

# Using the Possibilities of *in Vitro* Culture for the Preparation of Dietary Supplements from Alfalfa Tissues



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**Abstract:** *In vitro* methods have environmental advantages of preparing bio additives as harmful chemicals such as fertilizers, herbicides, pesticides are used. In addition, obtaining biomass does not depend on seasonality and a long growing season. In this series of experiments, an accelerated method for obtaining a sterile alfalfa culture was developed by adding 1% potassium humate to the explant culture. From 4 varieties of alfalfa, 3 cell lines were selected, characterized by heterogeneity of callus tissues. 1 - line morphogenic structures; 2 - line - without meristematic foci and brown and dark brown in color, which were not further differentiated; 3- line forming polymerogenic tissues. In addition, it was possible to induce denser tissues from loose callus by passaging on the Risting medium in the Ray khan variety. Structured tissues with meristematic foci induced on Risting medium were lyophilized and the dried biomass was prepared for further biochemical analyses. Thus, we have optimized the conditions for obtaining biomass from alfalfa culture and carried out the selection of cell lines, and we assume that callus lines, upon receipt of positive biochemical analyzes, can be used as feed additives.

**Keywords:** Alfalfa, Bio Additives, *In Vitro*, *Medicago Sativa L.*

## I. INTRODUCTION

Alfalfa is a leading forage crop and one of the best sources of protein that is widely cultivated worldwide. Alfalfa has been used in the pharmaceutical, cosmetic and food industries since ancient times, and it is known that alfalfa culture occupies a worthy place in the list of the Council of Europe as a source of natural food spices. Alfalfa is rich in easily digestible protein and minerals, as well as vitamins and dietary fiber.

In addition, the amino acid composition of alfalfa concentrate obtained after the production of the food additive

"Alfalfa Complex" is being studied, where the rich composition of the necessary complex of amino acids, minerals and proteins has been confirmed by classical and spectral methods [1].

The area of alfalfa cultivation is about 32 million hectares worldwide and is increasing with the development of the livestock market. [2].

Alfalfa has a high concentration of organic acids and prevents harmful effects used in diets due to the protein's ability to neutralize short-chain fatty acids, causing acid damage and ulcers. Alfalfa can be used to combat acidotic effects associated with high consumption, for example, in the environment of the rectum of horses [3].

Because of alfalfa protein being a source of "ideal" protein, the plant is successfully commercialized in many countries of the world [4].

Alfalfa leaf proteins are divided into two types: insoluble green fraction rich in lipids, chlorophyll and carotenoids, white fraction (soluble) contains 65% Ru Bis CO - ribulose-1,5-diphosphate carboxylase/oxygenase [1].

Undoubtedly, the classical methods of obtaining biologically active additives from alfalfa are still the leading technologies in many countries of the world.

Plants produce a large number of secondary metabolites and for their rapid production, cell and tissue culture can be effectively used, which can serve as a source of biologically active compounds [5].

It is also very important that alfalfa is a source of secondary metabolites, where saponins and flavonoids are of particular importance [6].

In one article it is difficult to describe and cover the data on the merits of alfalfa, which is rightly called the "queen" of cultivated plants.

However, the spread and widespread cultivation of alfalfa depends on climatic conditions [7].

Currently, modern breeding widely applies biotechnological approaches based on *in vitro* cultivation of plant cells.

The cellular biomass of plants can be fully used for food, whereas only parts of the plant are used when growing on a plantation. One of the advantages of using cell mass is the absence of pigments and lignified structures in the biomass. The advantage of cell culture is the exclusion of various kinds of ballast substances from the diet, as well as the exclusion of antibiotics, insecticides, herbicides and xenobiotics [8].

Also, the cell culture of *Medicago sativa L.* cells has functional features that determine different sensitivity to the action of abiotic stressors, which is expressed in different levels of cell viability:

Manuscript received on 03 October 2022 | Revised Manuscript received on 13 October 2022 | Manuscript Accepted on 15 November 2022 | Manuscript published on 30 November 2022.

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high (85%) during hypothermia and low (25%) during hypothermia. hyperosmotic action [9]. Genetic improvement of alfalfa by biotechnology methods was carried out in early works, where regeneration processes,

the specifics of the varietal response to cultivation conditions, optimization of cultivation modes in *in vitro* conditions, etc. were studied in detail.

Currently, the introduction of target genes, i.e., the transformation of alfalfa and obtaining valuable traits of a high-protein feed culture, are relevant. Researchers are interested in the fact that alfalfa protein is equated with the protein of mother's milk.

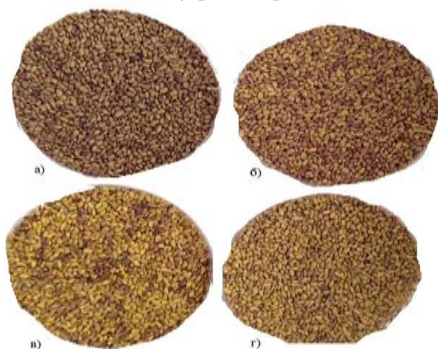
Thus, biotechnological methods make it possible to obtain environmentally friendly products for the use of alfalfa both as a food additive and as a feed additive.

The purpose of this series of experiments is to use the possibility of cell and tissue culture to create a dietary supplement from alfalfa biomass (*Medicago sativa* L.).

### II. MATERIALS AND METHODS

The objects of research are 3 varieties of alfalfa Ray khan, Lazur Naya, Shortandinskaya 2, which zoned in Northern Kazakhstan and 1 variety of alfalfa – Orai, which zoned in Southern Kazakhstan. The seeds of the varieties are shown in fig. 1.

Cultivation of plant cells in vitro was carried out according to the generally accepted method (R. G. Buteyko, 1999; E.A. Kalashnikova et al., 2006) [10, 11].



**Fig. 1. Seeds of Alfalfa Varieties used in Experiments: a) Raykhan; b) Orai; c) Lazurnaya; d) Shortandinskaya 2**

The seeds were sterilized in 4 stages: Stage 1) The seeds were stirred for 3-5 minutes in a soapy solution, then washed 3-5 times in distilled water; 2) Alfalfa seeds were kept in potassium permanganate for 5's by rinsing with sterile distilled water; 3) The material under study was soaked in 70% ethyl alcohol for 10' and washed 5 times with distilled water; 4) Sterilization of seeds in a solution of sodium hypochlorite.

Then the studied material was incubated in a camera for growing plants. For intensive plant growth, a 1% solution of potassium humate was added.

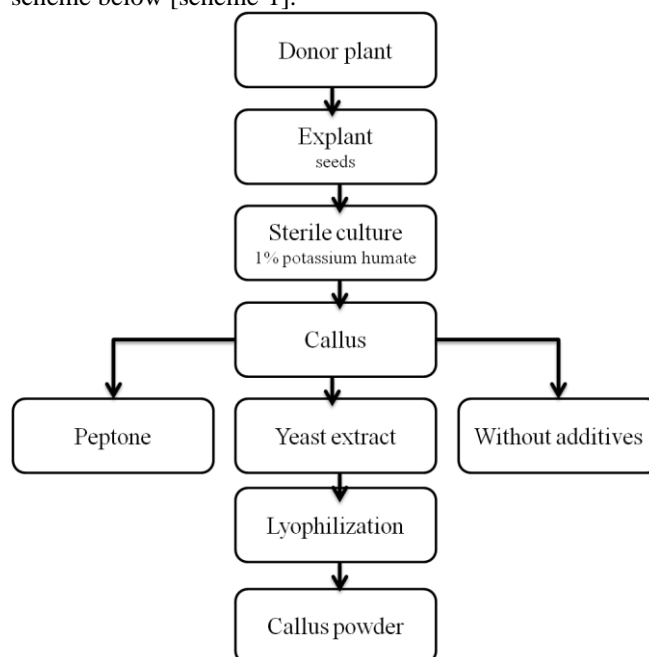
After the appearance of 2-3 green alfalfa leaves, explants were transplanted to MS medium with 2,4-D to form defibrinated cells, and MS medium with modifications – MSP + peptone, MS + yeast extract was also used. The callus was sub cultivated on the Risting medium.

### III. RESULT AND DISCUSSION

In our early research, the influence of various types of explants on the induction of callus tissues – the first leaf,

epicotyl, hypocotyl and the apex of the process, cotyledon node, root crown of alfalfa, etc. were studied. [12, 13].

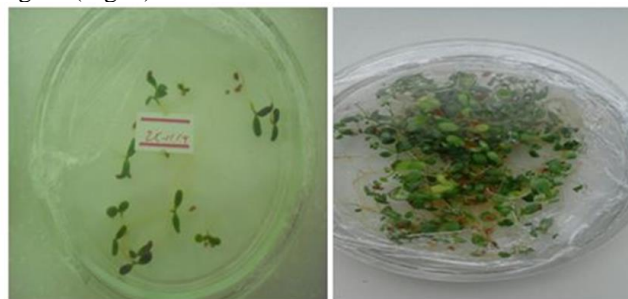
Experimental studies were carried out according to the scheme below [scheme-1].



**Scheme 1 - Scheme for obtaining callus powder from alfalfa in in vitro condition**

Detailed experiments were carried out to study the regeneration potential of alfalfa, for example, the highest average number of regenerated shoots per explant was 6.33 - 8.5 seedlings per explant after explants of cotyledon nodes were treated with BAP 0.40 mg/l, TDZ 0.55 mg/l [14, 15].

As a result of our research, we found that with the addition of 1% potassium humate, compared with an aqueous solution and a hormone-free MS medium, the intensity of the appearance of alfalfa seedlings is several orders of magnitude higher (Fig. 2).



**Fig. 2. Germination of Alfalfa Seeds: a) in an Aqueous Solution; b) 1% Potassium Humate Solution**

The next stage of the work is the production of a large amount of cell mass to obtain bio additives from alfalfa and the use of nutrient media with various additives, as well as increasing the morphogenic potential of the callus mass.

Callus tissues are often watered, unstructured and have a low ability to form cambial tissues, since conducting bundles are not formed sufficiently. At the same time, callus cells lose their ability to structure, which ultimately leads to a complete loss of morphogenic potential.

The probability that the protein content of such tissues will decrease is very high. Therefore, it is necessary to induce morphogenic cells with a well-developed cambial layer and conducting bundles.

**Table I. Morphogenic Ability of Alfalfa Varieties**

Varieties of alfalfa	Callus genesis, % on MS medium, loose		Morphogenic potential on the Risting medium, %
Raykhan	43,1	57	87
Orai	29,8	32,6	-
Lazurnaya	4,5	9,6	12

To increase the morphogenic potential of the selected varieties, callus tissues were sub cultivated on a Risting medium. When passion Ing loose calluses, the formation of morphogenic tissues in the Ray khan seeds on the Risting medium was 87% (Fig.3). In addition, in the Ray khan and Lazur Naya varieties, polyembryonies was induced on the Risting medium. Unfortunately, due to the high infectability of seeds of the Orai variety, significant results were not obtained.



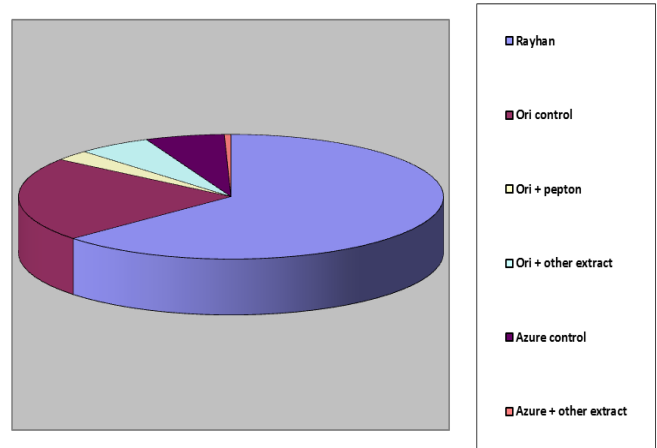
**Fig. 3. Sub cultivation and morphogenesis in alfalfa tissue culture: a) the beginning of structuring and growth; b) 100% regeneration; c) intensive growth of the Raykhan variety**

Starting from third and fourth passages 3 lines were selected: The first line is characterized by a high yield of morphogenic structures; The second line is without pronounced meristematic foci and brown in color, then these cell masses do not pass to secondary differentiation; The third line is formation of polymerogenic tissues (Fig.4).



**Fig. 4. Formation of heterogeneous callus and bipolar embryogenic tissues in vitro culture (Medicago sativa L.)**

One of the important points of preparation of the supplement is to increase the protein qualities of alfalfa calluses so that the drug is not inferior to the traditional green mass of alfalfa. The next stage of experimental research is the enrichment of the nutrient medium with protein components. Fig. 5 shows the results of these experiments, where it can be seen that the callus-forming ability of alfalfa culture with the addition of peptone and yeast extract decreases equally in the two tested varieties, but the positive point is that the ability to callus genesis is preserved.



**Fig. 5. Callus-Forming Ability of Alfalfa Culture on Ms Medium with Additives**

Further, all types of callus tissues induced on modified media are lyophilized, and the dried biomass is prepared for further biochemical analyses, the biomass of three lines is shown in fig. 6.



**Fig. 6. Callus Lines After Lyophilization From 3 Selected Alfalfa Lines**

Thus, the possibilities of preparing a dietary supplement in cell and tissue culture have been determined, the main advantage of which is the absence of the need to grow alfalfa on a plantation. In addition, alfalfa leaves are known to cause bloating in the gastrointestinal tract. When using other methods of preparing biological products and bio additives, there is a danger of excessive consumption of preservatives, dyes and flavors.

#### IV. CONCLUSION

The results of our experimental studies show the possibilities of using cell and tissue culture for the production of bio additives. Due to the fact that alfalfa is becoming widespread as an active additive in the food industry and in animal feed, it is necessary to use cost-effective and environmentally efficient methods. When inducing cell mass in *in vitro* culture, no harmful chemicals are used - fertilizers, herbicides, pesticides, etc. The reduction of the above substances and the improvement of environmental indicators is the most important task of using biotechnological methods in crop production.

#### REFERENCES

- Livia A., Sorin I., Claudia M et al. Alfalfa concentrate – a rich source of nutrients for use in food products. Agriculture & Food journal. – ISSN 1314-8591, Vol. 5, 2017. p. 66-73 <https://www.researchgate.net>. [CrossRef]

- Zhipeng G., Tingting Zh., Zhao C. et al. Occurrence, Distribution, and Genetic Diversity of Alfalfa (*Medicago sativa* L.) Viruses in Four Major Alfalfa-Producing Provinces of China. *Frontiers in Microbiology* journal. – Volume 12, 2022. p. 1-16 <https://doi.org/10.3389/fmicb.2021.771361>
- Ali Al-Snafi, Hanaa S., Hussein Al-Saedy et al. *Medicago sativa*: A potential medicinal plant. *International Journal of Biological and Pharmaceutical Sciences Archive*, 01(02), 2021. p. 22-33 <https://doi.org/10.30574/ijbpsa.2021.1.2.0302>
- F. Nofouzi, M. ÇağrıOğur, S. Khabbazi, Ali Ergul., Improvement of the in vitro regeneration and Agrobacterium-mediated genetic transformation of *Medicago sativa* L., *Turkish Journal of Agriculture and Forestry*, 2019,109-10,p. 96-104 <https://doi.org/10.3906/tar-1804-52> [CrossRef]
- Salih, A.M., Al-Qurainy, F., Khan, S. et al. Mass propagation of *Juniperus procera* Hochst. Ex Endl. From seedling and screening of bioactive compounds in shoot and callus extract. *BMC Plant Biol* 21, 192 (2021). <https://doi.org/10.1186/s12870-021-02946-2> [CrossRef]
- Nuraniye E., Burak D., Nazire G. K. D., Esra U. Comparative study of in vitro antioxidant, acetylcholinesterase and butyrylcholinesterase activity of alfalfa (*Medicago sativa* L.) collected during different growth stages // *De Gruyter*. – 2018. – P. 963-967 <https://doi.org/10.1515/chem-2018-0088> [CrossRef]
- Yong-Zhong L., Guang L., Guijun Y., Hui L., Neil C. T. Morphological Features and Biomass Partitioning of Lucerne Plants (*Medicago sativa* L.) Subjected to Water Stress // *Agronomy*, 10(3), 322. – 2020. p. 1-10 <https://doi.org/10.3390/agronomy10030322> [CrossRef]
- Cposob polucheniya pishchevogo belkovogo produkta (in Russian). Pat. 2043410. Ros. Federaciya MPK C12N 5/04, A01H 4/00, C07K 2/00, Mazin V. V., Patentobladatel'(i): Mazin V. V. - № 5058443/13, zayavl. 11.08.1992; opubl.: 10.09.1995
- L.A. Volkova; V.V. Urmantseva; A.B. Burgutin; A.M. Nosov. Stress. Protective Effect of Furostanol Glycosides on Cultured *Medicago sativa* Cells *in vitro* under Hypothermia and Hyperosmotic Stress // *Russian Journal of Plant Physiology*. – V. 69. - 2022 [CrossRef]
- Butenko R.G. *Biologiya kletok vysshih rastenij invitro i biotekhnologii na ih osnove* (in Russian). Uchebnoe posobie, 1999.M, FBK-PRESS, 160 s
- Kalashnikova E.A., Kochieva E.Z., Mironova O.YU. *Praktikum po sel'skohozyajstvennoj biotekhnologii* (in Russian). – M.: Kolos, 2006
- M.B. Botayeva, Zhamekova A., S.S. Bekkuzhina. Using possibilities of in vitro methods for enrichment of fodder balance of alfalfa. ISSN 2311-2158. *The Way of Science*. 2016 № 4 (26). 51-54.
- Bekenova A., Bugubaj G., Bekkuzhina S. *Isol'zovanie vozmozhnostej kul'tury kletok i tkanej dlya polucheniya biopreparata v kul'ture lyucerny* (in Russian). Moskva OOO «Konvert». – 2019. C. 178-180 s. ISBN 978-5-6042581-7-0
- Nesrin O. In vitro organogenesis and regeneration of Alfalfa (*Medicago sativa* L.) domestic cultivar cv. kayseri, *Fresenius Environmental Bulletin* (in Russian). – 2013. – Vol. 22(9a):2770-2774
- Farzad N., Muhammet Ç., Saber D., Ali E. Improvement of the in vitro regeneration and Agrobacterium-mediated genetic transformation of *Medicago sativa* L., 2019, Volume 43, Issue 1, 96 - 104 doi:10.3906/tar-1804-52 [CrossRef]

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Retrieval Number: 100.1/ijaent.104721191122

DOI: [10.35940/ijaent.10472.1191122](https://doi.org/10.35940/ijaent.10472.1191122)

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- Somatic embryogenesis and plant regeneration from upland switchgrass cultivars // *Research on Crops*. – 2020. – Vol. 21(2). №3410 – P.1-6

- Method choice for processing sugar sorghum juice to prevent the formation of 5-hydroxymethylfurfural in the syrup during storage Eurasia // *Biosci*. – 2020. № 14. – P. 1273-1280

- High frequency direct shoot regeneration from Kazakh commercial potato cultivars // *Peer. J*. – 2020. Vol. 20

- Studies on callus induction of *Cistanche deserticola* from Kazakhstan deserts // *Journal of Biotechnology*. – 2019. Vol. 305 IF

- Possibilities of use of Gamete Breeding for Selecting of Plants Resistant to Water Deficiency // *International Journal of Advanced Engineering and Nano Technology (IJAENT)* ISSN: 2347-6389. – 2016. Vol.3

- Using possibilities of in vitro methods for enrichment of fodder balance of alfalfa // *The Way of Science International scientific journal*. - № 4(26). – 2016.



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### Publication:

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- Bekenova A., Shvidchenko V.K., Kiyan V.S. Infection of spring wheat cultivated in northern Kazakhstan // *The II International Science Conference «Issues of practice and science»*, September 27 – 29, 2021. London, Great Britain. P. 10-13

- Bekenova A.B., Bugubay G., Bekkuzhina S. Ispol'zovanie vozmozhnostei kul'tury kletok i tkanej dlya polucheniya biopreparata v kul'ture lyucerny (in Russian) // *Vishaya shkola MBA Integral Russko-Ital'yanskii mezhdunarodnyi universitet, Sovremennaya mirovaya ekonomika: problem i perspektivy v epokhu razvitiya cifrovyykh tekhnologii i biotekhnologii / Sbornik nachnykh statej mezhdunarodnoi nachnoi konferencii* (29-31 marta 2019 g.)



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- Bekenova A.B., Hasenova B.B., Gadzhimuradova A.M. Razrabotka skhem selektsii i adaptatsiya tekhnologii sozdaniya novykh form rasteniya yarovoj myagkoj pschenicy na osnove metoda somaklonal'noj variabel'nosti rastenij (in Russian) // Sovremennaya mirovaya ekonomika: problemy i perspektivy v epohu razvitiya cifrovoy tekhnologii i biotekhnologii // Cbornik nauchnykh statej po itogam raboty vtorogo mezhdunarodnogo kruglogo stola. 16-15 maya 2019g. CHast' 2. - Moskva OOO «Konvert», -2019



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<http://dx.doi.org/10.17582/journal.aavs/2019/7.s1.21.27>

- Ermagambet B.T, Kassenova J.M, [Kuhar E.V](#), Bailina G. E., The effect of potassium humate on the body of animals in the form of a feed additive //Book of Abstracts Fifth International Conference of CIS IHSS on Humic Innovative Technologies «Humic substances and living systems» (HIT-2019) October 19-23, 2019, Moscow, Russia. – M., 2019. – P. 99. / [www.humus.ru/hit-2019](http://www.humus.ru/hit-2019).

- Elena Kukhar, Ainura Smagulova, Ainash Daniyarova, Aliya Baydusenova, Vladimir Kiyani. *Candida parapsilosis* as a causative agent of onychomycosis in patient with cirrhosis of the liver // J. Fungi 2020, Volume 6, Issue 4, 313; doi:10.3390/jof6040313. [www.mdpi.com/journal/jof](http://www.mdpi.com/journal/jof)

- Biological properties of *Phoma macrostoma* related to non-dermatophyte onychomycosis / Medical Mycology Case Reports, Vol. 27, P. 55-58, March 2020. <https://doi.org/10.1016/j.mmcr.2020.01.005>.

- [Elena V. Kukhar](#), Vladimir S. Kiyani. Chapter 9: *Phoma* as an infectious pathogen in medical practice. – P. 161-179. In Book "Phoma: Diversity, Taxonomy, Bioactivities, and Nanotechnology" Mahendra Rai, Beata Zimowska, György János Kövics. Editors. ISBN 978-3-030-81217-1; ISBN 978-3-030-81218-8 (eBook). <https://doi.org/10.1007/978-3-030-81218-8>

- Gulnar Aidarkhanova, Serik A. Kubentayev, Yelena V. Kukhar, Alina S. Urazalina. Current state of populations, resources, and biological activity *Pulsatilla patens* (Ranunculaceae) in Northern Kazakhstan //BIODIVERSITAS Vol.23, N.5, May 2022. P. 2311-2320/ DOI: 10.13057/biodiv/d230509

- [Yelena Kukhar](#), Ainura Smagulova, Vladimir Kiyani. Generalized Dermatophytosis of Combined Etiology in a Circus Tiger (*Panthera tigris altaica*) // International Journal of Veterinary Science, 2022, 11(4): 552-556. <https://doi.org/10.47278/journal.ijvs/2022.159>



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Information about the main publications:

Tussipkan Dilnur and Manabayeva Shuga A. Alfalfa (*Medicago sativa* L.): Genotypic diversity and transgenic alfalfa for phytoremediation //Frontiers in Environmental Science. – 2022, V. 10, p.1-13. 10:828257. doi: 10.3389/fenvs.2022.828257 Web of Science Q2. IF-4,581. Percentile - 78%. CiteScore – 4.2

Tussipkan Dilnur and Manabayeva Shuga A. Employing CRISPR/Cas technology for the improvement of potato and other tuber crops // Frontiers in Plant Science. – 2020, V. 12, p.1-16. doi: 10.3389/fpls.2021.747476 Web of Science Q1, IF – 5,754; Percentile - 95%. CiteScore – 7.8

Abeuova L.S., Kali B.R., Rakhimzhanova A.O., Bekkuzhina S.S., Manabayeva Sh.A. High frequency direct shoot regeneration from Kazakh commercial potato cultivars //PeerJ. –2020. Vol. 2020, Issue 7, 2020, Article number e9447. [doi.org/10.7717/peerj.9447](https://doi.org/10.7717/peerj.9447) Web of Science Q2, IF – 2,984; Percentile – 83%. CiteScore – 4.6

Information about available patents

Manabaeva S.A., Abeuova L.S., Ramankulov E.M. Transient gene expression of recombinant human GFP and G-CSF proteins in *N. benthamiana* plants // Innovative patent of the Republic of Kazakhstan. No. 31056. 03/16/2016.

Manabaeva S.A., Rakhimzhanova A.O., Ramankulov E.M. A method for obtaining herbicide-resistant transgenic cotton plants of the "Turkistan" variety // Innovative patent of the Republic of Kazakhstan. No. 31284. 06/20/2016.

Manabayeva S.A., Zhumabek A.T., Ramankulov E.M. Method for transient expression of the gene of recombinant protein  $\beta$ -1,4 endoglucanase E1 in *N. benthamiana* plants using a viral vector based on the TBSV genome. RK patent. No. 32592. 12/12/2017