresses of authors:** borovayasveta@mail.ru; alex.klykov@mail.ru; boginskaia98@gmail.com

DOI https://doi.org/10.3986/fag0038

**Received:** January 12, 2024; accepted February 21, 2024.
**Key words:** common buckwheat; in vitro; heavy metals; selective media; survival

## ABSTRACT

The research goal was to study the morphogenesis of common buckwheat plantlets obtained using different stressors *in vitro*. The research object was common buckwheat variety Izumrud – single-node cuttings (1.0-1.5 cm in length; two-three lowest internodes) of the plantlets that were obtained by subculturing on a hormone-free MS medium and of the lines that showed tolerance to $CuSO_4 \times 5H_2O$ (161 and 184 mg/l) at the Laboratory of Agricultural Biotechnology. Selective media were prepared based on the Murashige and Skoog medium supplemented with a zinc salt ($ZnSO_4 \times 7H_2O$) at concentrations of 808, 909, 1010, 1111, 1212, and 1313 mg/l. To evaluate the morphological characteristics of the studied genotypes, their parameter values were calculated relative to the control and expressed as percentage. The genotypes were compared in the following groups depending on the exposure to a certain stressor: group 1 – the genotypes obtained without exposure to copper, group 2 – the genotypes obtained on the media containing the copper salt at a concentration of 161 mg/l, and group 3 – the genotypes obtained on the media containing the copper salt at a concentration of 184 mg/l. Plants from different groups demonstrated dissimilar growth rates under the toxic conditions caused by the studied concentrations of zinc. The morphological parameter values of the copper-tolerant plants (groups 2 and 3) were significantly higher than those of the plantlets obtained without exposure to copper. The plantlets tolerant to $CuSO_4 \times 5H_2O$ (161 and 184 mg/l) showed a heightened resistance to the selective factor (Zn) as evidenced by their high regeneration and survival rates (95.7-100%).

## INTRODUCTION

Adaptability is the most important characteristic that enables plants to adjust to heterogeneous environments. It is well known that a response to stress is an active process aiming to achieve homeostasis under the suboptimal conditions of a given environment (Koyro et al., 2018). The adaptation of plants to numerous stresses, which occur successively or simultaneously, might induce reprogramming at the molecular, biochemical, cellular, and physiological levels (Zhang et al., 2020). Research in this area has been generating considerable interest in breeders. Innovations in the biotechnologies and biological control aimed at improving plant resistance to heavy metals have been gaining in popularity as well. These advances might become an effective method for increasing the yield and quality of agricultural crops.

Common buckwheat (*Fagopyrum esculentum* Moench) is a diploid annual plant species characterized by a broad range of stress resistance and plasticity and widely spread in Russia and other countries in Asia, Europe, and the Americas (Kreft, 2007; Chrungoo et al., 2016; Germ et al., 2016). Common buckwheat is an important food crop in the mountainous regions of India and China due to its short growing period, ability to grow at high altitudes, and high-quality protein in grain.

There is a lack of sufficient research on the adaptive responses of common buckwheat plants to metal stress coordinating their growth and development *in vitro*. E.N. Barsukova et al. have conducted a large number of experiments to study how heavy metals influence the tissue culture of *F. esculentum* (Barsukova, 1997, 2003, 2008; Barsukova et al., 2019, 2020; Klykov et al., 2019). They have discovered that buckwheat is highly tolerant to copper and zinc salts *in vitro* (Barsukova, 2003). The researchers have noted the varietal specificity of the studied plants in the resistance of their seeds germinated in Petri dishes to lethal and sublethal doses of copper and zinc. It has been found out that zinc ions produce a more adverse effect and aftereffect on the growth and development of callus cells and microshoots compared to copper ions (Barsukova et al., 2011). Additionally, the tolerance of cellular structures to ion stress is determined by their genotypes. As a result of the conducted research, promising buckwheat variety Ussurochka was created using the methods of tissue culture and hybridization. This variety is characterized by increased yield and rutin content (Klykov et al., 2017). Thus, more information on the biological processes that allow plants to survive under stress conditions is required to create productive and stress resistant varieties with the highest quality of produce.

Our research goal was to study the *in vitro* morphogenesis of the common buckwheat microclones that were obtained using various stressors.

## MATERIALS AND METHODS

Common buckwheat variety Izumrud (created in FSBSI "FSC of Agricultural Biotechnology of the Far East named after A.K. Chaiki") was used as the research object, namely single-node cuttings (1.0-1.5 cm in length; two-three lowest internodes) of the plantlets that were obtained by subculturing on a hormone-free MS medium and of the lines that showed tolerance to $CuSO_4 \times 5H_2O$ (161 and 184 mg/l) at the Laboratory of Agricultural Biotechnology.

To create selective conditions, the MS medium was supplemented with a zinc salt ($ZnSO_4 \times 7H_2O$) at the following experimental concentrations (variants): 808, 909, 1010, 1111, 1212, and 1313 mg/l. The aseptic single-node cuttings were grown on the MS medium with the standard amount (8.6 mg/l) of zinc sulfate (the control) and the experimental selective media with zinc for thirty-three days. Twenty test tubes were used in each experimental variant with three repetitions. The survived genotypes were subcultured (two passages) on the nutrient MS media. The duration of each passage was thirty-three days.

To study the adaptability of the *F. esculentum* plants obtained using various stressors *in vitro*, we carried out the comparative evaluation of their responses to the stress caused by cultivation on the selective media with zinc (808-1313 mg/l of $ZnSO_4 \times 7H_2O$). The following parameters were evaluated: relative plant height of the studied specimens (calculated relative to the control (%)) and the presence of rhizogenesis (% in each variant). The survival rate of the microclones was calculated in each variant as the ratio of the survived specimens to the total number of the microclones cultured on the MS and expressed as percentage. The studied parameters were compared in the following groups of stressors: group 1 – the genotypes obtained without exposure to copper, group 2 – the genotypes obtained on the media containing 161 mg/l of $CuSO_4 \times 5H_2O$, and group 3 – the genotypes obtained on the media containing 184 mg/l of $CuSO_4 \times 5H_2O$.

Microsoft Excel 2010 and PAST4.03 were employed for the input, processing, and statistical analysis of the research data.

## RESULTS AND DISCUSSION

*F. esculentum* plants from different groups of stressors demonstrated dissimilar regeneration rates at the studied toxic levels of zinc (808-1313 mg/l $ZnSO_4 \times 7H_2O$). The relative parameter "plant height" in the first group, where the regenerants were not exposed to copper, varied within 2.7-14.4% among the experimental variants and was by 6.9-37.0 times lower than the control (Figure 1).

The specimens obtained on the selective media with copper showed more intense growth. The height of the plants from the second group (161 mg/l of the copper salt) was 6.1-28.6%, which was by 3.5-16.4 times lower than the control. The height of the plants from the third group (184 mg/l of the copper salt) was 5.7-17.8% (lower by 5.6-17.5 times). The lowest plant height in all the groups was observed on the selective media at zinc salt concentrations of 1111-1313 mg/l. It should be noted that the height of the plants tolerant to copper (the second and third group) exceeded the values of the specimens obtained without exposure to copper by 1.2-2.7 times. The most significant difference (by 2.4-2.7 times) was observed among the variants with copper salt concentrations of 1111-1212 mg/l.

During the first subculturing of the mircoplants under the non-selective conditions, the discovered tenden-

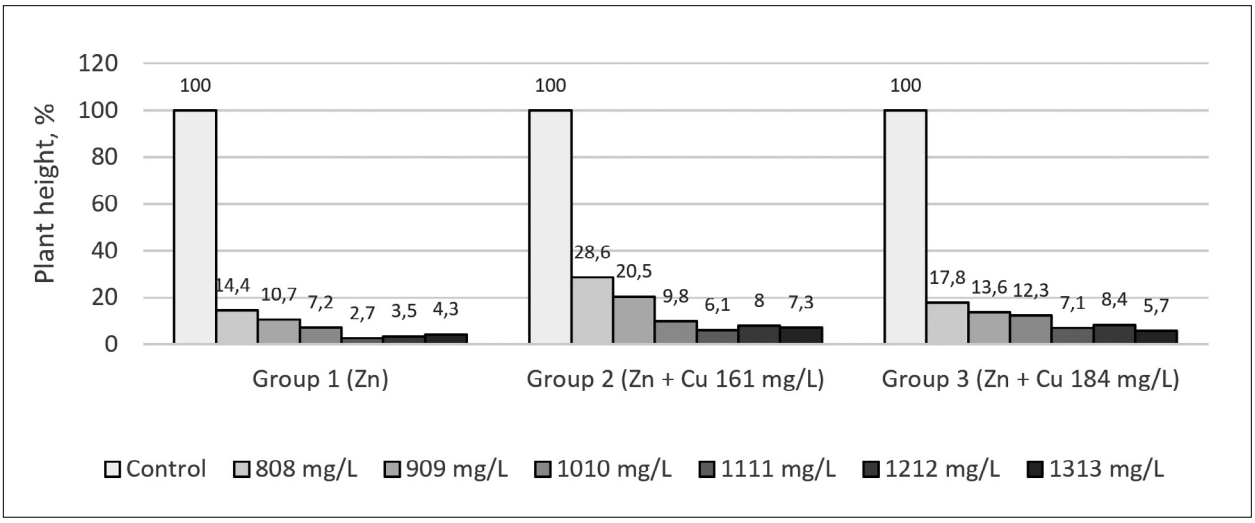


***Figure 1.*** *Height of plants from different groups of stressors on the 21st day of cultivation on the media with zinc (% relative to the control)*

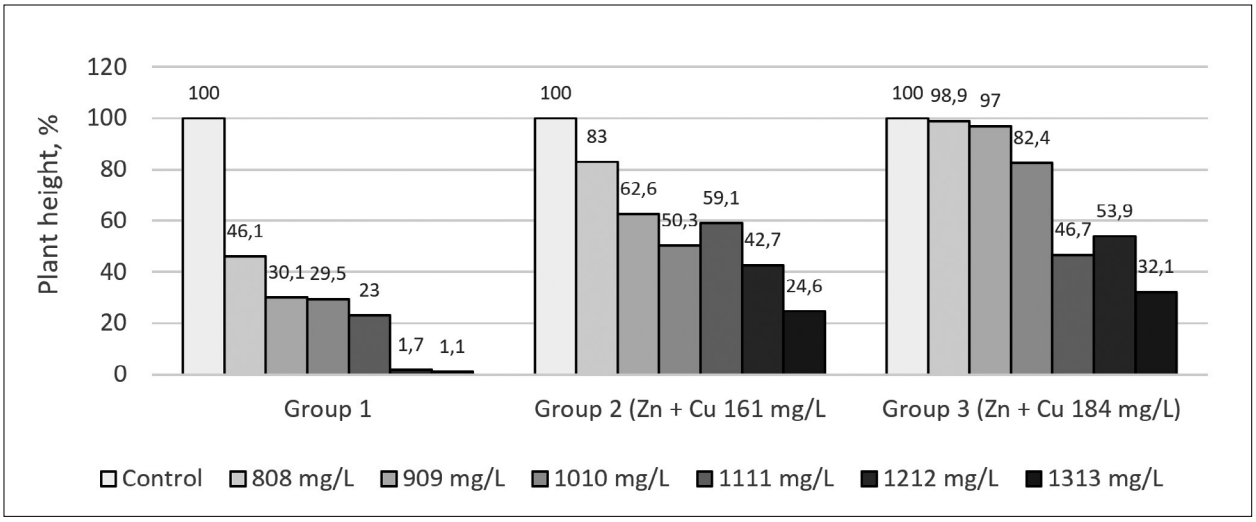


***Figure 2.*** *Height of plants from different groups of stressors, the first subculturing on the MS (% relative to the control)*

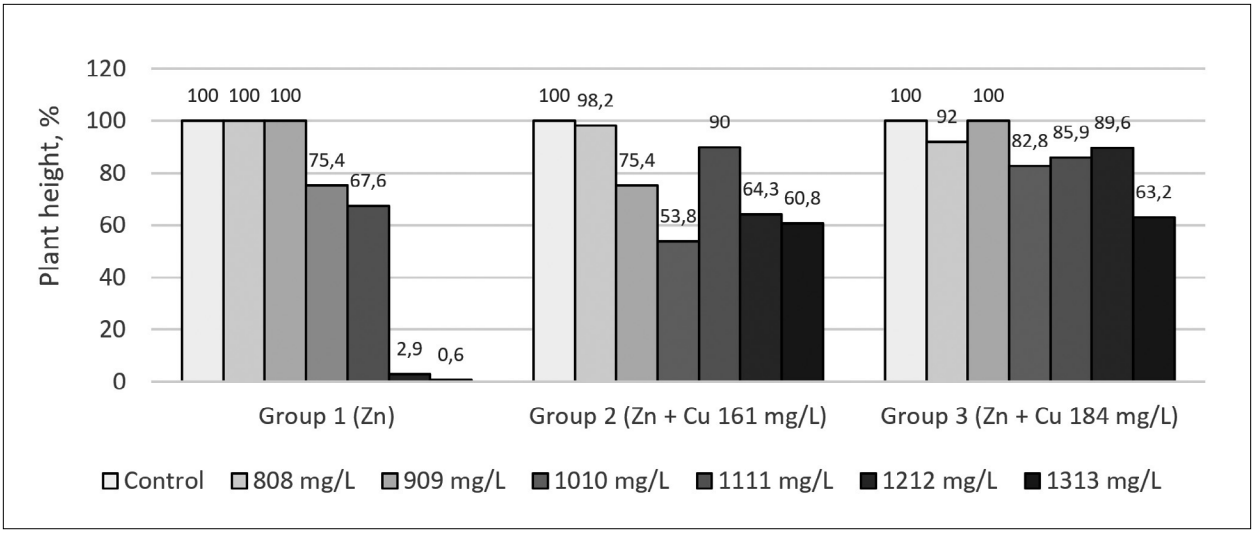


***Figure 3.*** *Height of plants from different groups of stressors, the second subculturing on the MS (% relative to the control)*

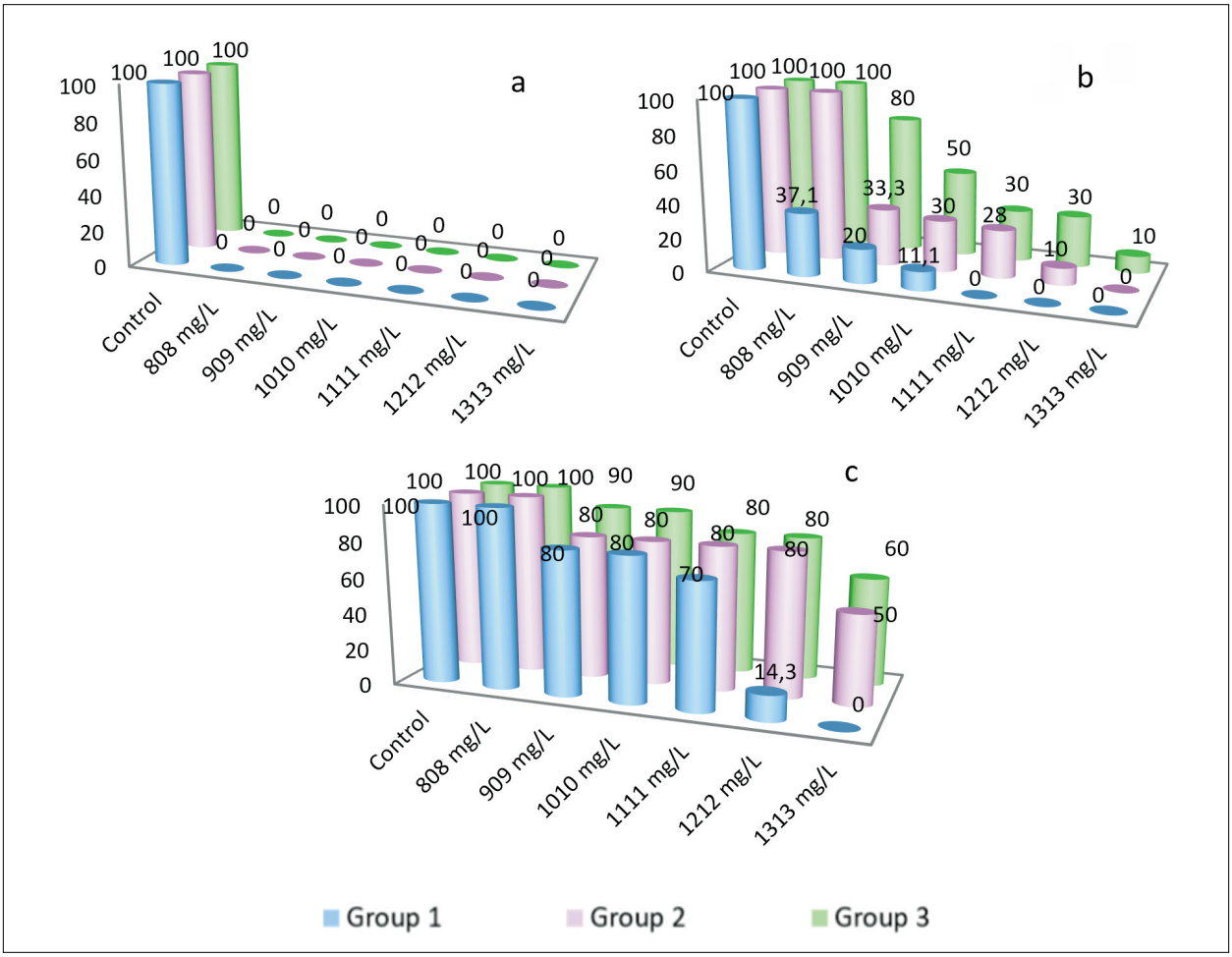


***Figure 4.*** *Characteristics of the rhizogenesis in plants from different groups of stressors (a – on the 21st day of cultivation on the medium with zinc, b – the first subculturing on the MS, c – the second subculturing on the MS)*

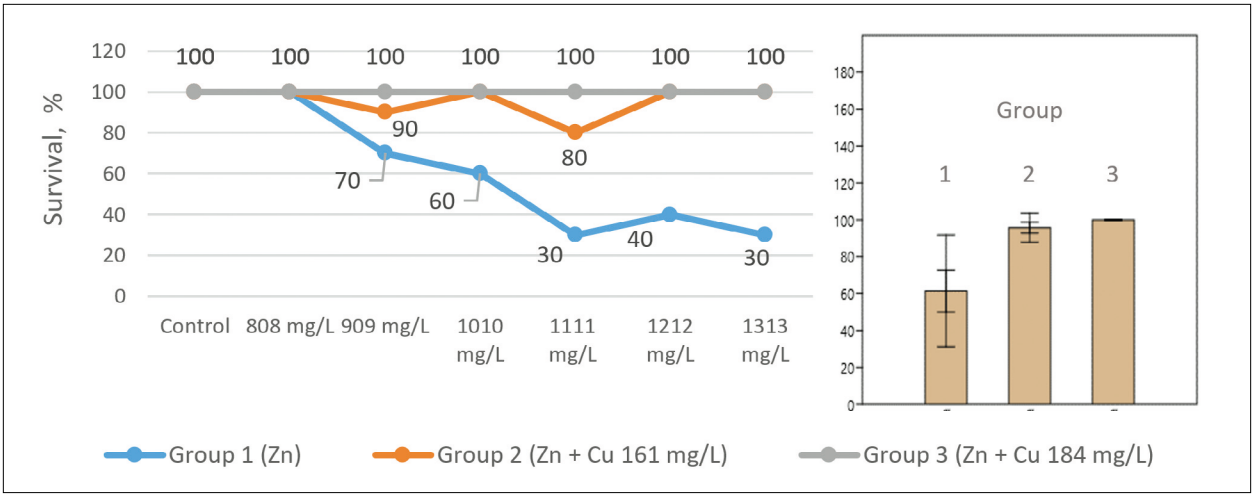


***Figure 5.*** *Survival rate of plants from different groups of stressors on the selective media with zinc*

cies remained. However, differences between the relative height of the plants from the first and second-third groups increased significantly, especially in the variants with 1212-1313 mg/l (by 22.3-31.7 times) (Figure 2).

During the second subculturing on the MS, the plants from the second and third group had an increased regeneration rate. Their height reached 53.8-100.0% of the control in all the experimental variants. The microclones from the first group had substantial height after 808-1111 mg/l of the zinc salt (67.6-100%) but suffered considerably after 1212-1313 mg/l (0.6-2.9%) (Figure 3).

The high toxicity effected root formation – rhizogenesis was absent in the three groups in all variants with zinc on the 21$^{st}$ day of cultivation (Figure 4a).

During the first subculturing on the MS without the toxicant (Figure 4b), roots developed in 11.1-37.1% of the plants from the first group in the variants with zinc salt concentrations of 808-1010 mg/l (depending on the dose of zinc), in 10-100% of the plants from the second group in the variants with zinc salt concentrations of 808-1212 mg/l, and in 10-100% of the plants from the third group in all the experimental variants. During the repeated subculturing, roots developed in the second and third group in all the studied variants. No roots formed in the first group in the variant with a zinc salt concentration of 1313 mg/l (Figure 4c).

The survival rate of the plants obtained as the result of the complex effect of copper and zinc (the second and third group) (80-100% in the variants) exceeded significantly the survival rate of the plants from the first group (30-100%), which was not exposed to the toxicity of copper ions (Figure 5). The regenerants obtained using the selective factors were characterized by high values of the morphological parameters and survival rate (95.7-100% in general). Manifestations of the toxic stress were considerably lower during the second subculturing.

It is well known that an increase in resistance to one stressor might lead to an increase in tolerance to another [Swaaiy et al., 1986; Zinchenko et al., 2013). This was confirmed by our experiment. The genotypes exposed to the severe ion stress (184 mg/l of copper) demonstrated the highest survivability under the toxic conditions. The difference in the responses of the studied specimens to the selective factor (zinc) might be due to genomic mutations, which could occur under the influence of heavy metals in the selected groups of buckwheat because the level of stress resistance is a genetically controlled and heritable trait (Barsukova, 2017).

## CONCLUSION

Common buckwheat is highly adaptable to high doses of zinc (808-1313 mg/l of $ZnSO_4 \times 7H_2O$) in a nutrient medium *in vitro* (survival rate of 30-100%). The genotypes obtained by subsequently using the selective factors (copper and zinc) were characterized by high values of the studied morphological parameters and an increased survival rate. The highest rate of post-stress regeneration was noted in the buckwheat regenerants tolerant to the complex effect of the heavy metals. Manifestations of zinc toxicity considerably decreased during the second-third subculturing.

## REFERENCES

Barsukova E.N. Adaptive properties of selection samples of buckwheat tolerant to heavy metal ions // Agrarian Russia. 2017. No. 8. P. 12-15.

Barsukova E.N. Micropropagation of buckwheat regenerants in vitro. In: Collection of research papers "On the scientific support of agricultural production in Primorie region": Far Eastern Branch of the Russian Academy of Sciences. Khabarovsk, 1997. P. 27-31.

Barsukova E.N. Morphogenetic potential of common buckwheat callus under the influence of ion stress in vitro. In: Proceedings of the 3rd International symposium "Food biotechnology: problems and perspectives in the 21st century", October 8-10, 2008. Vladivostok: TGEHU. 2008. P. 105-107.

Barsukova E.N. Survivability of common buckwheat plants under the influence of copper and zinc ions] // Ways of improving the efficiency of scientific research in the Russian Far East: collection of scientific papers / Far Eastern Branch of the Russian Academy of Sciences. V. 1: Breeding and plant science. Novosibirsk, 2003. P. 129-136.

Barsukova E.N., Fisenko P.P., Efremova O.S., Romashova M.V., Khokhlova N.I. Implementing biotechnological methods in the Primorsky Scientific Research Institute of Agriculture // Innovations in the agrarian science of The Far Eastern region: Scientific papers of the Russian Academy of agricultural sciences. Vladivostok: Dal'nauka, 2011. P. 66-76.

Barsukova E.N., Klykov A.G., Chaikina E.L. Usage of the tissue culture method for the development of new forms of *Fagopyrum esculentum* Moench // Russian Agricultural Science. 2019. No. 5. P. 3-6. https:/doi.org/10.31857/S2500-2627201953-6.

Barsukova E.N., Klykov A.G., Fisenko P.V., Borovaya S.A., Chaikina E.L. Usage of the method of biotechnology in the selection of buckwheat plants in the Far East // Vestnik of Far Eastern Branch of Russian Academy of Sciences. 2020. No. 4. P. 58-66. https:/doi.org/10.37102/08697698.2020.212.4.010.

Chrungoo N.K., Dohtdong L., Chettry U. Phenotypic plasticity in buckwheat // Molecular breeding and nutritional aspects of buckwheat. London: Elsevier, Academic Press, 2016. P. 137-149. https:/doi.org/10.1016/B978-0-12-803692-1.00010-9.

Germ M., Gaberščik A. The effect of environmental factors on buckwheat // Molecular breeding and nutritional aspects of buckwheat. – London: Elsevier, Academic Press, 2016. P. 273-281. https:/doi.org/10.1016/B978-0-12-803692-1.00021-3.

Klykov A.G., Barsukova E.N., Chaikina E.L., Anisimov M.M. Prospects and results of selection of *Fagopyrum esculentum* Moench for increased flavonoid content // Vestnik of Far Eastern Branch of Russian Academy of Sciences. 2019. No. 3. P. 5-16. https:/doi.org/10.25808/08697698.2019.205.3.001.

Klykov A.G., Parskaya N.S., Barsukova E.N. Selection of buckwheat to increase routine content // Agrarian bulletin of Primorie region. 2017. V. 8, No. 4. P. 24-29.

Koyro H.-W., Huchzermeyer B. Coordinated Regulation of Photosynthesis in Plants Increases Yield and Resistance to Different Types of Environmental Stress // Plant Metabolites and Regulation Under Environmental Stress. Academic Press, 2018. P. 281-309. https:/doi.org/10.1016/B978-0-12-812689-9.00014-5.

Kreft I. Buchweizen in Slowenien // Das Buchweizen Buch: mit Rezepten aus aller Welt. 2. Aufl. Islek ohne Grenzen EWIV: Arzfeld, 2007. P. 71-79.

Swaaiy A., Jacobsen E., Keil I., Feenstra W. Selection, characterization and regeneration of hydroxyproline-resistant cell lines of *Solanum tuberosum*: tolerance to NaCl and freezing stress // Physiol. Plant. 1986. V. 68, No 3. P. 359-366.

Zhang X., Huang B. Drought priming-induced heat tolerance: Metabolic pathways and molecular mechanisms // Priming-Mediated Stress and Cross-Stress Tolerance in Crop Plants. Academic Press, 2020. P. 149-160. https:/doi.org/10.1016/B978-0-12-817892-8.00009-X.

Zinchenko M.A., Dubrovnaya O.V., Bavol A.V. In vitro selection of wheat for complex resistance and analysis of obtained form // Izvestia of Samara Scientific Center of the Russian Academy of Sciences. 2013. V. 15, No. 3. P. 1610-1614.

## IZVLEČEK

**Prilagodljivost rastlin ajde *in vitro***

Cilj raziskave je bil preučiti morfogenezo rastlin navadne ajde, pridobljenih *in vitro* z uporabo različnih stresnih dejavnikov. Raziskovali so rastline navadne ajde, sorta Izumrud – potaknjence z enim nodijem (od dveh ali treh najnižjih nodijev), dolžine 1,0-1,5 cm, pridobljenih s subkultiviranjem na mediju MS brez hormonov. Uporabljene so bile linije, ki so pokazale toleranco na $CuSO_4 \times 5H_2O$ (161 in 184 mg/l), v Laboratoriju za kmetijsko biotehnologijo.

Selektivno gojišče je bilo pripravljeno na osnovi gojišča Murashige in Skoog, dopolnjenega s cinkovo soljo ($ZnSO_4 \times 7H_2O$) v koncentracijah 808, 909, 1010, 1111, 1212 in 1313 mg/l. Za oceno morfoloških značilnosti preučevanih genotipov so bile njihove vrednosti parametrov izračunane v primerjavi s kontrolo in izražene v odstotkih. Genotipe so primerjali po skupinah, odvisno od izpostavljenosti določenemu stresorju: skupina 1 - genotipi, pridobljeni brez izpostavljenosti bakru, skupina 2 – genotipi na gojišču, ki je vsebovalo bakrovo sol v koncentraciji 161 mg/l, in skupina 3 – genotipi na gojišču, ki je vsebovalo bakrovo sol v koncentraciji 184 mg/l.

Rastline iz različnih skupin so pokazale različne stopnje rasti v toksičnih razmerah, ki jih povzročajo koncentracije cinka. Morfološke vrednosti parametrov rastlin, odpornih na baker (skupini 2 in 3), so bile bistveno višje od vrednosti pridobljenih brez izpostavljenosti bakru. Rastline, odporne na $CuSO_4 \times 5H_2O$ (161 in 184 mg/l), so pokazale povečano odpornost na selektivni faktor (Zn), kar dokazuje visoke stopnje regeneracije in preživetja (95,7-100%).