Agar System (Agaroponics) for Modeling Abiotic and Biotic Effects on a Plant Organism (Manuals)

T.H. Garagezov*, M.H. Mamedova, I.M. Huseynova

Institute of Molecular Biology & Biotechnologies, Azerbaijan National Academy of Sciences, 2A Matbuat Avenue, Baku AZ1073, Azerbaijan; E-mail: qaragezovt@mail.ru

A method of using agaroponics for studying the resistance and adaptability of wheat plants to stress effects is proposed. The possibility of modeling the increasing salt stress effect is shown.

***Keywords**: Agaroponica, stressor, salt stress gradient, modeling, wheat*

The modern physiology of plants is characterized by a system-structural approach, and its correctness largely determines the success of further manipulations with experimental material.

The use of model systems at the level of a native plant is required in physiological, biochemical, biophysical and molecular-genetic studies.

The basis and success of the experimental approach depend on the stages of obtaining and cultivation of the initial material.

In natural habitats or in modeling natural conditions, plants are exposed to numerous stresses, and the response to them as a whole may differ from responses to individual effects or the sum of individual stresses. In this case, one stress can simulate the action of another (Kawa et al., 2016). At present, approaches based on the plant cultivation in the presence of certain factors, using water and sand cultures have been used. However, water and sand cultures, in spite of their simplicity, have a number of shortcomings, which ultimately have a negative impact not only on the interpretation of the results, they do not completely clarify the potential of the genotype in tests-experiments either (Besslavskaya et al., 1973; Grozdinskiy et al., 1973; Zhurbinskiy, 1968; Hyuit, 1960; Ivanova, 2004). For example, when using an aquatic culture, germination and cultivation of non-aquatic plants under the conditions of a given stressor effect occur in the presence of additional water stress, which, in its turn is aggravated by other types of stress. Water culture has shortcomings due to changes in metabolic processes during root uptake, difficulties in maintaining a certain concentration and reaction of the nutrient solution associated with simultaneous and uninterrupted supply of the root system with a solution of mineral elements, an increase in the toxicity of the stressor in aqueous solutions, the necessity for aeration and the complexity of determining the norm of requirement in the air supply, because of the low solubility of oxygen in water. In fact, the obligatory replacement of nutrient solutions, which are necessary to maintain balance and equilibrium of nutrients, as an additional stage of manipulation, itself is a stress factor. This problem cannot be solved using the method of fluid solutions (Hyuit, 1960, Chernavina, 1978), since, ideally, additional equipment is required to monitor all parameters, and ultimately, all this together is a laborious process accompanied by an increasing stress.

One of the shortcomings of water cultures is a difficulty in providing sterility of nutrient solutions. This is important if the cultivated objects are needed for molecular genetic analysis. The use of various components of some antibiotics used for the inhibition of protein synthesis, which prevents bacterial contamination, can also significantly affect the results of the analysis.

Sand culture used in vegetation experiments is more preferable compared to aquatic culture, although it has the same drawbacks. The most important among them is the weak retention of nutrients on an inert substrate, their uneven distribution throughout the volume and, as a result, the weak buffer capacity of the sand mixture, the inability to observe and control the root system, the probability of the development of the bacterial factor (Zhurbitsky, 1968; Hyuit, 1960).

Existing methods of plant cultivation, such as hydroponics, agregatoponics, chemoponics, aerponics, are mainly intended for industrial cultivation of plants. For scientific research purposes, the most suitable method is the cultivation in agar medium. For the first time this method was developed to study the degree of adaptivity by modeling the increasing impact of salt stress. (Karagezov et al., 2012).

The presented research is devoted to the development of methodological approaches for determining the degree of resistance and adaptability of different varieties and forms of wheat to stressors with the aim of ranking them according to these characteristics. The plant cultivation method, proposed by us is based on the cultivation of plants in agar medium under sterile conditions (Fig. 1).

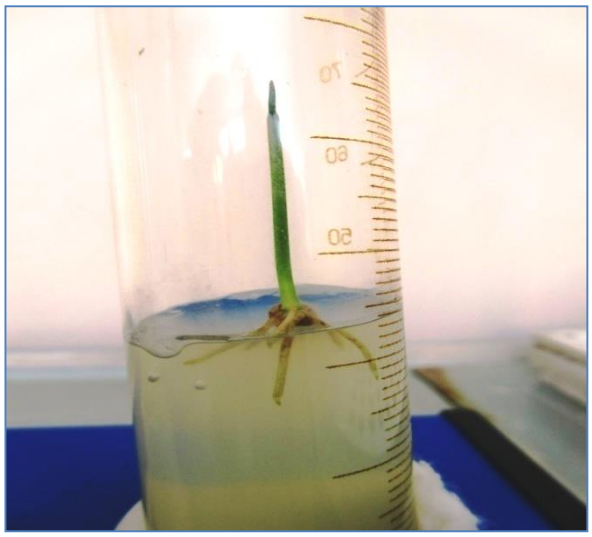


Figure 1. Cultivation of wheat plants in agar medium under salt stress in the initial period of development.

Agar medium contains the mineral nutrient medium Murasige and Skoog (M-S) (Murashige, 1962). As a basis for culture media, it is possible to use any nutrient media recommended for plant cultivation, based on the biological characteristics of the study object and the experimental tasks.

The choice of the MS medium is explained by the fact that of all media recommended for the cultivation of cereal plants under *in vitro* conditions, this medium has the most optimal and balanced composition of nutrients, and a sufficient buffer capacity throughout the entire cultivation period. The use of this medium allows also the coordination of results of *in vivo* experiments with studies of adaptive responses at the cellular level using *in vitro* model systems.

In addition, the presence of $CaCl_2$ in this medium makes possible modelling the effect of NaCl in studies on the resistance to NaCl, taking into account its toxicity. The concentration of $CaCl_2$ can vary depending on the content of the acting stressor, but the molar ratio of NaCl to CaCl2 of 5: 1 is a required condition (Mamedova et al., 2010).

Composition and preparation of the mineral part of the nutrient medium:

1. Microelements: NH_4NO_3 - 1650 mg / l; KNO3 - 1900 mg / l; $MgSO_4$ x $7H_2O$ -370 mg / l; KH_2PO_4 -170 mg / l. The macroelements are prepared in 10-fold concentration in 1L volume. 100 ml of stock solution per 1 liter of nutrient medium is used. It is possible to increase the multiplicity to 20, in this case 50 ml of the solution is used.

2. Microelements: H_3BO_3 -6.2 mg / l; $MnSO_4$x $4H_2O$-22.3 mg / l; $ZnSO_4$ x $4H_2O$-8.6 mg / l; KJ – 0.83mg / l; Na_2MoO_4 x $2H_2O$ – 0.25 mg / l; $CuSO_4$ x $5H_2O$-0.025 mg / l; $CoCl_2$ x $6H_2O$ - 0.025 mg / l.

3. $CaCl_2$ (440 mg / l) is added to the medium. It is convenient to prepare a concentrated solution (44g / 100ml H_2O) and to add 1ml of the solution to the medium.

4. Iron chelate: 557 mg of $FeSO_4$ x $7H_2O$ is dissolved in cold water, 775 mg / l of Na_2EDTA is added, volume is brought to 100 ml and heated in a boiling water bath for 10-15 minutes. In the nutrient solution, 5 ml chelate is added per 1 liter. For the cultivation of wheat plants, it is recommended to use both the described above medium M-S, and the ½ and ¼ parts of this medium (Murashiqe and Skoog, 1962).

5. After preparation of the liquid medium, its pH is adjusted to 5.6-5.8 with 0.1N NaOH or 0.1N HCl.

To prepare 0.6-0.8% solution half volume of molten agar was added into the half volume of nutrition medium. The medium is poured into culture vessels and autoclaved at 0.8 atm. for 30 minutes.

The volume of vessels used for growing plants is selected depending on the plant species and the duration of cultivation. We used graduated 100 ml cylinders, in which 50-60 ml medium was added, sufficient for the cultivation of wheat plants for 2 months. The proposed system acquires an exceptional advantage in studying the stressor effect with an increasing gradient of the acting factor, for example, secondary salinization.

Preparation of the agar medium with an increasing gradient of the active factor or with combined stressor effects

When the studies were performed under increasing stressor concentrations, a 2 or 3 component medium was also prepared as described above, but in this case, the medium containing the greatest stressor concentration was poured first into the culture vessel. After autoclaving and solidification of the medium, the remaining one-time constituents pre-autoclaved in separate volumes and cooled to about 40°C were added to the vessels with the medium under aseptic conditions. Fig. 2 shows the variants of the modeling scheme with the gradient of the increasing stress factor.

Taking into account the possibility of slight natural diffusion of NaCl, under an increasing gradient of the active factor, conditions are created for the smooth transition of growing roots from one concentration of NaCl in agar to another. This is a positive side of the proposed method, since it excludes a sharp change in the strength of the stress effect as an additional factor. Depending on the tasks of the experiment, if plants are not studied in the seed germination phase in the presence of the stressor, and for equalizing the conditions, it is pos-

sible to layer the agar medium without NaCl of various heights to the first phase of the salt medium.

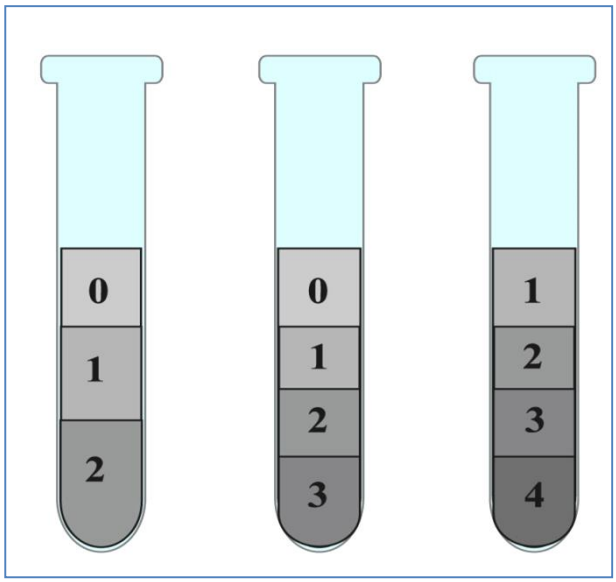


Figure 2. Scheme of the variants with the use of salt stress gradient. Agar medium without stressor. 1;2;3;4-agar media (blocks) with increasing concentration of the stressor factor.

Seed preparation: The process of plant seed preparation is their sterilization for the subsequent placement in the agar medium.

Seed sterilization: Plant seeds were thoroughly washed with running water with the addition of detergent, and kept in a weak solution of $KMnO_4$ for 10-15 minutes. All other manipulations related to the sterilization process were carried out under aseptic conditions. The first stage of sterilization involved incubating the seeds in a 10-12% solution of H_2O_2 for 10 minutes, washing with sterile water 2-3 volumes from the volume of the sterilizing solution. The second stage consisted of the sterilization with 70% ethyl alcohol for 5 minutes followed by washing with 3 volumes of sterile water. The third and final stage was sterilization with Na hypochlorite with the addition of Tween-20 detergent and careful washing with 5-6 volumes of sterile water. The period of incubation in sterile water between new portions should be at least 5 minutes. The duration of sterilization and concentration of the sterilizing agent must be selected experimentally to avoid both the excess effect of the sterilizer and its insufficient effect. The optimal concentration, when using Na hypochlorite with 5% active substance, the sterilization period was 15-20 minutes. The main condition was the stabilization of the temperature of the sterilizing solution (20-25°C).

Prepared in this way sterile seeds are planted in vessels with agar medium. When placing seeds on the surface of the agar medium, it is desirable to add ~ 1ml of sterile water in order to create the necessary humidity for rapid swelling and seed germination. After 2-3 days, the seeds germinated at a temperature of 23-25^{0}C, in the dark and after germination they were transferred to light with the necessary intensity of illumination, humidity and temperature.

In the initial period of plant growth and development, the regulation of moisture does not matter, since it remains in the vessels to a sufficient degree. When seedlings reached the level of a cotton plug, it was removed, and the plants in the vessels were grown till the agar dried. Usually this period (depending on the volume of the agar block) is 40 to 60 days.

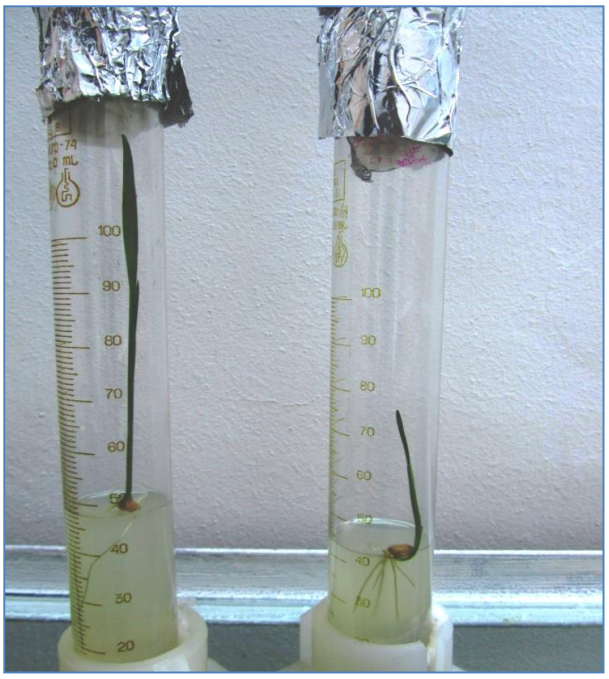
It is possible to maximally prolong the time of the experiment to study the influence of various factors at certain stages of ontogenesis. In this case, as the agar block dries, it is replaced by a neutral substrate. The most acceptable is the use of perlite. In this case, only the degree of wetting of perlite by the nutrient solution is monitored in the same proportions as those used in the preparation of the agar medium. In this case, the approach used is a successive change of agaroponics by agregatoponics.

The advantage of the methodical approach with the use of agar is that the growth processes of the vegetative part of plants and root system can be observed and controlled simultaneously (Fig. 3).

Growth and development of roots depend on the environmental conditions. Survival under heterogeneous soil conditions is determined by the plasticity of the roots to avoid unfavorable factors, such as salinity. Root resistance to salt and other stressors is a component of the overall resistance of the organism, and in some cases, depending on the type of stressor, the plant roots which are the structural and functional part of the body, are the first subjected to stress. The complexity of the root system responses to a stressor was confirmed by transcriptomics and proteomics (Koussevitzky et al, 2008; Rasmussen et al, 2013; Rivero et al, 2014; Sewelam et al, 2014).

The proposed culture system allows perfect controlling and modeling the relationship between the development of the plant vegetative part and the root system, in the presence of single, double and multiple stress, and studying the resistant variability to counteract stress combinations (Fig. 4).

Figure 3. Development of the root system of wheat plants using agaroponics.



1 **2**

Figure 4. Development of wheat plants in agar medium: 1 - without stress factor; 2- in the presence of 100 mM NaCl.

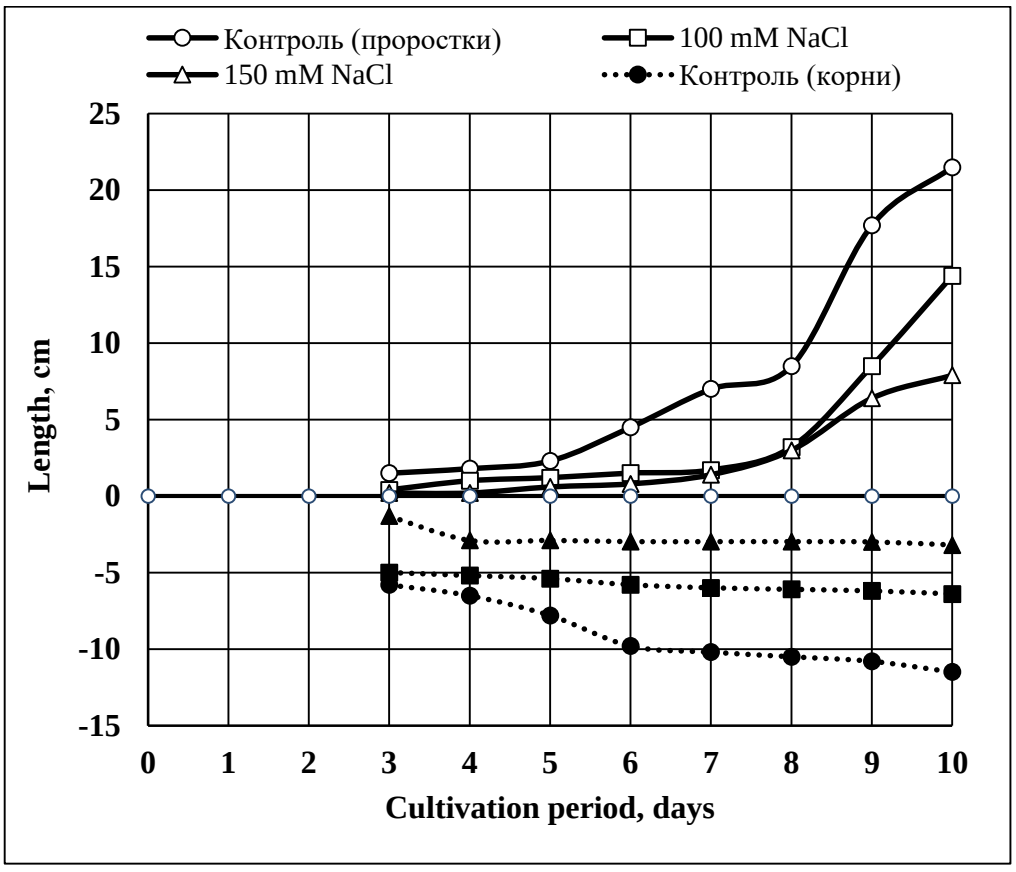


Figure 5. Response of durum wheat plants to salt stressor.

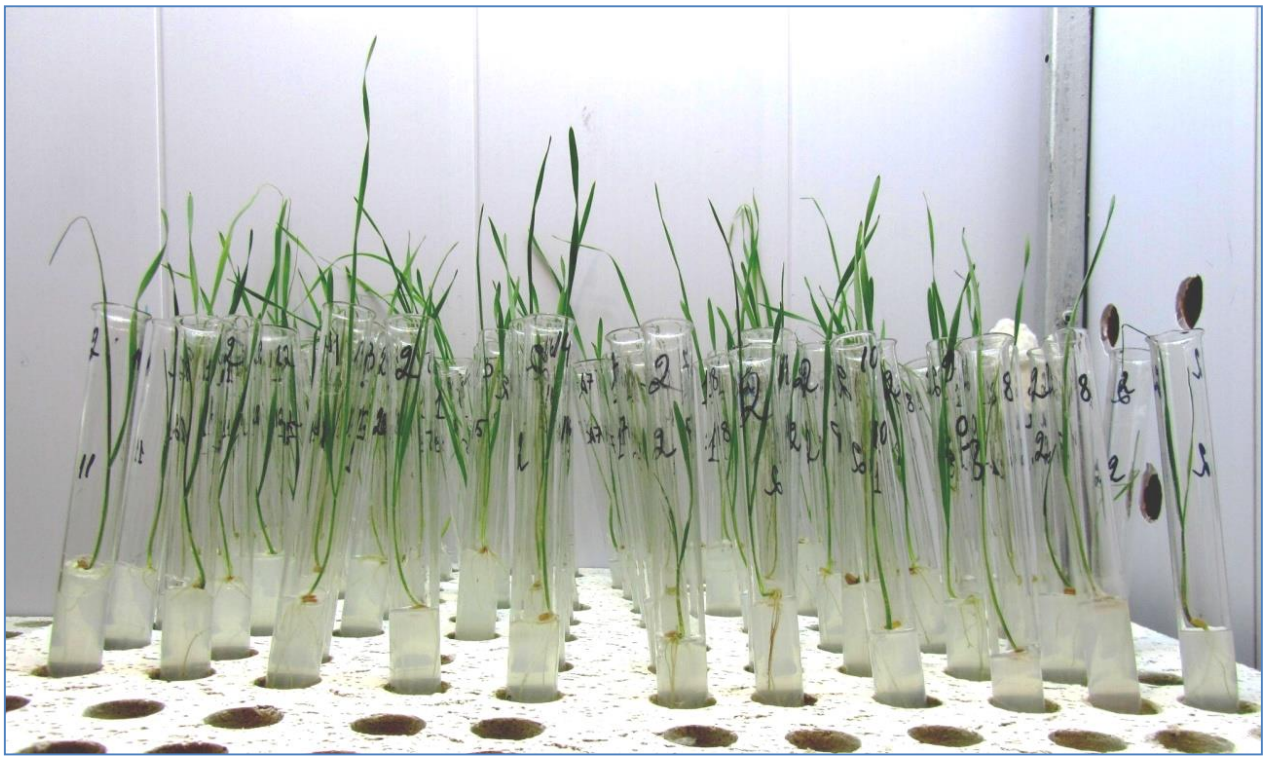


Figure 6. Cultivation of wheat genotypes under different levels of salt stress.

When adapting to constantly changing conditions, plants need to adapt their development program. These processes largely depend on changing conditions. The level of plasticity of the root system can facilitate the response to stress (Pierik et al., 2014). Recently, the studies of root adaptations have become increasingly important.

So, in particular, the root morphology and their functionality can be influenced by a number of factors - mineral supply, osmotic stress, light, salinity (Giehl et al., 2014, Malamy, 2005; Galvan-Ampudia et al., 2011; Kellermeier et al., 2014).

Figure 5 presents (as an example) the results of the study of the dynamics of durum wheat development under salt stress. The results of 10-day cultivation allow us to get an idea of the separate influence of the salt stressor on the growth indices of the vegetative and root system of plants, depending on the level of the stress factor.

This methodological approach makes it possible to establish the limits of resistance to the level of salt stressor, to establish the correlation of the responses of the vegetative and root parts of the plant organism and to determine the response, adaptability and resistance to salt stress.

The described method was used to study the reaction to salt stress of more than 18 genotypes of durum and bread wheat from the genebank of the Institute of Plant Resources of the National Academy of Sciences (Fig. 6).

Based on the obtained data, the genotypes are ranked according to the degree of resistance to salt stress. Thus, this method can be applied in a wide range of studies on the influence of various factors on the growth and adaptive processes of plants. It is particularly valuable in modeling the growing effects, including combined effects on the root system and allows performing researches and observations on the same object.

This experimental approach can also be used to study the pathogen-host interaction both simultaneously in the root and vegetative relationships, or separately, to study the genetic control of the virulence of populations of the causative agent of diseases, in the selection of varieties with horizontal resistance, for the selection of genotypes with host stability in order to increase its variability amplitude (Korobova et al., 2013; Tyryshkin et al, 2000).

Obviously, this method is applicable not only for working with cereals, but also for other plants when studying the action of various stressors. Primary stability diagnostics, combined with studies of adaptive capabilities, as a test is important and necessary for selection processes.

ACKNOWLEDGEMENT

This work was supported by the grant (EIF-KETPL-2-2015-1 (25) -56 / 35/3-M-10) of the Science Development Foundation under the President of the Republic of Azerbaijan

REFERENCES

Besslavskaya S.S., Borodulina F.Z., Gavrilenko V.F. et al. (1973) Small Practicum on Plant Physiology. M.: 84 p. (in Russian).

Galvan-Ampudia C.S., Testerink C. (2011) Salt stress signals shape the plant root. *Curr. Opin. Plant Biol.*, **14:** 296–302

Giehl R.F., Gruber B.D., von Wirén N. (2014) It's time to make changes: modulation of root system architecture by nutrient signals. *J. Exp. Bot.*, **65:** 769–778.

Grodzinskiy A.M., Grodzinsky D.M. (1973) Brief reference on plant physiology. Kiev: 593 p. (in Russian).

Hyuitt E. (1960) Sand and aquatic cultures in the study of plant nutrition. M.: 96 p. (in Russian).

Ivanova V.B. (2004) Practicum on Plant Physiology. M.: 144 p. (in Russian).

Karagezov T.G., Asadova S.Sh., Ibrahimova U.F., Mamedova M.G. (2012) Methodical approach for determining the degree of adaptability of wheat plants by modeling the increasing impact of salt stress. *Transactions of the Azerbaijan Research Institute of Crop Husbandry*, **33:** 101-106 (in Russian).

Kawa D., Magdalena M.J., Hector M.S., Anneliek ter H., Michel A.H., Christa T. (2016) Phosphate-dependent root system architecture responses to salt stress. *Plant Physiol*., **17:** 690 - 706.

Kellermeier F., Armengaud P., Seditas T.J., Danku J., Salt D.E., Amtmann A. (2014) Analysis of the root system architecture of Arabidopsis provides a quantitative readout of crosstalk between nutritional signals. *Plant Cell*, **26:** 1480–1496.

Korobova L.N., Gurova T.A., Lugovskaya O.S. (2013) Diagnostics of resistance of spring wheat and barley varieties to common root rot using conductometric method. *Agricultural Biology*, **No 5:** 100-105 (in Russian).

Koussevitzky S., Suzuki N., Huntington S., Armijo L., Sha W., Cortes D., Shulaev V., Mittler R. (2008) Ascorbate peroxidase 1 plays a key role in the response of *Arabidopsis thaliana* to stress combination. *J. Biol. Chem.*, **283:** 34197–34203.

Malamy J.E. (2005) Intrinsic and environmental response pathways that regulate root system architecture. *Plant Cell Environ*., **28:** 67–77.

Mamedova M.H., Asadova S.Sh., Qaragezov T.H. (2010) Nature of physiological reactions of various wheat genotypes under sudden and salinity condition. *Proceedinngs of ANAS (Biological Sciences)*, **65 (5-6):** 201-204.

Murashiqe T., Skoog F.A. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*., **15(13):** 473-497.

Potapov N.G., Kosulina L.G., Krendeleyeva T.E. (1978) A large practicum on plant physiology. M.: 408 p. (in Russian).

Pierik R., Testerink C. (2014) The art of being flexible: how to escape from shade, salt, and drought. *Plant Physiol.*, **166:** 5–22

Rasmussen S., Barah P., Suarez-Rodriguez M.C., Bressendorff S., Friis P., Costantino P., Bones A.M., Nielsen H.B., Mundy J. (2013) Transcriptome responses to combinations of stresses in Arabidopsis. *Plant Physiol*., **161:** 1783-1794.

Rivero R.M., Mestre T.C., Mittler R., Rubio F., Garcia-Sanchez F., Martinez V. (2014) The combined effect of salinity and heat reveals a specific physiological, biochemical and molecular response in tomato plants. *Plant Cell Environ*., **37:** 1059–1073.

Sewelam N., Oshima Y., Mitsuda N., Ohme-Takagi M. (2014) A step towards understanding plant responses to multiple environmental stresses: a genomewide study. *Plant Cell Environ*., **37:** 2024–2035.

Tyryshkin L.G., Loktionova O.E., Salimzhanova O.V. (2000) Somaclonal variability of wheat in resistance to harmful organisms. *Plant Protection News*. St. Petersburg-Pushkin, **2:** 16-19 (in Russian).

Zhurbitsky Z.I. (1968) Theory and Practice of the Vegetative Method. M.: 78 p. (in Russian).

Bitki Orqanizminə Biotik Və Abiotik Təsirin Modelləşdirilməsi Üçün Ağar Sistemi (Agaroponika) (Metodiki Tövsiyə)

T.H. Qaragözov, M.H. Məmmədova, İ.M. Hüseynova

AMEA Molekulyar Biologiya və Biotexnologiyalar İnstitutu

Aqaroponikadan istifadə etməklə duz stresinin modelləşdirmə metodu təsvir edilmişdir. Buğda bitkisinin rezistentlik və adaptivliyinin öyrənilməsində stressor təsirin artan qradient üzrə modelləşdirilməsinə yanaşmalar nəzərdən kecirilib

Açar sözlər: *Aqaroponika, stressor, duz stresinin qradienti, modelləşdirilmə, buğda*

Агаровая Система (Агаропоника) Для Моделирования Абиотических И Биотических Воздействий На Растительный Организм (Методическая рекомендация)

Т.Г. Гарагезов, М.Г. Мамедова, И.М. Гусейнова

Институт молекулярной биологии и биотехнологий НАН Азербайджана

Предложен метод использования агаропоники для изучения резистентности и адаптивности растений пшеницы к стрессорным воздействиям. Показана возможность моделирования нарастающего солевого стрессорного фактора.

Ключевые слова: *Агаропоника, стрессор, градиент солевого стресса, моделирование, пшеница*