Journal of Life Sciences & Biomedicine, vol. 3(76), No 1, p. 05-16 (2021)

Effect of climate change on health in Azerbaijan

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Received: April 09, 2021; Received in revised form: April 09, 2021; Accepted: April 05, 2021

It is known that humanity is facing a new and unusual problem, such as global climate change of anthropogenic origin. The causes of this problem, its consequences for the environment, economy, human health, and other areas of life are already being noted. In recent decades, 90 percent of the causes of climate change have been associated with man-made pollution, especially the release of large amounts of "greenhouse gases" (carbon dioxide, methane, water vapor, nitrogen oxides, etc.) into the atmosphere and deforestation. The concentration of "greenhouse gases" in the atmosphere has never been higher in the history of mankind. In particular, the amount of carbon dioxide emitted into the atmosphere as a result of the use of hydrocarbon fuels is increasing rapidly in line with fuel consumption. The essence of the "greenhouse effect" known to science since the first half of the XIX century is that the thermal energy released from the Earth's surface heated by solar energy (50% of solar energy is absorbed by the Earth) is absorbed by atmospheric air and "greenhouse gases". It plays the role of a kind of "polyethylene cover". As a result, the temperature of the Earth's surface and oceans is gradually rising, and the Arctic, Greenland and some large mountain glaciers are degrading. It was found that from 1899 to 2007, the temperature of the Earth's atmosphere increased by more than 1°C, and the temperature of ocean waters increased by 0.8°C. Such global climate change is accompanied by a number of serious cataclysms on the planet - rising sea and ocean levels, river floods, floods, storms, hurricanes, torrential rains, severe heat, drought, forest fires, desertification, and in some places swamps accompanied by natural disasters. Observations show that an increase in ocean temperature by 1.0-1.5°C leads to a rapid decline in several oceanic species, including some fish species. It is predicted that by the end of the XXI century, the level of ocean water will rise by 30-45 cm. This means, first of all, the flooding of large areas, a number of islands and island countries, the world's largest coastal cities, the degradation of agriculture, the threat of food shortages. The economic consequences of natural disasters as a result of global warming include the destruction of houses due to flooding of coastal areas, the lack of drinking water, the deterioration of living conditions of the population accompanied by the failure of engineering facilities and infrastructure. Such natural phenomena are already registered in our country (in recent years, due to the rise in the level of the Kura River, groundwater in the regions, damage to the infrastructure of some mountain rivers, landslides, etc.). The intensification of global climate change increases the expected risk of such events. Therefore, there must be a limit to the impact of human activities on the environment. Global warming of 2°C should be considered an undesirable limit. If at 2°C by the middle of the century, 500 million people will suffer from a shortage of drinking water, at 3°C their number will reach 3 billion. The initiative of Inter-Academy Partnership (IAP) to address the climate change impact on health is a worthy scientific effort. The effects of global climate change, and in particular, its impact on health, are already being felt in many different ways and forms around the world. Research by the World Health Organization (WHO) in 53 countries in the European region shows that the direct health effects of climate change include abnormally high or low temperatures, as well as diseases caused by high temperatures during floods, storms, and forest fires. It is accompanied by an increase in the number of malignant tumors, mental disorders, trauma, and death. According to the WHO, climate change now

https://doi.org/10.29228/jlsb.1
Available online 30 June 2021

causes more than 150,000 premature deaths (excluding predicted deaths) each year. Climate warming, as well as an increase in the number of infectious and parasitic pathogens (acute intestinal infections, viral hepatitis, hemorrhagic fever, etc.), the expansion of the range of some natural foci of infection (tick-borne encephalitis, skin leishmaniasis, malaria, etc.), goes along. Moreover, COVID-19 is also spreading due to a compromised immune system. Thus, the world needs the decarbonization of the world economy and change of financial power from grey to green to initiate the resilience of people and communities to provide a safe and healthy environment for the future generations.

Keywords: *Adaptation, agriculture, climate change, COVID-19, decarbonization, diseases, food security, global warming, health, mitigation*

1. INTRODUCTION

1.1. Climate change and health – A global agenda

One of the WHO's initiatives is the Health and Climate Change Country Profile Project, which is monitoring national and global progress on climate change and health. The Project aims are:

- Increasing responsiveness of the health impacts of climate change;
- Supporting evidence-based decision making to support the flexibility of health systems;
- Supporting health participation in national and international climate processes such as the United Nations Framework Convention on Climate Change (UNFCCC)
- Promoting actions that improve health while reducing greenhouse gas emissions.

WHO's report entitled, "The WHO Health and Climate Change Survey: Tackling Global Progress" emphasizes the neglected link between climate change and health (WHO, 2018). This report emphasized that the rising global temperature was extremely disturbing the social and environmental determinants of health. The global burden of disease increases due to climate change.

A global network of science academies representing more than 130 academies (Inter-Academy Partnership - IAP) and Association of Academies and Societies of Sciences in Asia (AASSA) took the initiative of addressing the challenge of climate change impact on health and will make a wide-ranging global associated report by the end of 2021.

2. CLIMATE CHANGE AND HEALTH IMPACT IN AZERBAIJAN

2.1. Climatic features of Azerbaij*an*

Azerbaijan's geography covers a various collection of landscapes, from wetlands to high mountains, deserts to fertile valleys. The center of the country is taken up by a broad valley, centered around the Kura River. This valley is bordered to the north by the Greater Caucasus Mountains, and to the south by the Lesser Caucasus Mountains, and opens in the east to the Caspian Sea. The highest point in Azerbaijan is Bazarduzu Dagi, at 4,467 m (14,656 ft), and the lowest point is - 28 m (-92 ft), in the Caspian Sea.

The Caspian Sea is home to many species of fish, and the shores hold important wetlands, where numerous species of birds live. Even though the Caspian Sea is called a sea, it's actually the largest lake in the world.

The issue of well-organized use of climate resources in agricultural production is one of the central tasks to solve the food problem. To implement it, it is necessary to study the features of our area in depth, to identify potential opportunities for more efficient and rapid development of agriculture. As we know, the existing 9 out of the world's 11 climate zones in Azerbaijan enable growing abundant agricultural products in different regions of it in all seasons. This assists to provide the population with agricultural products around the year. Today 3 large enterprises in Republic realize the processing of agricultural products that meet ecological standards (Humbatova et al., 2020). Besides these enterprises, several family farms deal with the processing of homemade ecological products. They are mainly engaged in

the desiccation of fruits (plums, apricots, figs, apples, cornel, hirnic, and other wild plants), confuter and jams, soaps, juices, and compotes.

In general, Azerbaijan is a mountainous country. Therefore, the study of the spatial and temporal distribution of natural factors, quantitative relationships of individual elements of the climate, the distribution of agroclimatic indicators depending on altitude, indented relief, exposure of slopes is of great scientific and practical importance. Due to its location in the southern hemisphere, the territory of Azerbaijan receives a lot of sunlight and heat. The duration of sunlight in the Kura-Araks lowland is 2200-2400 hours per year, and the annual amount of PAR (photosynthetic active radiation) is more than 64 kcal/cm^2. The maximum value of these indicators is observed in the Arzaboyu plain (more than 2800 hours and more than 76 kcal/cm^2, respectively). The annual flow of solar energy in the area is also well expressed. At a time when solar energy is most concentrated (April-October), the total PAR is 50-54 kcal/cm^2 in the Kura-Araks lowland and 59-60 kcal/cm^2 in the plains of the Nakhchivan Autonomous Republic (eco.gov.az/az/hidrometeoro-logiya).

In summer, the lighting conditions on the eastern slopes are better than on the western slopes. Plants are more affected by frost on the eastern slopes. As the heating effect of solar radiation increases, plants in the mountains require a lower total temperature than in the plains in the interfacial period. It is of practical importance to take this effect into account when relocating crops to mountainous areas.

Much of the area is characterized by high thermal regimes, low rainfall, and in some places very low rainfall. Almost the entire Kura-Araks lowland, the Absheron Peninsula, and the Arazboyu plain receive only 110-350 mm of rainfall per year. Thus, the amount of precipitation in the mountainous area increases with altitude. Accordingly, the role of precipitation in humidity is increasing due to the decrease in air temperature. At first glance, it seems that the total amount of precipitation in many areas is enough to meet the plant's need for moisture. However, the annual rainfall regime is such that at a time when the plant is growing intensively and transpiration is increasing, it is not sufficiently supplied with moisture.

2.2. Previous Academy Publications on Climate Change and Health

Unfortunately, ANAS did not publish any report on Climate Change and Health but there are a number of papers on this issue, published by academy institutions. Publications on the subject of climate change and health by the Azerbaijani scientists have been listed.

Agayev I., Vahabov E., Jalilov V. et al. (2020) Epidemiological situation and spatial distribution of visceral leishmaniasis in the Republic of Azerbaijan. *J. Parasit. Dis.,* **44:** 639–645 (2020).

Aliyev K., Gasimov I. (2020) Life (DIS) satisfaction and intention to emigrate in Azerbaijan: Mediating role of institutional trust. *Economic and Social Development: Book of Proceedings*, **3:** 306-313.

Allakhverdiev A.R., Allakhverdieva A.A., Babayev E.S. (2020) Functional state of the brain of elderly women at rest and in mental stress under varying geomagnetic conditions. *Hum. Physiol.,* **46:** 408–416.

Guliyeva A.E., Lis M. (2020) Sustainability Management of Organic Food Organizations: A Case Study of Azerbaijan. *Sustainability*, **12(12):** 5057.

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Neudert R., Allahverdiyeva N., Mammadov N., Didebulidze A., Beckmann V. (2020) Diversification of livestock-keeping smallholders in mountainous rural regions of Azerbaijan and Georgia. *Land*, **9(8):** 267.

Temel T. (2004) Malaria from the gap: need for cross-sector co-operation in Azerbaijan. *Acta Tropica*, **89(2):** 249-259.

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2.3. Effect of climate change in Azerbaijan

One of the global problems that the world faces is climate change. The world community is increasingly concerned about climate change and its impact on the living world. Azerbaijan ranked 111, according to the 2019 Global Climate Risk Index report which was released by the Germanatch, a public policy group (Eckstein et al., 2019). The major contribution to this climate change is the greenhouse gas emissions (CO_2, CH_4, N_2O, and H_2O), though Azerbaijan's contribution to global greenhouse gas emissions is negligible and insignificant.

Unstable weather conditions are felt not only in Azerbaijan but also in a number of countries around the world and create problems. Increased attention to these problems is reflected in the holding of a number of international events, including scientific and practical conferences. According to a recent assessment by the Intergovernmental Panel on Climate Change, the average global temperature has risen by 0.8 degrees over the past 100 years. The increase in temperature is mainly due to anthropogenic factors. Anthropogenic factors are based on gases that create a thermal effect: carbon, methane, nitrogen oxide, nitrogen oxide 1, and chlorine-fluorine compounds. The last 100 years of space observations show that both the intensity and frequency of storms and blizzards have increased. Warm winds, hurricanes, and rains intensified. At the same time, the number of floods has increased. If the surface of the ocean is used to heat up to a depth of 1,000 meters, now the heat reaches a depth of 2,000 meters. This causes the hot streams to heat up even more. That is, climate change is a key factor in the growth of all these natural disasters.

Azerbaijan has also been affected by global climate change. Over the past 100 years, the average annual temperature in Azerbaijan has increased by 0.4-1.3ºC. The temperature-rise is unevenly distributed depending on the regions. In the last 10 years, the number and intensity of floods in small mountain rivers in the territory of Azerbaijan have increased.

Heatwaves and natural fires are other consequences of climate change. Valleys, where normal temperatures used to be around 35~40ºC, are now having a temperature of 45ºC on several days during the season. Desertification is one more thoughtful effect of climate change in Azerbaijan.

2.4. Impact of climate change on human health

Climate change is the supreme hazard to worldwide health in the 21st century. The possessions of global warming consist of their effects on human health. The experimental and expected increased climate change-related impacts will auxiliary intensify the effects on human health. Climate change is affecting the safety of shelter, air and water quality, food availability, and nutrition levels in the food that impacts human health. As climate change grows, scientists assume an increase in related health issues. Cold and heatwaves, droughts, storms, floods, land sliding, and natural fires can cause injury, illness and death directly. Besides, due to the environmental and ecological conditions, the quality and yield of crops, and availability of food items there could be decreased. Also, the availability of clean drinking water can be decreased which will lead to the spread of water-borne and vector-borne diseases. Altogether, climate change could result in poverty, hunger, food insecurity, and ill-health.

2.4.1. Impact of climate change on agriculture and food security

The impact of climate change on the world is already clear, and signs of this are being observed in Azerbaijan. Compared to other sectors of the economy, agriculture is the most dependent on climatic conditions. Agricultural production is sensitive to weather conditions and is therefore directly affected by climate change. Currently, non-seasonal weather conditions are observed in Azerbaijan. The most severe period of the winter season in February of this year was above normal in the Republic, and on some days in the lowlands was 15-20%.

In 2018, the average annual precipitation was 480 mm, and in 2019 - 360 mm. In 2020, the downward trend and uneven distribution of precipitation continued. The current situation requires a reconsideration of production models in agriculture, the application of modern cultivation

technologies, and irrigation methods. The main crop in the country is cereals. In areas where there is normally enough rainfall in the autumn, there is a serious lack of rainfall during the planting season. Due to the lack of moisture in the soil, most of the seeds sown did not germinate, and the germinated seeds did not develop due to drought. They cause serious damage when feeding on them. During severe climate change, productivity decreases, crop losses increase, and product quality declines. More than 80 percent of crop production in the country is produced on irrigated lands. In this regard, the reduction of water resources due to climate change has put serious pressure on agriculture.

Impacts of climate change on crop production in Azerbaijan are:

• *Lacking of irrigation water, on the contrary, increasing demand for water from crop products;*

• *Decreasing rainfall in remote areas, resulting in lower productivity;*

• *Changing in the vegetation period of plants with changes in the seasonal cycle and changes in the timing of the product on the market;*

• *Increased spring drought;*

• *Creating favorable conditions for the development of plant pests.*

• *Decreasing in the number of irrigations due to water scarcity in the irrigation system and resulting in lower productivity (especially in cotton and grain)*

Impacts of climate change on livestock in Azerbaijan are:

• *Decreasing productivity in natural pastures and increased risk of erosion*

• *Increasing infectious diseases and, accordingly, preventive measures,*

• *Expanding of desertification, reduction of natural pastures and hayfields,*

• *Decreasing biodiversity and vegetation in natural pastures.*

The "Strategic Roadmap for the Production and Processing of Agricultural Products in Azerbaijan" envisages preventive measures to reduce the impact of climate change on agriculture.

2.4.2. Risk of infectious and vector-borne diseases

Climate changes have an actual deep effect on the life-cycle and growth of infectious organisms. Hence, the transmission of water and food-borne diseases is at a peak after the rainy and flooding season. Malaria vectors in Azerbaijan include *Anopheles maculipennis* (the Caucasus), *An. sacharovi* (Kura-Araksin and Lenkoran lowlands) and *An. Persiensis* (Lenkoran lowland) in 2011 (WHO Azerbaijan, 2011). Malaria was once common in Europe and happened almost as far north as the Arctic Circle. Recurrent outbreaks have occurred in Eastern Europe, Azerbaijan, Tajikistan, and Turkey. However, none of these outbreaks was associated with climate change but rather with hydro-agricultural development schemes, movement of infected cases, and the cessation of malaria-control activities (Githeko et al., 2000; Temel, 2004). Azerbaijan successfully interrupted malaria transmission in 2013, meeting its national goal laid out in the 2008–2013 strategic plan. Now the country focuses on preventing malaria re-introduction. In 2015, the national strategy for the prevention of malaria reintroduction for the years 2015–2020 was adopted (WHO Reports Azerbaijan, 2020a). According to the WHO's report, Azerbaijan effectively broke up malaria transmission in 2013, meeting its national goal laid out in the 2008–2013 strategic plan. Now Azerbaijan focusses on avoiding malaria reintroduction. The national strategy for avoidance of malaria reintroduction for the years 2015–2020 was adopted in 2015.

Leishmaniasis is a neglected and poorly reported disease with an underestimated burden in most countries of the Region. The regional incidence of VL and cutaneous leishmaniasis (CL) could be estimated at less than 2% of the global burden. Cases of anthroponotic CL, which is caused by *L. tropica*, could be found in Azerbaijan (Agayev et al., 2020). Cholera, diarrhea, hepatitis A, typhoid are also typical water-borne diseases in Azerbaijan. However, we do not have exact data for these diseases.

2.4.3. Impact of high levels of pollens, allergens and "smog"

Increased temperatures contribute to higher levels of pollens and allergens in the atmosphere, which cause airway inflammation, asthma symptoms, and increased healthcare utilization among individuals. Transport, industrial emissions and crop burning residues have become major causes of pollution, especially in the capital city Baku (Krzyżanowski et al., 2005; Lacey et al., 2017).

2.4.4. Impact of heatwaves and natural fires

Although now heat waves are more common in the cities of the country due to climate change, most natural fires happen in the mountain areas because of the extremely hot weather conditions. This leads to the loss of wildlife and vegetation.

2.4.5. Impact on the development of chronic diseases

According to the WHO's report, during the last ten years, circulatory system diseases have increased by 5%, respiratory system diseases 11%, endocrine disorders 2.5 times (including diabetes 3.7 times), nervous system diseases by 15%, malignant neoplasms by 30%. During this period, the global prevalence of noncommunicable diseases has risen by 14%. As in most countries, noncommunicable diseases are the leading cause of morbidity and mortality in Azerbaijan. Noncommunicable diseases accounted for 50% of all diseases, including 17.6% circulatory system diseases, 15% respiratory system diseases, 15% endocrine disorders, 2.7% malignant neoplasms (WHO Reports Azerbaijan, 2020b). During 10 years (2007-2017) cirrhosis, Alzheimer's disease, and lung cancer increase significantly in Azerbaijan, although, ischemic heart disease and stroke are always on the top of the chronic diseases, which cause the most deaths (www.healthdata.org/azerbaijan).

Food insecurity and malnutrition, higher temperature, heatwaves and pollution, and weakened health systems due to climate change are directly related to global diabetes epidemics according to the 2012 report of the International Diabetes Federation (International Diabetes Federation, 2012).

2.4.6. Impact on the risk of development of cancer

Climate change is already increasing the number of toxic chemicals, especially carcinogens after extreme weather events such as hurricanes and wildfires in nature. Increased exposure of humans to these toxic chemicals can lead to various types of cancer such as liver cancer, breast cancer, and lung cancer. Moreover, various air pollutants have also been shown to be causing lung cancer in humans (National Institute of Environmental Health Sciences, 2019). In addition to increasing cancer risk, climate change is also affecting cancer survival (Nogueira et al., 2020). The frequency of stomach cancer and lung cancer is higher in Azerbaijan (www.healthdata.org/azerbaijan). The high incidence of these types of cancer is recognized as a consequence of adverse changes in the environment.

2.4.7. Impact on mental health

One of the health-related fields is the impact of environmental changes on mental health, which has not been adequately investigated in Azerbaijan. However, few works have been done to study the effect of the geomagnetic conditions (Allakhverdiev et al., 2020). Depression, anxiety, psychological distress, post-traumatic stress, aggression, complicated grief, complex psychopathology, sleep disorders, sexual dysfunction, social avoidance, irritability, drugs, etc. are the most common mental health forms, which climate change affects.

2.5. Adaptation and mitigation

Azerbaijan has already identified social and economic development and poverty reduction as its priorities. The country's mitigation and adaptation strategies for climate change are reflected in the following long-term State Programs:

- State Program on the Use of Alternative and Renewable Energy Sources (2004);
- Azerbaijan 2020-FUTURE CONCEPT OF DEVELOPMENT;
- State Program on Socio-Economic Development of the Region in the Republic of Azerbaijan for 2008-2015 (2008-2015);
- State Program on Poverty Reduction and Sustainable Development in the Republic of Azerbaijan for 2008-2015 (2008-2015);
- Strategic Roadmap for Agricultural Production and Processing in the Republic of Azerbaijan, 2016;
- To achieve the Sustainable Development Goals, The National Coordinating Council for Sustainable Development (NCCSD) of Azerbaijan was established (2016).

Preventive measures against the consequences of climate change should also focus primarily on the development of technologies that increase the opportunities for sustainable and efficient use of land and water resources, optimization and adaptation of the agricultural sector. For that, we need to do:

- ✓ Soil monitoring and comparative research to study the consequences of climate change. Development of mathematical forecasting models based on complex and multidisciplinary research;
- ✓ Conducting research in the field of maintaining soil fertility, prevention of salinization, water and wind erosion;
- ✓ Development of an improved system of agro-technical and phytomeliorative measures to prevent soil erosion;
- ✓ Application of irrigation water-saving irrigation systems in the conditions of an arid zone, development of the concept of efficient use of existing water resources;
- ✓ Study of the possibility of adaptation of new varieties of drought- and salt-resistant created in the field of plant breeding in different soil-climatic zones.

For successful development of action, all the stakeholders including the government agencies and academy institutions must join hands to meet the climate change and health challenges in Azerbaijan.

2.5.1. What needs to be done regarding adaptation and mitigation

We need to create coordination among various institutions related to climate change in Azerbaijan, there is an urgent need to address the problem as one of the uppermost importance plan items.

2.6. Recent initiatives by government of Azerbaijan for climate change mitigation and adaptation

2.6.1. International agreements

- ➢ The Republic of Azerbaijan acceded to the UN Framework Convention on Climate Change in 1995 and ratified the Kyoto Protocol in 2000.
- ➢ The Doha Annex, adopted for the second period of implementation of the Kyoto Protocol, was ratified by the Milli Majlis (The National Parliament) of the Republic of Azerbaijan on April 14, 2015, and signed by the President of the country.
- ➢ The Paris Agreement was signed in April 2016 and ratified by the Milli Majlis on October 28, 2016. By the Paris Agreement, the Republic of Azerbaijan submitted its National Contributions to the Secretariat of the Convention and aims to reduce greenhouse gas emissions by 35% by 2030 compared to the base year (1990) as its contribution to global climate change mitigation initiatives.

2.6.2. Climate change mitigation measures

Although Azerbaijan has not made quantitative commitments to reduce emissions from the Kyoto Protocol, the country has taken several important steps in recent years, including the introduction of low-carbon, energy-efficient, renewable energy and waste management technologies, as well as forest expansion and deforestation.

In addition to national mitigation initiatives, Azerbaijan successfully cooperates with several international organizations through the implementation of various projects. Thus, more than 30 projects related to climate change mitigation technology and capacity building have been implemented (eco.gov.az).

2.6.3. Prevention of intrusion of the Caspian Sea

The main factors determining the climate of the Caspian Sea - the geographical location of the sea, the nature of atmospheric circulation, the impact of surrounding land areas - are the Aral-Caspian lowlands in the east, the Caucasus Mountains in the west and water exchange between different parts of the sea. The main characteristic of the Caspian climate is the predominance of anticyclone weather conditions, sharp temperature changes throughout the year, cold, and windy winters in the North Caspian and hot in the South Caspian, and hot, dry, and calm summers throughout the Caspian.

The water of the Caspian Sea is of oceanic origin and the average salinity is 12.85‰ (promille) (average salinity of ocean water is 35‰). Low salinity is since the sea is closed and fed mainly by river currents. The Caspian Sea has more carbonates and sulfates than ocean waters and fewer chlorides. Strong fluctuations in the Caspian Sea occur, especially during strong north and south winds in autumn and winter.

Observations are carried out on water level, water temperature, salinity, color, transparency, wave height, length and period, air temperature, humidity, amount of cloudiness, wind speed and direction, atmospheric pressure, precipitation in

the Caspian observation network to study the hydrometeorological conditions of the Caspian Sea. Studies covering the hydrometeorological regime of the Caspian Sea are carried out at 4 observation times at the stations and points located in the coastal zone and open sea. Thanks to the continuous information received from the observation network, it is possible to analyze the long-term hydrometeorological conditions of the Caspian Sea. The National Hydrometeorology Department exchanges information in cooperation with CASPCOM, the coordination center of the Caspian littoral states.

Climate change affects all hydrometeorological parameters as well as the sea level.

2.7. Covid-19 pandemic and climate change

The first Covid-19 case was identified in Azerbaijan on February 29, 2020. Until September 29, 2020; more than 40,061 positive cases of Covid-19 have been identified and 588 deaths were caused by this infection. Although, the complete lockdown for several months has harshly affected the economy of Azerbaijan it also had a positive influence on greenhouse gas emissions and the environment. The air quality index in major cities in Azerbaijan becomes better. The health system in Azerbaijan has been challenged. Moreover, people suffering from non-Covid diseases were able to get mental problems due to the long complete lockdown for several months. We believe that a climate-smart approach that may also offer a better human immune system is likely to provide better health for future generations against the pandemic.

3. CONCLUSIONS AND RECOMMENDATIONS

In a world of numerous "what if" scenarios of immediate climate change, it becomes difficult to make health policies for the future, because of the improbability of expecting environmental change and human decisions. To be familiar with the difficulty of this issue, an ad hoc Interagency Working Group on Climate Change and Health (IWGCCH) bring together to develop research and science needs, including research on mitigation and adaptation strategies. This research includes basic and applied science, technological innovations and capacities, public health infrastructure, communication and education. Attention is also given to the possible structure of the climate change and health research agenda and the use of these research results for applications and decision making. Azerbaijan being a developing country with an oil-based economy has been most severely affected by climate change and its subsequent events and this is seriously affecting the health of the people. To address the challenge of climate change impact on health, it is authoritative to take measures towards mitigation, and to mitigate we need to identify the priority scientific fields. The challenges that science needs to be addressed is the impact of climate change on human health are: 1) Asthma, Respiratory Allergies, and Airway Diseases; 2) Cancer; 3) Cardiovascular Disease and Stroke; 4) Foodborne Diseases and Nutrition; 5) Human Developmental Effects; 5) Heat-Related Morbidity and Mortality; 6) Neurological Diseases and Disorders; 7) Vectorborne and Zoonotic Diseases; 8) Waterborne Diseases; 9) Weather-Related Morbidity and Mortality; 10) Human immune system; 11) Pandemics. All these aspects become more predominant because of the increased human exposure to climate change. Adaptation and mitigation adaptation may significantly diminish these risks (WHO Reports, 2016). Research should address the relationship between climate change and the factors which affect human health.

A division on "Climate Change and Health" in the Azerbaijan National Academy of Sciences and the government should be created to prepare a scientific, economic, and political agenda for the adaptation and mitigation strategies. The specific areas which could be determined by such divisions should receive extreme consideration.

ACKNOWLEDGMENTS

We thank the Division of Biological and Medical Sciences of Azerbaijan National Academy of Sciences, its Institutions and the staff members. We also thank the staff members of the Ministry of Health, the Ministry of Ecology and Natural Resources, the Ministry of Agriculture, the Food Safety Agency, and the Management Union of Medical Territorial Units who involved in the preparation of this report.

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İqlim dəyişikliyinin Azərbaycanda sağlamlığa təsiri

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Bəşəriyyətin antropogen mənşəli qlobal iqlim dəyişikliyi kimi yeni və qeyri-adi bir problemlə üzləşdiyi məlumdur. Bu problemin səbəbləri, ətraf mühit, iqtisadiyyat, insan sağlamlığı və həyatın digər sahələri üçün nəticələri artıq hiss olunur. Son onilliklərdə, iqlim dəyişikliyi səbəblərinin yüzdə 90-ı texnogen çirk-

lənmə ilə, xüsusən atmosferə çox miqdarda "istixana qazları" (karbon qazı, metan, su buxarı, azot oksidləri və s.) və meşələrin qırılması ilə əlaqələndirilir. Atmosferdəki "istixana qazları"nın qatılığı bəşəriyyət tarixində heç vaxt bu qədər yüksək olmayıb. Xüsusilə, karbohidrogen yanacaqlarının istifadəsi nəticəsində atmosferə atılan karbon dioksid miqdarı yanacaq istehlakına uyğun olaraq sürətlə artır. XIX əsrin birinci yarısından bəri elmə məlum olan "istixana effekti"nin mahiyyəti ondan ibarətdir ki, günəş enerjisi ilə qızdırılan yer səthindən çıxan istilik enerjisi (günəş enerjisinin 50%-i Yer tərəfindən udulur) atmosfer havası və "istixana qazları" tərəfindən udulur. Bu bir növ "polietilen örtük" rolunu oynayır. Nəticədə, Yer səthinin və okeanların temperaturu tədricən yüksəlir və Arktika, Qrenlandiya və bəzi böyük dağlarda buzlaqlar əriyirlər. 1899-cu ildən 2007-ci ilə qədər Yer atmosferinin istiliyinin 1°C-dən çox, okean sularının temperaturunun isə 0,8°C artdığı göstərilmişdir. Bu cür qlobal iqlim dəyişikliyi planetdə bir sıra ciddi kataklizmlərlə müşayiət olunur - dəniz və okean səviyyəsinin yüksəlməsi, çay daşqınları, daşqınlar, fırtınalar, qasırğalar, leysan yağışlar, şiddətli istilər, quraqlıq, meşə yanğınları, səhralaşma və təbii fəlakətlər nəticəsində bəzi yerlərdə bataqlıqlar əmələ gəlməsi ilə müşayiət olunur. Müşahidələr göstərir ki, okean temperaturunun 1,0-1,5°C-yə yüksəlməsi bəzi balıq növləri də daxil olmaqla bir neçə okean növünün sürətlə azalmasına səbəb olur. XXI əsrin sonlarında okean suyunun səviyyəsinin 30-45 sm artacağı proqnozlaşdırılır. Bu, ilk növbədə, geniş ərazilərin, bir sıra adaların və ada ölkələrinin, dünyanın ən böyük sahil şəhərlərinin su basması, kənd təsərrüfatının deqradasiyası, ərzaq çatışmazlığı təhlükəsi deməkdir. Qlobal istiləşmə nəticəsində baş verən təbii fəlakətlərin iqtisadi nəticələrinə sahil ərazilərini su basması, içməli suyun olmaması, mühəndis qurğularının və infrastrukturun sıradan çıxması ilə müşayiət olunan əhalinin yaşayış şəraitinin pisləşməsi səbəbindən evlərin dağıdılması daxildir. Bu cür təbiət hadisələri artıq ölkəmizdə qeydə alınmışdır (son illərdə Kür çayının səviyyəsinin qalxması, bölgələrdəki yeraltı suları, bəzi dağ çaylarının infrastrukturuna ziyan vurması, sürüşmə və s.). Qlobal iqlim dəyişikliyinin intensivləşməsi bu kimi hadisələrin gözlənilən riskini artırır. Buna görə də insan fəaliyyətinin ətraf mühitə təsirinin bir həddi olmalıdır. Qlobal istiləşmə 2°C arzuolunmaz bir sərhəd sayılmalıdır. Əsrin ortalarında qlobal istiləşmə 2°C oland 500 milyon insan içməli su çətinliyindən əziyyət çəkirsə, 1lobal istiləşmə 3°C olarsa onların sayı 3 milyarda çatacaqdır. İqlim dəyişikliyinin sağlamlığa təsirini həll etmək üçün Akademiyalararası Tərəfdaşlığın (IAP) təşəbbüsü layiqli bir elmi səydir. Qlobal iqlim dəyişikliyinin təsiri və xüsusən də onun sağlamlığa təsiri onsuz da dünyada bir çox fərqli şəkildə və formada hiss olunur. Ümumdünya Səhiyyə Təşkilatının (ÜST) Avropa bölgəsindəki 53 ölkədə apardığı araşdırmalar göstərir ki, iqlim dəyişikliyinin sağlamlığa birbaşa təsirlərinə anormal dərəcədə yüksək və ya aşağı temperatur, həmçinin daşqın, fırtına və meşə yanğınları zamanı yüksək temperaturun yaratdığı xəstəliklər daxildir. Bunlar da bədxassəli şişlər, zehni pozğunluqlar, travma və ölüm sayında artım ilə müşayiət olunur. ÜST-yə görə, iqlim dəyişikliyi hazırda hər il 150.000-dən çox erkən ölümə səbəb olur (proqnozlaşdırılan ölümlər istisna olmaqla). İqlim istiləşməsi, həmçinin yoluxucu və parazitar patogenlərin (kəskin bağırsaq infeksiyaları, viral hepatit, hemorajik qızdırma və s.), bəzi təbii infeksiya ocaqlarının (gənə ensefaliti, dəri leyşmaniozu) genişlənməsi, malyariya və s.), sayının artması birlikdə hərəkət edirlər. Üstəlik, COVID-19, zəifləmiş bir immunitet sistemi səbəbiylə də yayılır. Belə ki, dünya, gələcək nəsillər üçün etibarlı və sağlam bir mühit təmin etmək üçün, insanların və icmaların dayanıqlı inkişafını təmin etmək üçün dünya iqtisadiyyatın dekarbonlaşdırılmasına və maliyyə gücünün bozdan yaşıla keçməsinə ehtiyac duyur.

Açar sözlər: *COVİD-19, əkinçilik, qida təhlükəsizliyi, qlobal istiləşmə, xəstəliklər, iqlim dəyişikliyi, karbohidrogensizləşmə, sağlamlıq, təsirlərin azaldılması, uyğunlaşma*

Влияние изменения климата на состояние здоровья населения Азербайджана

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Известно, что человечество столкнулось с новой и необычной проблемой, такой, как глобальное изменение климата антропогенного происхождения. К настоящему времени уже выявлены причины этой проблемы, ее последствия для окружающей среды, экономики, здоровья человека и других сфер жизни. 90 процентов причин, приводящих к изменению климата в последние десятилетия, были связаны с антропогенным загрязнением, особенно с выбросом в атмосферу большого количества «парниковых газов» (углекислого газа, метана, водяного пара, оксидов азота и т. д.) и вырубкой лесов. В истории человечества концентрация «парниковых газов» в атмосфере никогда не была так высока, как сейчас. В частности, количество выбрасываемого в атмосферу углекислого газа, образующегося в результате использования углеводородного топлива, быстро увеличивается вместе с расходом топлива. Суть «парникового эффекта», известного науке с первой половины XIX века, заключается в том, что тепловая энергия, выделяемая с поверхности Земли, нагретой солнечной энергией (50% солнечной энергии поглощается Землей), поглощается атмосферным воздухом и «парниковыми газами», образуя прослойку, играющую роль своеобразной «полиэтиленовой оболочки». В результате, температура поверхности Земли и океанов постепенно повышается, а ледяные массивы Арктики, Гренландии и некоторых крупных горных ледников деградируют. Выяснилось, что с 1899 по 2007 годы температура атмосферы Земли увеличилась более чем на 1°C, а температура воды в океане - на 0,8°C. Такое глобальное изменение климата сопровождается рядом серьезных катаклизмов на планете: повышением уровня моря и океана, наводнениями, штормами, ураганами, проливными дождями, сильной жарой, засухой, лесными пожарами, опустыниванием, а в некоторых местах образованием болот, формирующихся в результате стихийных бедствий. Наблюдения показывают, что повышение температуры океана на 1,0–1,5°C приводит к быстрому сокращению численности ряда океанических видов, в том числе, некоторых видов рыб. Прогнозируется, что к концу XXI века уровень воды в океане поднимется на 30-45 см. Это означает, прежде всего, затопление больших территорий - ряда островов и островных стран, крупнейших прибрежных городов мира-, деградацию сельского хозяйства, угрозу нехватки продовольствия. К экономическим последствиям стихийных бедствий в результате глобального потепления можно отнести разрушение домов из-за затопления прибрежных территорий, нехватку питьевой воды, ухудшение условий жизни населения, сопровождающееся выходом из строя инженерных сооружений и инфраструктуры. Подобные природные явления уже регистрируются в нашей стране (в последние годы из-за подъема уровня реки Кура и грунтовых вод в регионах, повреждается имеющаяся там инфраструктура, а также инфраструктура, расположенная вдоль некоторых горных рек, возникает опасность схождения оползней и т. д.). Усиление глобального изменения климата увеличивает ожидаемый риск таких событий. Следовательно, должен быть предел воздействия человеческой деятельности на окружающую среду. Глобальное потепление на 2°C следует рассматривать как нежелательный предел. Если к середине века температура воздуха увеличится на 2°C, то от нехватки питьевой воды будут страдать 500 миллионов человек, при 3°C их число достигнет 3 миллиардов. Инициатива Межакадемического партнерства (IAP) по решению проблемы воздействия изменения климата на здоровье человека является достойным научным почином. Последствие глобального изменения климата и, в

частности, его влияние на здоровье человека уже отмечено во всех странах мира и проявляется в различных формах. Исследования Всемирной организации здравоохранения (ВОЗ) в 53 странах европейского региона показывают, что прямым последствием изменения климата, включающего аномально высокие или низкие температуры, наводнения, штормы и лесные пожары, является увеличение количества злокачественных опухолей, психических расстройств, травм и летального исхода. По данным ВОЗ, изменение климата в настоящее время вызывает более 150 000 преждевременных смертей ежегодно (не считая прогнозируемых). При потеплении климата, увеличивается количество возбудителей инфекционных и паразитарных заболеваний (острые кишечные инфекции, вирусные гепатиты, геморрагическая лихорадка и др.), одновременно с этим расширяется ареал некоторых природных очагов инфекции (клещевой энцефалит, кожный лейшманиоз, малярия и т. д). Более того, распространение COVID-19 также связано с ослаблением иммунной системы. Таким образом, миру нужна декарбонизация мировой экономики и изменение финансовой мощи с серой на зеленую, чтобы стимулировать жизнестойкость людей и живых сообществ и обеспечить безопасную и здоровую окружающую среду для будущих поколений.

Ключевые слова: *Адаптация, болезни, глобальное потепление, декарбонизация, здоровье, изменение климата, продовольственная безопасность, сельское хозяйство, смягчение последствий, COVİD-19*

Journal of Life Sciences & Biomedicine, vol. 3(76), No 1, p. 17-23 (2021)

Comparative studies on genome organization and evolution of some fish and crustacean species

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Received: October 22, 2020; Received in revised form: March 18, 2021; Accepted: April 05, 2021

Cellular functions are carried out by complexes of coordinately functioning proteins. Understanding genome organization and gene functions in diverse organisms can reveal new insights into the evolution of the coordinated gene expression mechanisms. It is suggested that gene sets of different species may be mostly similar, while regulatory mechanisms of the gene expression are expected to be evolved in the species-specific manner. In this work, the genome-wide peculiarities of organization, transcription and evolution of 5 fish and 4 crustacean species were explored. The interspecies BLAST comparison of annotated protein sets revealed that inter-species protein diversity of crustaceans varies in much wider range. Moreover, in some cases, comparing with the crustaceans-crustaceans homology, the crustaceans-fish protein conservation seems to be higher. A search for possible traces of the mitochondrial DNA (mtDNA) in the nuclear genome of 8 crustacean and fish species discovered that only one crustacean and one fish species (*Armadillidium vulgare* and *Carassius auratus*) have quite long (≥500 bp) insertions of mtDNA in the nuclear genome, including the almost complete insertion of the organelle DNA in the *C. auratus* nuclear genome. Exploring the promoter architecture of 8 crustacean and fish nuclear genes revealed that (1) most of protein genes have, at least, one putative bidirectional promoter, and (2) hundreds of genes in these genomes are organized closely in the Head-to-Head manner with a potential BDP between them. It is concluded that BDPs may play a key role in the coordinated transcription of the crustacean and fish genes involved in the same cellular processes.

Keywords: *Fish, crustaceans, mtDNA, bidirectional promoter, Head-to-Head genes*

INTORDUCTION

Our current knowledge suggests that the aquatic vertebrates have been evolved from heavy benthic microphages to floating, mobile, and omnivorous. Vertebrates including the first fishes were probably originated about 530 million years ago during the Cambrian explosion. The evolution of fishes seems to occur in freshwater, while crustaceans mostly evolved in marine habitats (Wagele, 1992). The bony fish species (*Osteichthyes*) with about 27 000 living species represent more than 50% of all known vertebrate species (Spaink et al., 2013). Crustaceans (crabs, lobsters, crayfish, shrimps, prawns, krill, woodlice and barnacles) diversified over the 455 million years ago form a large, diverse arthropod taxon. To date, 67,000 crustacean species have been described. Decapods (crabs, shrimp and lobsters), the most definitely recognizable crustaceans, include over 15 000 living and 3000 fossil species from 233 families (Wolfe et al., 2019; Stillman et al., 2008).

Cellular functions are carried out by complexes of coordinately functioning proteins. Therefore, the evolution of closely related species could involve the coordinately gains or losses of such gene groups.

https://doi.org/10.29228/jlsb.2
Available online 30 June 2021

Therefore, understanding genome organization and gene functions via comparative genomic and proteomic studies in extremely diverse organisms can reveal new insights into the evolution of the coordinated gene expression mechanisms (Martin and Fraser, 2018). In particular, it is supposed that gene (protein and non-coding RNA) sets of different species may be mostly similar, while regulatory mechanisms of the gene expression are expected to be evolved in the species-specific manner. The comparative genomic studies are very important for both fundamental science and practical use (the development of new medicines, sustainable aquaculture strategies, etc). In the last years, the great advances in genomic studies of bony fish species and some crustaceans were achieved (Spaink et al., 2013; Martin and Fraser, 2018). To date, the nuclear genomes of about 300 fish and over 10 crustacean species have been sequenced (https://www.ncbi.nlm.nih.gov/genome/?term=animals).

In general, all known eukaryotic genetic systems consist of the nuclear genome and semi-autonomous mitochondrial genome; plants have also the plastid genome. Mitochondrial functions were conserved almost in all eukaryotes studied and it supposed that these organelles are of the endosymbiotc α-proteobacterial origin. The mitochondrial genome encodes only about 10% its proteins and most of mitochondrial functions are encoded in the nuclear genome, synthesized in the cytosole and transported to the organelles. In comparison with plants, animals have smaller mitochondrial genome (Adams et al., 2002; Burger et al., 2003; Herrmann et al., 2003). The current findings suggest that during the evolution most of the organellar genes were transferred to the nucleus and, to date, such a transfer process was discovered mostly in plants (Shahmuradov et al., 2003; Barbrook et al., 2006; Noutsos et al., 2007). Moreover, the organelle-to-nucleus gene transfer seems to be presently continued. Presently (Sheppard et al 2008).

Transcription is the first, decisive phase of the genome expression. Genome transcription (when, where and how) is regulated by RNA polymerases, transcription factors and promoters. Protein coding genes are transcribed by RNA Polymerase II (Pol II; Solovyev et al., 2010; Danino et al., 2015). Previously, it was thought that promoters are unidirectional: they can initiate transcription only on a single strand of DNA. But, recently it was revealed that a single promoter can initiate transcription in both directions (Wei et al., 2011; Duttke et al., 2015; Bagchi and Iyer, 2016; Weingarten-Gabbay et al., 2019). Moreover, it was found that most genes are transcribed from alternative promoters. Using alternative promoters is regulated in a cell/tissue-specific manner, depending on the development stage and/or environmental signals. The alternative transcription initiation seems to be one of main principles of the RNA metabolism (Chen et al., 2016).

In this work, the genome-wide peculiarities of organization, transcription and evolution of some fish and crustacean species were explored. Results of these studies are presented below.

MATERIALS AND METHODS

For the inter-species comparison of protein sets, promoter studies and search for possible splinters of mitochondrial DNA (mtDNA) in the nucleus 4 crustacean (*Armadillidium vulgare*, *Eurytemora affinis*, *Hyalella azteca* and *Daphnia pulex*) and 5 fish species (*Carassius auratus*, *Neolamprologus brichardi*, *Oryzias latipes*, *Salmo salar* and *Cyprinus carpio*) with sequenced and annotated nuclear genome were selected (https://www.ncbi.nlm.nih.gov/genome/browse#!/overview/).

The pairwise comparison of DNA and protein sequences was performed by **BLAST** package (Altschul et al., 1997) and **BLAN** computer program (I.Shahmuradov, unpublished). To search for the nuclear copies of the mtDNA sequences based on the BLAST comparison of nuclear and mitochondrial genomes, we applied the **TRANSFER** computer program (I.Shahmuradov, unpublished)

Search for bi-directional Pol II promoters (BDPs) and exploration of the mutual location of neighbor protein genes in a genome was done by **TSShm** and **BDPGfinder** computer programs (I. Shahmuradov, unpublished).

Table 1. Summary of the reciprocal interspecies BLAST comparisons of all annotated proteins from 4 crustacean and 5 fish species

	Avu^{Cr}	**Eaf^{Cr}**	**Haz^{Cr}**	**Dpu^{Cr}**	**Cca^{Fish}**	**Cau^{Fish}**	**Nbr^{Fish}**	**Ola^{Fish}**	**Ssa^{Fish}**
Avu^{Cr}, 19051*		199/246	430/437	326/356	224/652	221/990	212/332	193/399	230/857
Eaf^{Cr}, 30425*	246/199		277/229	492/400	342/733	269/1341	242/388	226/532	291/1037
Haz^{Cr}, 22749*	437/430	229/277		415/407	248/693	217/1211	215/365	198/503	249/890
Dpu^{Cr}, 30611*	356/326	400/492	407/415		418/1257	392/2225	372/750	350/997	403/2166
Cca^{Fish}, 63928*	652/224	733/342	693/248	1257/418		43432/69140	11469/8768	11115/12174	1578/29117
Cau^{Fish}, 96703*	990/221	1341/269	1211/217	2225/392	69140/43432		17985/7352	16930/9402	22184/15784
Nbr^{Fish}, 31372*	332/212	388/242	365/215	750/372	8768/11469	7352/17985		12284/15342	9884/21312
Ola^{Fish}, 44766*	399/193	532/226	503/198	997/350	12174/11115	9402/16930	15342/12284		12664/19639
Ssa^{Fish}, 97555*	857/230	1037/1037	890/249	2166/403	29117/15782	15784/22184	21312/9884	19639/12664	

Species selected for pairwise BLAST comparison: **Avu** – *A. vulgare*, **Eaf** – *E. affinis*, **Haz** – *H. azteca*, **Dpu** – *D. pulex*, **Cca** – *C. carpio*, **Cau** – *C. auratus*, **Nbr** – *N. brichardi*, **Ola** – *O. latipes*, **Ssa** – *S. salar*. **Cr**: crustaceans. Full-length homology level: ≥80%. ***** – number of analyzed protein sequences for every species

The **TSShm** program searches for CpG, non-CpG /TATA and non-CpG/TATA-less promoters in animal DNA sequences. Depending on the promoter class, the TSShm has the highest prediction accuracy among analogous methods (90-98%). The **BDPGfinder** program identifies the close H2H gene pairs that may be associated with BDP(s) by analyzing the genome-wide TSShm results and genome annotation files in the GenBank format. Hereinafter, a DNA region with a pair of TSSs on the opposite strands of DNA and at distance less than 300 bp is termed as BDP.

RESULTS AND DISCUSSION

To study trends in evolution of fishes and crustaceans, we performed the pairwise interspecies BLAST comparison of all annotated to date proteins from 4 crustacean and 5 fish species, including crustaceans *A. vulgare* (19051 proteins), *E. affinis* (30425), *H. azteca* (22749) and *D.pulex* (30611), fishes *C. auratus* (96703), *C. carpio* (63928), *N.* (31372), *O. latipes* (44766) and *S. salar* (97555). Results of the analysis are summarized in the Table 1. Although thousands of proteins from 5 fish species show the full-length high (≥80%) level evolutionary conservation, the inter-species protein diversity of crustaceans seems to be high. Moreover, in some cases, comparing with the crustaceans-crustaceans homology, the crustaceans-fish protein conservation seems to be higher (see Table 1: marked in grey). The biological relevance of these findings remains to be further investigated.

Further, we performed a search for possible traces of the mtDNA in the nuclear genome of 4 crustacean (*A. vulgare, E. affinis, H. azteca* and *D. pulex*) and 4 fish species (*C. auratus, N. brichardi, S. salar* and *C. carpio*). For this purpose, an intra-species **BLAST** comparison of the DNA sequences of the nuclear and mitochondrial genomes for each species was performed, and the obtained BLAST results were analyzed using **BLAN** and **transfer** programs. Contrary to the previously discovered facts on higher plants (Adams et al. 2002; Shahmuradov et al. 2003; Shahmuradov et al., 2010), it was revealed that only one crustacean (*A. vulgare*) and one fish (*C. auratus*) species have long (≥500 bp) insertions of mtDNA in the nuclear genome (Table 2).

Table 2. mtDNA insertions in the nuclear genomes of analyzed crustacean and fish species

Organism	**Length of mitochondrial genome, bp**	**Length of the mtDNA insertion, bp**
A.vulgare	13939	2545 1168
C.auratus	16580	15658

In particular, for the first time, almost complete insertion of the mtDNA was found in the fish nuclear genome. The results of this study and previous studies on the existence of the striking differences in the number and total length of DNA sequences of mitochondrial origin in the nuclear genomes suggest

that the Mitochondrion-to-Nucleus transfer in crustacean and fish species occurred after these species separated from a common ancestor.

In any genome, the neighbor genes may be organized in Tail-to-Head (T2H) or Tail-to-Tail (T2T) or Head-to-Head (H2H) manner (Fig. 1). In particular, the non-stop transcription of the closely located T2H neighbor genes may produce chimeric transcripts (proteins). The closely located H2H genes might coordinately transcribed from the BDP between them. The H2H fashion of location of close gene pairs seems to be very important in a sense of the coordinated transcription (expression) of genes via the BDP(s).

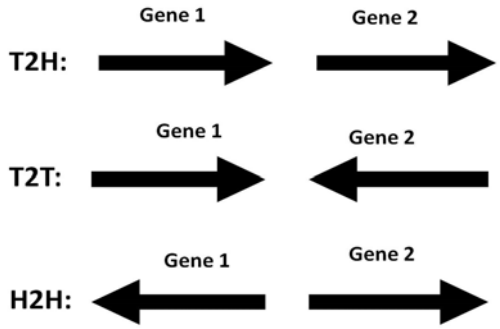

Figure 1. Schematic presentation of the T2H, T2T and H2H genes.

In this study, for 3 crustacean (*A. vulgare, E. affinis* and *H. azteca*) and 5 fish (*C. carpio, C. auratus, N. brichardi, O. latipes* and *S. salar*) species, we performed (1) the genome-wide analysis of the mutual location of the neighbor genes, (2) a search for putative BDPs for all annotated protein genes and (3) an identification of potential H2H gene pairs with BDP between them. Results of these studies are summarized in Table 3. Initially, using the **TSShm** tool, we performed search for CpG, non-CpG/TATA and non-CpG/TATA-less promoters in [-1000:+100] regions (+1 corresponds to the gene start) of these genes. Then, applying the **BDPGfinder** tool, we explored the putative BDPs for every gene analyzed. At least, 1 putative BDP was identified for 73-83% of the protein genes in 8 species (see Table 3) where most of these promoters belong to the CpG class (data not shown).

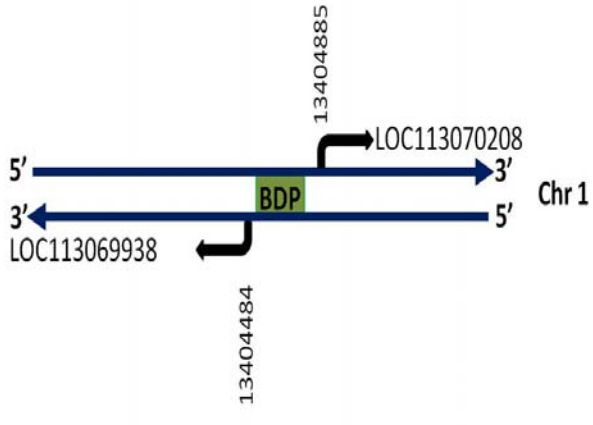

Figure 2. Bi-directional CpG-island promoter between neighbor genes LOC113069938 and LOC113070208 located on the opposite strands of the chromosome 1 of *C. auratus* (gold fish). These genes encode succinate-CoA ligase [ADP/GDP-forming] subunit alpha (mitochondrial; protein ID: XP_026098902.1) and CDGSH iron-sulfur domain-containing protein 2-like (protein ID: XP_026099249.1), respectively. Inter-genes distance – 401 bp, inter-TSSs distance - 196 bp.

Table 3. Some peculiarities of the genomic organization and promoter architecture of protein coding genes in 3 crustacean and 5 fish species

	T2H	T2T	H2H	BDPs	BDPGs
Avu^{Cr}, 6152*	1975 /55 ^{a}/0^{b}	1073/20 ^{a}/58^{b}	739/18^{a}/66^{b}	5121; 83%	13
Eaf^{Cr}, 19743*	11504/615 ^{a}/676^{b}	3337/105 ^{a}/206^{b}	3339/341 ^{a}/555^{b}	14935; 76%	300
Haz^{Cr}, 17842*	7890/408 ^{a}/179^{b}	3510/397 ^{a}/238^{b}	3003/309 ^{a}/140^{b}	14425; 81%	303
Cca^{Fish}, 24424*	12986/272 ^{a}/507^{b}	5720/161 ^{a}/279^{b}	5718/637 ^{a}/592^{b}	18785; 77%	550
Cau^{Fish}, 39645*	21063/700 ^{a}/375^{b}	9311/701 ^{a}/755^{b}	9293/1671 ^{a}/960^{b}	3096; 78%	1416
Nbr^{Fish}, 18486*	9298/157 ^{a}/148^{b}	4310/328 ^{a}/195^{b}	4315/717 ^{a}/312^{b}	14117; 76%	599
Ola^{Fish}, 22040*	11390/496/204^{b}	5284/405 ^{a}/613^{b}	5340/997 ^{a}/831^{b}	17331; 79%	883
Ssa^{Fish}, 6719*	3517/124 ^{a}/78^{b}	1610/91 ^{a}/121^{b}	1606/224 ^{a}/230^{b}	4904; 73%	175

* – number of analyzed protein genes for every species. ^{a} – pairs of genes located at distance of ≤1000 bp and ≥50 bp; ^{b} – number of overlapping gene pairs. The designation of the species is the same as in table 1.

At last, H2H pairs of the neighbor and non-overlapping genes at distance from 50 bp to 1000 bp were investigated for an existence of putative BDP(s) between them. It was found that the number of H2H genes with shared BDP varies in quite diapason in the crustacean and fish species explored (Table 3).

In particular, excepting the S. salar, all fish species are significantly enriched in H2H pairs with putative BDP. An example of the adjacent H2H pair with a putative BDP between them is illustrated in Fig. 2. Summarizing these results, it can be concluded that BDPs may play a key role of in the coordinated transcription of genes involved in the same cellular processes.

ACKNOWLEDGEMENTS

This work was supported by Science and Technology Center of Ukraine, (STCU) Project #6417 ("Molecular-genetic studies of the contamination effects on some animal species in the Caspian Sea") and partially by Azerbaijan National Academy of Sciences.

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Bəzi balıq və xərçənq növlərinin genomlarının təşkili və təkamülünün müqayisəli tədqiqi

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Hüceyrə funksiyaları razılaşdırılmış şəkildə işləyən zülal kompleksləri tərəfindən həyata keçirilir. Müxtəlif orqanizmlərdə genomun təşkilini və gen funksiyalarını aydınlaşdırılmaqla genlərin razılaşdırılmış expressiyası müxanizmlərinin takamülündə yeni məqamları aşkar etməyə imkan verə bilər. Güman olunur ki, müxtəlif növlərdə gen dəstlərinin çoxu oxşar ola bilər, lakin gen ekspressiyasının tənizmlənmə mexanizmləri növ-spesifik yönümdə təkamül edir. Bu işdə 5 balıq və 4 xərçəng növündə genomun təşkili, transkripsiyası və təkamül xüsusiyyətləri araşdırılmışdır. Tədqiq olunmuş xərçəngkimilər və balıqların annotasiya olunmuş zülal dəstlərinin növlərarası BLAST müqayisəsi xərçənglərdə zülalların müxtəlifliyinin daha geniş diapazonda dəyişdiyini aşkar etmişdir. Üstəlik, bəzi hallarda, xərçəng-xərçəng oxşarlığı ilə müqayisədə, xərçəng-balıq zülallarının konservativlik dərəcəsi daha yüksəkdir. 8 xərçəngkimilər və balıq növlərinin nüvə genomunda mitoxndri DNT-sinin (mtDNT) izlərinin axtarışı göstərmişdir ki, yalnız bir xərçəng və bir balıq növünün (*Armadillidium vulgare* və *Carassius auratus*) nüvə genomunda mtDNT-sinin uzun (≥500 nc) insersiyaları mövcuddur. O cümlədən, *C. auratus* növünün nüvə genomunda organella DNT-sinin, demək olar ki, bütöv insersiyası vardır. 8 xərçəng və balıq növünün nüvə genlərinin promotor arxitekturasını araşdırarkən, (1) zülal genlərinin çoxunun, ən azı, bir ikiistiqamətli promotorunun (İİP) olduğu və (2) bu genomlarda yüzlərlə cüt genlərin yaxın qonşuluqda Baş-Başa yerləşmişləri və onların arasında potensial İİP mövcuddur. Belə bir nəticəyə gəlinmişdir ki, İİP-lar xərçəng və balıqlarda eyni hüceyrə prosesində iştirak edən genlərinin razılaşdırılmış transkripsiyasında mühüm rol oynaya bilər.

Açar sözlər: *Balıq, xərçəngkimilər, mtDNT, ikitərəfli promoter, baş-başa genlər*

Сравнительные исследования организации генома и эволюции некоторых видов рыб и ракообразных

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Функции клетки осуществляются благодаря согласованной работе комплексов функционирующих белков. Уточнение организации генома и функций генов у различных организмов создаст возможность для выявления новых аспектов в понимании эволюции механизмов скоординированной экспрессии генов. Предполагается, что наборы генов разных видов могут быть в основном похожими, в то время как, механизмы регуляции экспрессии генов, как ожидается, будут развиваться видоспецифичным образом. В данной работе исследованы полногеномные особенности организации, транскрипции и эволюции 5 видов рыб и 4 видов ракообразных. Межвидовое сравнение аннотированных наборов белков с помощью BLAST показало, что межвидовое белковое разнообразие ракообразных варьирует в гораздо более широком диапазоне. Более того, в некоторых случаях, по сравнению с гомологией ракообразные-ракообразные, консервация белка ракообразные-рыба оказывается более высокой. Поиск возможных следов митохондриальной ДНК (мтДНК) в ядерном геноме 8 видов ракообразных и рыб показал, что только у одного вида ракообразных и одного вида рыб (*Armadillidium vulgare* и *Carassius auratus*) довольно длинные (≥500 п.н.) вставки мтДНК в ядерный геном, включая почти полную вставку ДНК органелл в ядерный геном *C. auratus*. Изучение промоторной архитектуры 8 ядерных генов ракообразных и рыб показало, что (1) большинство генов белков имеют, по крайней мере, один предполагаемый двунаправленный промотор, (2) сотни генов в этих геномах организованы близко друг к другу в манере «голова к голове» с потенциальным двунаправленным промотором (ДНП) между ними. Сделано заключение, что ДНП могут играть ключевую роль в координированной транскрипции генов ракообразных и рыб, участвующих в одних и тех же клеточных процессах.

Ключевые слова: *Рыба, ракообразные, мтДНК, двунаправленный промотор, голова-к-голове гены*

Journal of Life Sciences & Biomedicine, vol. 3(76), No 1, p. 24-31 (2021)

The possibility and prospect of breeding wild silkworm of the Giant Peacock moth (*Saturnia pyri, Denis & Schiffermüller,* 1775), as a new branch of sericulture in Azerbaijan

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Received: December 10, 2020; Received in revised form: March 31, 2021; Accepted: April 06, 2021

It is known that in modern times there is a great demand for wild silkworm silk and its products, especially in the field of biomedicine and nanotechnology, among domestic and foreign consumers. The presence of large areas of various forests and favorable conditions in Azerbaijan provides great opportunities for the cultivation of wild silkworms of the *Saturniidae* family for industrial purposes. In this regard, there is a need for a detailed study of the productivity of the breed, which actually lives in the green spaces of our country, i.e., the study of its biological and technological parameters in laboratory conditions. The article describes breeding experience of the Giant Peacock moth (lat. *Saturnia pyri*) in laboratory conditions at constant temperature (20-25°C) and humidity (80-85%) on the leaves of cherry plant (*Prunus avium* L., 1755). The prospects and possibilities for the future expanded cultivation and production of specific silk, which will be the first in Azerbaijan and the entire Transcaucasia, have also been assessed.

***Keywords**: Wild silkworm, Saturnia pyri eggs, silkworm hatching, silkworm molting, cocoon, sericulture*

INTRODUCTION

Within the framework of the state program for the development of silk industry, specialists from our Sheki Regional Scientific Center of the National Academy of Sciences of Azerbaijan participated in various scientific events, analyzed the latest research articles to study methods for the improvement of sericulture and searched for methods to restore the former glory of Azerbaijani silk. In the articles of famous foreign scientists as well as young researchers, works devoted to non-traditional wild species of silkworms, about the aspects of using their silk in biomedicine, in smart and nano technologies were most frequently mentioned (Volova and et al., 2009),(Vepari and Kaplan, 2007), (Kasoju and et al., 2009).

Based on this, we began a detailed study of the possibility of breeding and obtaining silk of a local species of wild moth in laboratory conditions. Giant peacock moth, Bombyx pyri or Saturnia pyri - insects of the order Lepidoptera, family Saturniidae, genus Saturnia as a wild species that actually lives on the territory of our country (Efendi, 1971), became the object of our research. Although quite common in Azerbaijan, this species is very rare in Europe, for example, included in the Red Book of Ukraine and a number of southern regions of Russia (Shapoval, 2010) This moth has got its name due to the peculiarities of its colors: on each wing of the moth there is a disc-shaped ocular spot, similar to the spots on the tail of a peacock. *S. pyri* belongs to one of the largest species among all lepidopteran insects in Azerbaijan, and in the common folk this moth is called Butterfly-Soul (azerb. Ruh kəpənəyi), probably due to its large size and due to the fact that it mainly flies at night.

https://doi.org/10.29228/jlsb.3
Available online 30 June 2021

LABORATORY RESEARCH

We were able to find a pair of mated Giant Peacock moths at night from May 28 to May 29, 2019 in the mountain village of Bash Shabalyd (azerb. Baş Şabalıd) in the Sheki district. These two moths were captured with a light trap and were carefully transferred into a cardboard box covered with gauze (Kumar and Shukurova, 2018). After fertilization, the male was taken away for further research, and the female was inactive. On May 30, 2019, the fertilized female started to lay the first group of eggs which consisted of 6 eggs, this process lasted until June 3, 2019 when the total number of eggs was 63. On June 4, the female moth died and was withdrawn for a detailed examination.

RESULTS OF VISUAL EXAMINATION OF MOTHS

Female *Saturnia pyri:* the length of body was 40 mm, the length of forewing – 85 mm and the wingspan – 170 mm. The body of the male was a bit larger – 45 mm, the length of forewing – 70 mm and the wingspan – 150 mm (Fig. 1). The inner (basal) part of the forewings of both moths is dark brown but the outer part is light grey, and both parts of the hindwings are light grey. There are dark straight inner lanes and light brown unevenly serrated outer lanes which divide all the wings into unequal transverse parts.

Between the lanes on each wing there is a large eyespot of a dark blue color. On the upper part of the eyespot the hair scales sharply decrease – this small part is transparent, above that there is a slightly wide rufous (reddish brown) strip in the shape of a crescent, then there is a white crescent above it and another rufous thin one on the top of that. On the bottom of the eyespot there is a light brown crescent. Finally, the outermost border of the whole oval is a black line (Zolotarev and et al., 1940).

A light stripe runs along the edge of the wings, which gradually becomes slightly darker (cream color) towards the border of the wings. At the base of the wing there is a wide black strip that stretches to the apex, then breaks off. The body is massive, covered in thick hairs, divided into three parts: head, thorax and abdomen.

There are eyes, antennae and mouth on the head (Fig. 2 and 3). The antennae are bipectinate and have a feather-like shape, they are composed of segments with long slender lateral processes on both sides. The processes on the antennae of female moth are thinner and have denticles, on the contrary, the processes of male are quite conspicuous, and they resemble a feather. Therefore, the female is easily distinguished from the male due to the structure of the antennae. However, the length of the antennae of both moths was almost the same. (Symonds et al., 2012).

Fig. 1. Female *Saturnia pyri*; wings in a wide (1) and seated position (2).

The organs of vision are located on the both sides of the head – two large dark compound eyes, each of which consists of many separate simple eyes – ommatidia. The mouthparts of the moths are underdeveloped (Mikhailov and Gershenson, 1958).

The thorax of *Saturnia pyri* consists of three segments, fused together: prothorax, mesothorax,

and metathorax. Each of these segments has a pair of jointed legs that are covered in scales; the legs end up with small paws. There are claws on the paws which enable the moth to cling to the surfaces (Fig. 4).

Fig. 2. A specific eyespot on the wings.

Fig. 3. *Saturnia pyri* antennas: male (1) female (2).

Fig. 4. Corpus (1) and leg (2).

The mesothorax and metathorax carry a pair of well-developed wings which are made up of two chitinous layers, the veins that pass through the wings support them by providing mechanical strength.

The abdomen is multi-segmented, it consists of nine segments linked together by soft flexible tissues. It should be mentioned that the abdomen of male looked thicker because the segments were more closely linked and the scales were longer which gave an additional volume, also the striped color of abdomen was more distinguished. On the contrary, the abdomen of female was more elongated. At the end of the abdomen are the anus and external genitalia. The end of the abdomen of female is blunt and is covered in hairs. The end of the male abdomen is conical and has genital appendages with setae. So, this is their distinguishing feature.

Moreover, several moth eggs of different days from different fragments were also taken away for the examination. The eggs were examined for 10 days, right up to their hatching.

RESULTS OF EGG EXAMINATION

The eggs of *S. pyri* are oval, slightly flattened, they have an average length of 2.5 mm and an average width of 1.5 mm (Fig. 5). Due to the small amount of eggs, they were weighed in groups and it was possible to approximately calculate the mass of one egg (6 mg) that appeared in the first egg-laying, the egg weight in all subsequent egg-laying was approximately 4 mg.

Fig. 5. Eggs after laying (1) and hatching (2).

Upon a detailed examination, the eggs have a greenish-white chitinous shell but the coating substance which glues the eggs to each other is greyish-brown, so that is why the eggs are greyish-brown in appearance. It was seen under the microscope that

the eggshells were penetrated by many filamentous thin tubules where the gas exchange was likely to occur, also there was a point hole (micropyle) through which the sperm penetrated, and the egg was fertilized. The egg was filled with greenish semi-liquid contents – egg white. The color of the egg changes and becomes lighter with the development of the embryo because a transparent yolk membrane emerges under the shell, and eventually a third serous membrane appears under it. After hatching, the eggs are empty, dry and have a corroded pole of the shell.

The development of the eggs of *Saturnia pyri* began immediately after the egg laying and did not have a resting stage (diapause). We tried to work without tactile contact and sudden movements with the experimental subjects during our experiments on growing *S. pyri.* The first 4 days after egg laying – during the period called embryo overturning (blast kinesis), the eggs were kept at the room temperature (22-24°C). In the evening of June 3, the eggs in a plastic box with good ventilation and protection from other insects were placed in the garden, in a specially designated self-made structure – in a mini greenhouse, where the natural conditions for incubation were created in the open air. Our mini greenhouse was placed in the shade where the air temperature was 24-26°C, it could reach up to 30° C in the afternoon. In order to maintain the microclimate inside the greenhouse, we daily placed a fresh branch of cherries or pears and a 30 ml container with water. Air was well accessible to the eggs throughout the incubation period. All the time, the eggs remained perfectly still on that paper surface where they were laid by the moth (Sinitsky et al., 1952).

Age I. The first hatching took place on June 8, 2019 and this process lasted 3 days, so the caterpillars in the greenhouse were of different ages during the whole control time. The approximate number of hatched and viable ones: 50/63. The larvae, that hatched on the first two days (the majority) were about 6-7 mm long and weighed about 4 mg, while the ones hatched on the next day were 5-6 mm long (Fig. 6)

At the first age, the caterpillar was black and had four rows of warts where long light brown setae protrude. The branches of elm, oak, plum, cherry and pear were offered as food sources; the young shoots of cherry turned out to be optimal for them, but they also fed on elm, pear and plum branches (Klimiashvili- Nutsibidze, 1952).

From June 12 to June 15, the sleep of caterpillars was noted – this is a stationary state in which the front part of the caterpillar's body is raised, the old chitin capsule is sliding from the head and during this period which lasted 1-2 days, the feeding stops. It should be noted that further feeding was continued in the laboratory in a portable small greenhouse.

Fig. 6. *Saturnia pyri* at the first age (5-7 mm).

Age II. The first molting occurred from June 14 to June 16: the caterpillars achieved a gap on the dorsal side of the old skin by making wavy movements. Caterpillars of the second age had changed their body color to taupe, the warts throughout their body became orange and more conspicuous, and their head and anal segments acquired a brown color (Fig. 7).

Fig. 7. *Saturnia pyri* at the second age (26-30 mm).

About 1-2 hours after the molting, the caterpillars actively began to feed on young branches of

cherries and plums. They excreted a lot of granular feces. By the end of the second age, the length of caterpillars was ~ 26-30 mm. Since June 19, the activity of most of the caterpillars had stopped, they fell asleep.

Age III. The second molting took place on June 21 as a result of which the caterpillars partially shed the old skin and the old cranial capsule came off. Their new skin was light green with small black dots, yellow warts and quite long hairs. In addition, their head had solidified and hardened, so became a bit larger. In less than two and a half hours, the caterpillars started to eat again. The feeding continued with young branches of pears, cherries and plums. The length of the caterpillars by the end of this age reached ~ 35-40 mm (Fig. 8).

Fig. 8. *Saturnia pyri* at the third age (35-40 mm).

At that moment, there were caterpillars of two ages (II and III) in the experimental greenhouse. On June 23, 5 caterpillars of the third age were released on the plum tree for the control study. On June 26, the remaining caterpillars of the third age fell asleep and their sleep lasted two days.

Age IV. The third molting occurred from June 27 to June 28, 2019. During the fourth stage of development, the caterpillars acquired a green body color and sky blue warts with long hairs and spikes filled with liquid. Also, a long yellow stripe occurred on the side of the body. The length of the caterpillars was ~ 45-55 mm (Fig. 9). On July 3-5, the caterpillars of this stage stopped eating food, some of them strongly clung to the branches, others to the gauze that covered the box. They took a tense elongated shape; the color of their warts became slightly lilac.

Age V. From July 6-10, the old cranial capsule came off and the old skin was torn – so, the fourth molt had occurred. During this molt, three caterpillars could not completely get rid of the old skin since it did not fully tear on the back and caused the constriction of their body leading to their death.

Fig. 9. *Saturnia pyri* at the fourth age (45-55 mm).

The color of the caterpillars that passed to the fifth age gradually turned into yellowish-green, starting in July 12. Their massive legs and prolegs were clearly seen, the head width was about 9 mm and the labrum (upper lip) was quite large. Caterpillars grew rapidly, as they consumed a large amount of foliage: we provided them with 6-7 ripe branches of cherry (~30-35 cm in size) per day. It should be noted that the average length of their body at the fifth age was 90-100 mm, weight – 10.0-10.5 g (Fig. 10).

Another color change took place by July 16, right before pupation. The color of the caterpillars changed, starting from the head, and became yellowish orange, this time there was no molting. The caterpillars gradually stopped eating, their body became dull and sluggish, it felt like they shortened in length. They were moving anxiously and jerking the leaves, it was clear that they were looking for a convenient place to spin themselves a cocoon. As soon as they found such a place, they calmed down and began to excrete excrement and mucus, and then they started to spin their cocoons. The caterpillars began the process by pulling together several leaves around their body with the help of silk threads, thus forming a comfortable bed for themselves. Then, they weaved the inner surface of the pulled leaves and in 2 hours, the caterpillars literally were not visible.

Cocoon. There were several freshly spun cocoons in our laboratory on July 18 (Fig. 11). They were of an elongated oval shape (pear-shaped),

dark brown in color, with a diameter of ~ 22.7 mm and a length of ~54 mm. Their walls consisted of a loose outer shell and a dense inner shell which is formed by strongly glued loops of silk thread. In the future, we will continue experiments to determine the structure and shape of the threads, to find out the causes of some subjective facts.

Fig. 10. *Saturnia pyri* at the fifth age (90-100 mm).

Fig. 11. Cocoon (90-100 mm).

Controlled Silkworms: Out of 3 silkworms of the third age that were transplanted to the plum tree on June 23, just one specimen was found on July 6 (Fig. 12). That caterpillar was reddish green in color, quite large in size, its length was ~ 10.5 cm. On that day, it was going down the trunk of the tree, probably in search of a place to pupate. To protect it, the caterpillar was moved to a well-ventilated container with a dry plum branch.

2 hours later that evening, the body of the caterpillar contracted as it neither was eating nor excreting feces. It was clearly seen how a silk thread was secreted from a silk-secreting papilla, due to which the caterpillar was able to connect several leaves and began to weave the outer (main) part of the cocoon. In almost 4 hours, the caterpillar was no longer visible, only a dark brown, shaggy, pear-shaped cocoon was seen between the leaves.

Fig. 12. The control one (105 mm).

Examination of excrements: Excrements (feces) were in the form of prismatic hexagons with a star-shaped cross section. The size of the granule increased with the development of the caterpillar. The color of the excrements was dark blue green, they unfolded in warm water secreting a greenish tint and a specific smell.

CONCLUSIONS

In the article, we described the biological characteristics of *Saturnia pyri*, the period of growth and development of the caterpillar from hatching to pupation *in vitro*. Our experiment allows us to make the following conclusions:

1. It is possible to breed *Saturnia pyri* in laboratory conditions.
2. To provide food to the caterpillars, the leaves of cherry which grows well in our forests and gardens can be used.
3. The following feeding regimen must be observed, depending on the age of the caterpillars: at I – II ages, feeding must be 1-2 times a day, III age – 3 times a day, IV age – 4 times a day and V age – 5 times a day.
4. The optimal feeding rate by age per 100 caterpillars: I age 30-35 g, II age 180-200 g, III age 1000-1500 g, IV age 3500-4000 g, V age 14000 g.
5. To fully satisfy the stocks of fodder leaves for further mass breeding of this species, it is necessary to plant cherry plantations, which is very low-budget in the natural conditions of our country.
6. Throughout the entire feeding period, it is necessary to maintain the temperature within 20°-25° C, with a relative humidity of 80-85%.
7. The control caterpillar, which fed and grew in

the natural environment, had a shorter development cycle. The pupation process of this caterpillar was 10 days earlier than the experimental group.

8. To obtain the cocoons of good quality, the caterpillars should defecate for the last time before pupation. After that, they can be placed in separate paper bags with adequate ventilation, where they can weave their cocoons in a clean environment.
9. To maintain the best quality of the cocoons, they must be collected in 15 days after the beginning of the pupation.

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Vəhşi ipək qurdu olan armud saturniyasının (*Saturnia pyri, Denis & Schiffermüller,* 1775), Azərbaycanda ipəkçiliyin yeni bir qolu kimi yetişdirilməsi ehtimalı və perspektivləri

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Məlumdur ki, müasir dövürdə yerli və xarici istehlakçılar arasında, xüsusilə biotibb və nano texnologiyalar sahəsində, vəhşi (yabanı) ipəkqurdu ipəyinə və ondan hazırlanan məhsullara böyük tələbat var. Azərbaycanda geniş meşə ərazilərinin və əlverişli şəraitin olması *Saturnidae* ailəsinə aid vəhşi ipəkqurdularının sənaye məqsədləri üçün yetişdirilməsinə böyük imkanlar yaradır. Bununla əlaqədar olaraq, ölkəmizin yaşıl ərazilərində mövcud olan vəhşı ipəkqurdu növünün bioloji və texnoloji göstəricilərinin laboratoriya şəraitində araşdırılmasına, istehsal gücünün ətraflı öyrənilməsinə ehtiyac var. Məqalədə müəyyən temperatur (20-25°C) və nəmlik (80-85%) şəraitində, armud saturniyası (lat. *Saturnia pyri*) ipəkqurdunun gilas ağacı

(*Prunus avium* L., 1755) yarpaqları ilə laboratoriyada yetişdirilməsi təcrübəsi təsvir edilmişdir. Həmçinin, məqalədə ilk dəfə olaraq *Saturnia pyri* ipəkqurdunun və ondan alınan xüsusi ipəyin gələcəkdə Azərbaycanda və bütün Zaqafqaziya bölgəsində geniş istehsal mümkünlüyü və səmərəliliyi qiymətləndirilmişdir.

***Açar sözlər**: Vəhşi (yabanı) ipəkqurdu, Saturnia pyri yumurtaları, ipəkqurdunun yumurtadan çıxması, ipəkqurdunun qabıq dəyişməsi, barama, ipəkçilik*

Возможности и перспективы разведения дикого шелкопряда грушевой сатурнии (*Saturnia pyri, Denis & Schiffermüller,* 1775), как нового направления шелководства в Азербайджане

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Известно, что в настоящее время среди отечественных и зарубежных потребителей существует большой спрос на шёлк диких шелкопрядов, особенно в области биомедицины и нанотехнологий. Наличие больших лесных массивов и благоприятные условия Азербайджана создают большие возможности для выращивания в промышленных целях диких шелкопрядов, принадлежащих семейству *Saturnidae*. В связи с этим возникает необходимость изучения в лабораторных условиях биологических и технологических характеристик дикого шелкопряда, живущего в зеленых районах нашей страны, а также детального изучения его производственной мощности. В статье описывается опыт разведения грушевой сатурнии (лат. *Saturnia pyri*) в лабораторных условиях при постоянной температуре (20-25^{o}C) и влажности (80-85%) на листьях растения черешни (*Prunus avium* L., 1755). Также оценены возможности и перспективы расширенного выращивания грушевой сатурнии и производства в будущем «дикого» шелка, который будет первым в Азербайджане и во всем Закавказском регионе.

***Ключевые слова:** Дикий шелкопряд, яйца Saturnia pyri, вылупление шелкопряда, линька шелкопряда, кокон, шелководство*

Journal of Life Sciences & Biomedicine, vol. 3(76), No 1, p. 32-37 (2021)

The prescription of the vitamin D and biochemical tests in tumours of the female reproductive system

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Received: December 23, 2020; Received in revised form: March 17, 2021; Accepted: April 12, 2021

The results of a study conducted to compare the diagnostic role of vitamin D, calcium and phosphorus in patients with various tumours in the female reproductive system have been presented in this article. For the research, 50 women diagnosed with a tumour in the reproductive system were selected. The concentrations of vitamin D, parathormone (PTH), calcium and phosphorus were determined in all patients by immunoenzyme and biochemical methods. The concentration of vitamin D in patients with a malignant tumour was 2.2 fold higher than that in patients with a benign tumour. Moreover, the concentrations of the calcium and phosphorus in patients with a malignant tumour were significantly lower in comparison with the control group, while the concentration of PTH increased. Thus, these markers can be used for early diagnosis and screening of female genital tumours.

Keywords: *Cancer of the female reproductive system, vitamin D, parathormone, calcium, phosphorus*

INTRODUCTION

Vitamin D deficiency is widespread worldwide global public health problem. Determination of the level of vitamin D used not only in studies that are about prolonging life but also in other studies that confirm the presence of the pathological process.

Vitamin D plays an important role in the protective function of the immune system. Vitamin D deficiency is a serious health problem, which increases the risk of many diseases, as well as the risk of cancer formation. Statistics show that the formation of cancer in the female reproductive system is rapidly increasing among women (Siegel et al., 2017). Recently, large-scale studies show the role of vitamin D in the pathogenesis of certain types of oncological diseases, such as colorectal and lung cancer. Studies show that there is a link between a decrease in vitamin D concentration and an increase in the incidence of female genital cancer. Researches show, that calcitriol and other forms of vitamin D have a proapoptotic, antiproliferative, and antimetastatic effect on cancer tissue by altering the expression of many transcription factors of apoptosis and proliferation (Кобякова и др., 2015).

Recent investigations on the metabolism and biological effects of vitamin D bring to a radical change in the known information about its role and importance in the body (Захарова и др., 2013).

The hormonal system of active metabolites of vitamin D stimulates the synthesis and resorption of bone tissue (Lappe et al., 2004). During the sufficient concentration of vitamin D in the body, the calcium absorption reaches 30% in the intestine and 60-80% during the active growth of the child. Deficiency of vitamin D leads to a reduction of calcium absorption. Low levels of ionized calcium stimulate the secretion of parathyroid hormone, which in turn accelerates the reabsorption of calcium in the kidneys and its absorption from the small intestine (Turti et al., 2017; Golden and Abrams, 2014). Increased levels of parathyroid hormone have an adverse effect on the amount of phosphate in the blood, as it slows renal reabsorption, increases the loss of phosphorus in the urine, and as a result, its level in the blood decreases. Decreased levels of phosphorus and calcium in the body cause changes in bone mineralization (Dobnig et al., 2008; Maltsev et al., 2008). An elevated level of the parathyroid hormone considered an

https://doi.org/10.29228/jlsb.4
Available online 30 June 2021

early and very reliable indicator of vitamin D deficiency (Rastogi et al., 2013).

It should be noted that this hormone maintains the concertation of the calcium in the body and together with vitamin D metabolites the constant stabilization in the organism achieves. The study of phosphorus-calcium metabolism is not enough to assess the condition of vitamin D in the body, since the level of total calcium in the organism is stable. During Vitamin D deficiency and decreased intestinal absorption of calcium, bone resorption maintained for a long time, due to the parathyroid hormone.

In these studies, the effect of vitamin D and vitamin D receptors on endometrial, ovarian, cervical, vulvar, and vaginal cancers analyzed systematically.

Environmental studies show a positive correlation between vitamin D synthesis and decrease the risk for ovarian cancer (Gagel, 2006).

Vulvar cancer is the most common gynecological cancer, accounts for 3% to 5% of all genital cancer cases in women (Parkin et al., 2015). Taking into account the lymph node metastases among these patients, the recovery rate for five-year is 40% (Beller et al., 2006). Vitamin D receptor found in the non-pathological ovarian epithelium, as well as in ovarian tumours. It is important for ovarian function and affects the biosynthesis of estrogen receptors (Lurie et al., 2007). Vaginal carcinoma is a rare gynecological malignancy, accounts for only 1-2% (Parkin et al., 2015).

Preclinical and epidemiological evidence shows that the presence of vitamin D in the body reduces the risk of gynecological cancer [Holick, 2007; Walentowicz-Sadlecka et al., 2013).

Studies that investigate the effect of vitamin D and its receptor on tumour formation in the female reproductive system are few.

Studies show that women with a high level of vitamin D have a lower risk (15 to 25%) of the breast, colon, endometrial and rectal cancer. High level of vitamin D can increase the chances of surviving even after cancer diagnosticate (Reichrath et al., 1998). The metabolism of vitamin D in the body is closely linked with the levels of calcium, phosphorus and magnesium, as well as parathyroid hormone (PTH). PTH plays an important role in calcium-phosphorus metabolism by regulating the stable concentration of calcium and phosphorus in the extracellular fluid. Study of recent decades show that vitamin D deficiency may alter calcium-phosphorus metabolism and thus can lead to a number of degenerative changes in bone formation (Holick, 2007).

Vitamin D deficiency is now seen as a common problem in women and thus, there is a need to increase its level.

The aim of the study was to investigate vitamin D deficiency as well as calcium-phosphorus metabolism in women with various genital cancers.

MATERIALS AND METHODS

The fresh blood samples collected on a voluntary basis from 50 women diagnosed with genital cancer who had applied to the Oncology Clinic of the Azerbaijan Medical University and received treatment. The studied include 28 women diagnosed with uterine cancer, 25 with cervical cancer, and 7 with vulvar cancer. The diagnosis confirmed based on general instrumental examination methods and pathohistological opinion. The control group consisted of 20 healthy individuals.

Blood samples from case and control group taken to determine the concentration of vitamin D, calcium, PTH and phosphorus from the blood serum.

The concentration of vitamin D in blood serum was determined by immunoenzyme analysis with the reagent kit belonging to the company "Bioaktiva Diganostica". The concentration of calcium and phosphorus was analyzed by colorimetric method using "Human" kit.

Statistical analysis of the obtained results was calculated by the Student non-parametric method.

RESULTS AND DISSCUSION

The metabolic activity of vitamin D, regardless of the source of the vitamin (exogenous or endogenous) or its chemical form (cholecalciferol or ergocalciferol), achieved through a number of enzymatic pathways that differ from each other. Vitamin D enters the body through food (dairy products, eggs, fish and meat). In addition, under the influence of ultraviolet rays, vitamin D is synthesized from 7-hydroxy-cholesterol in the skin and

provides 90-95% of the body's need for vitamin D. Meanwhile, provitamin D undergoes enzymatic transformations in the liver and kidneys and converted into the active metabolite 1,25-dihydroxivitamin D3 (1,25 (OH)-D3). 25(OH)-D3 is stored in adipose tissue and is an accurate biomarker of the body's overall vitamin D status. Two major metabolites formed during the metabolism of 25(OH)-D3: 24.25-dihydroxycholecalciferol [24.25(OH)2-D3] or 25-dihydroxycholecalciferol [1.25(OH)2-D3] (Dobnig et al., 2008). The level of 25(OH)-D3 determined in the study.

The results show that concentration of vitamin D in control group varies from 11.5-39.8 nq/ml and in average reached to 25.82±0.29 ng/ml. In female genital cancer, the level of vitamin D varies between 5.80-24.60 ng/ml and in average reached to 11.51±0.18 ng/ml. According to statistics, level of vitamin D decrease in 2.2-fold (p<0.001) in patients with genital cancer (Figure).

Low levels of vitamin D caused by liver or kidney disease, taking certain medications, limited epidermal synthesis of cholecalciferol (living in low-radiation areas, low exposure to sunlight, etc.). Vitamin D and calcium metabolism is an important factor for bone health (Maltsev et al., 2012). The study showed that the concentration of PTH in female genital cancer increased by 32% (p<0.01) compared to the control group and in average reached to 29.6±0.5 pg/ml. The concentration of this hormone varies in the range of 14.8-48.9 pg/ml. Meanwhile, the concentration of PTH in the control group varies in the range of 13.5-24.6 pg/ml, and the average is 22.4±2.8 pg/ml. It is known that there is an inverse relationship between the secretion of PTH and the concetration of calcium in the blood. So, when calcium ions in the blood decrease, the secretion of the hormone increases. Vitamin D is an active regulator of calcium-phosphorus metabolism in the body. PTH maintains normal calcium levels in the blood, especially ionized calcium, by affecting the intestinal absorption function of calcium through the activated form of vitamin D (calcitriol) (Rastogi et al., 2013).

Vitamin D deficiency leads to serious disorders in calcium-phosphorus metabolism. Thus, vitamin D deficiency slows down the absorption of calcium from the intestine and disrupts the calcium-phosphorus metabolism in the body.

Significant change in concentration of potassium and phosphorus observed in the blood of patients with tumours in female reproductive system. Thus, the concentration of calcium in the blood serum of these patients is significantly reduced (22.9%) compared to the control group (p<0.01). The concentration of calcium in patients with cancer of the female reproductive organs is 7.98±0.16 mg/dl (control: 9.81± 0.16 mg/dl).

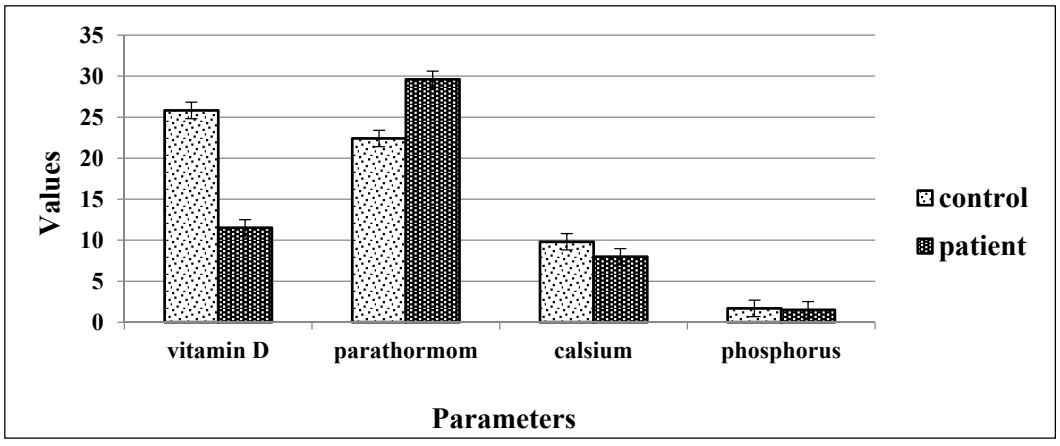

Fig. 1. Alteration of concentration of vitamin D and parathyroid hormone as well as calcium-phosphorus metabolism in patients with malignant tumors of the female reproductive system.

Table 1. Results of vitamin D, PTH and calcium-phosphorus metabolism in tumors of the female reproductive system

Groups	Results			
	Vitamin D3, nq/ml	Parathormon, pq/ml	Calcium, mq/dl	Phosphorus, mq/ml
Female patient with tumour in reproductive system (n=50)	11.51±0.18*** (5.80-24.00)	29.60±050 (14.80-48.90)**	7.98±0.16** (7.15±0.80)	1.52±0.26* (1.89-2.34)
Control (n=20)	25.82±0.29 (5.80-24.60)	22.40±2.80 (13.50-24.60)	9.81±0.16 (8.75-10.96)	1.69±0.35 (1.54-1,75)

Note: ***- p<0.001; ** - p<0.01, *** - p<0.001.

Deficiency of vitamin D also leads to a decrease in concentration of phosphorus. The concentration of phosphorus in the blood serum of patients is 1.52±0.26 mg/dl (1.69±0.35). According to the results of the comparative analysis, concentration of phosphorus decreases by 11.2% (p<0.005) compared to the control.

Based on the age, sex, and hormonal status the calcium intake prescribed as a protective method against decrease of calcium concentration in the body (Beller et al., 2006). In addition, the intake of vitamin D can increase the concentration of phosphorus in the body. Thus, the results show that vitamin D and its receptor play an important role in cancer of female reproductive system. In addition, vitamin D deficiency considered as a risk factor for cancer of the female reproductive system.

It is impossible to compensate for the physiological needs for vitamin D3 via food, therefore, its main source is the B-ultraviolet rays of the sun. Thus, these rays stimulate the synthesis of D3 from provitamin D (7-dehydrocholesterol) in the skin epidermis. This form of vitamin D regulates many physiological processes such as proliferation and differentiation of the epidermis. There are specific receptors for D3 (1.25(OH)2D that located in cells of various organs and tissues, including mitochondria. By possessing endocrine function, vitamin D regulates gene transcription in nearly 40 cells. Thus, vitamin D affects the transcription of the important genes which make 3% of the human genome and responsible for the synthesis of sex hormones and carbohydrate metabolism.

1.25 (OH) 2D is a biologically active form of vitamin D that binds to receptors located in the cell nucleus, activates the expression of specific genes in tissues, inhibits cell proliferation, induces apoptosis and differentiation, and prevents angiogenesis in cancer seeds. Experimental studies show that high doses of vitamin D suppress proliferation of epithelium and carcinogenesis. Vitamin D is involved in the process of carcinogenesis through two biological forms: $1.25(OH)_2D$ and 25(OH)D. 25 (OH) D enters cancer tissue and shows a certain anti-cancer effect. Later 1-α-hydroxylase promote the conversion of 25 (OH) D to 1, 25 (OH) and this conversion regulates cell proliferation, differentiation, and apoptosis. Calcium thought to have an anti-cancer effect in the presence of vitamin D, as calcium is one of the main mediators of vitamin D-induced apoptosis in cancer cells.

There is a metabolic relationship between Vitamin D and calcium, under normal physiological conditions. Calcium homeostasis plays an important role in the circulation of $1.25\ (OH)_2D$. Its level is inverse to the consumption of calcium. Reduction of the synthesis of $1.25\ (OH)\ _2D$ in response to a deficiency of trace element leads to a decrease in calcium absorption. In addition, $1.25\ (OH)_2D$ accelerates the release of calcium from cells into the bloodstream. On the other hand, the level of calcium in the blood affects the activity of 1-α-hydroxylase in the kidneys thereby increase the concentration of vitamin D.

Thus, vitamin D has a mechanism that differs from other classic vitamins and as a metabolic hormone has anti-proliferative, anti-apoptotic and anti-neongeon effects. The anti-cancer mechanisms of this vitamin in carcinogenesis not fully studied. The results show that maintaining optimal levels of vitamin 25 (OH)D in the blood is one of the most effective methods in the prevention of oncological diseases, including cancer of the female genital organs.

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Qadın cinsiyyət sisteminin şişləri zamanı vitamin D və biokimyəvi müayinələrin təyini

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Məqalədə qadın cinsiyyət sisteminin müxtəlif şişləri olan xəstələrdə vitamin D-nin, kalsium və fosforun diaqnostik rolunun müqayisəli öyrənilməsi məqsədilə aparılmış tədqiqat işinin nəticələri təqdim edilibdir. 50 qadın cinsiyyət sisteminin müxtəlif şişləri diqanozu qoyulmuş xəstə tədqiq edilibdir. Bütün xəstələrdə vitamin D-nin, parathormon (PTH), kalsium və fosforun qatılığı immunoferment və biokimyəvi metodlarla

təyin edilibdir. Bədxassəli şişlər olan xəstələrdə vitamin D-nin qatılığının xoşxassəli şişlər olan xəstələrlə müqayisədə 2,2 dəfə etibarlı artması müəyyən edilibdir. Bundan əlavə, bədxassəli şişlər olan xəstələrdə kalsium və fosforun qatılığının kontrola nsibətən əhəmiyyətli dərəcədə azalması, PTH-nun qatılığının isə əksinə artması aşkar edilibdir. Beləliklə, göstərilən bu markerlər qadın cinsiyyət orqanlarının şişlərinin erkən diaqnostikası və skrininqi üçün istifadə edilə bilər.

Açar sözlər: *Qadın cinsiyyət orqanlarının xərçəngi, vitamin D, parathormon, kalsium, fosfor*

Определение витамина D и биохимические исследования при опухолях женских половых органов

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В статье представлены результаты сравнительного исследования диагностической роли витамина D, кальция и фосфора у пациентов с различными опухолями женской репродуктивной системы. Исследовано 50 пациенток с различными опухолями женских половых органов. У всех больных концентрация витамин D, паратгормона (ПРГ), кальция и фосфора в сыворотке крови была определена иммуноферментным и биохимических методами. Установлено, что концентрация витамина D у пациентов со злокачественными опухолями по сравнению с пациентами с доброкачественными опухолями была достоверно выше в 2,2 раза. Также выявлено значительное уменьшение содержания кальция и фосфора, а также увеличение ПТГ у больных со злокачественными опухолями по отношению контролю. Таким образом, все эти маркеры могут быть использованы для ранней диагностики и скрининга при опухолях женских половых органов.

Ключевые слова: *Опухоль женских половых органов, витамин D, паратгармон, кальций и фосфор*

Journal of Life Sciences & Biomedicine, vol. 3(76), No 1, p. 38-44 (2021)

The histological and cytological analysis of muscles of lizards (*Reptilia, Squamata*)

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Received: April 06, 2021; Received in revised form: April 12, 2021; Accepted: April 21, 2021

All lizard cells possess microfilaments. However, there are many microfilaments in muscle cells, and these cells provide contraction. In our research, we have used three species (*Ophisops elegans* (Menetries, 1832), *Lacerta strigata* (Eichwald, 1831), *Tenuidactylus caspius* (Eichwald, 1831)) of lizards. In the embryonic period of lizard, myoblast cells are formed from the myotomes. Migration of myoblasts are essential to generate the skeletal muscles. When myoblasts reach their target area, they first form the muscle plate. The number of nuclei in the muscle plate is small and their length is short. Muscle plates are fused with myoblasts to form a large syncytium for producing myosymplast. During the further development of the embryo, related parts of membranes of myoblasts disintegrate and become the sarcoplasmic reticulum. As a result, a long myotube is formed. When we investigated skeletal muscles of hind limbs, according to their colour, three types of muscle fibers were found in the skeletal muscles of the lizard. Red fibers were observed in *Lacerta strigata* more commonly than that in other two lizards. In the same muscles, light fibers were observed in *Tenuidactylus caspius* more frequently. That allows them to move on vertical surfaces very quickly. *Ophisops elegans* had more intermediate colored fibers than the other two lizards. Diameter of myotube depended on species of lizards, muscle location, age of animal and its size. Thickness of epimysium, perimysium and endomysium, observed in the same lizard, also varied depending on type of muscles, the animal's age, weight and environmental conditions where it lived. Myofibrils of cardiomyocytes occupy up to half of total volume of the cytoplasm. In lizards, force of the smooth muscle is more than that of the skeletal muscle. Structural characteristics of skeletal muscle fibers are influenced by many factors such as species, genotypes, nutritional and environmental factors.

Keywords: *Muscle cell, myofibril, histological structure, Ophisops elegans*, *Lacerta strigata*, *Tenuidactylus caspius*

INTRODUCTION

The micromorphological structural features of the separate tissues and organs of a lot of species of herpetofauna have not been studied in detailed yet. One of the such kind of unstudied tissue is muscle tissue of lizard. This tissue plays a vital role in ensuring the dynamics of reptiles in nature, additionally maintains the balance of the biocenosis and trophic communication of animals. Considering that reptiles carry a number of parasitic and infectious diseases, the relevance of the study becomes even more obvious. The object of research - Caspian bent-toed gecko, is naturally distributed in the rocks of the semi-deserts, in all biotops where it can hide. They have become synanthropic animal by living in residential areas, gardens and fences. Spending this type of nocturnal lifestyle protects them against predators. Snake-eyed lizard lives in semi-desert areas with rocky, hard soils and

https://doi.org/10.29228/jlsb.5
Available online 30 June 2021

drought-resistant drought-tolerant plants. The caspian green lizard inhabits in pastures and bushes in the areas where are rich in plants. As it can be seen, the histological study of types of muscles of lizards, which live in different environmental conditions and with different physiological characteristics, worth to study scientifically and practically.

MATERIALS AND METHODS

Researches were conducted at the Department of Medical Biology and Genetics of AMU and the Central Scientific Research Laboratory of AMU in 2009-2020. Snake-eyed lizard - *Ophisops elegans* (Menetries, 1832), Caspian green lizard - *Lacerta strigata* (Eichwald, 1831) and Caspian bent-toed gecko - *Tenuidactylus caspius* (Eichwald, 1831) spread in the territory of the Azerbaijan Republic widely (Najafov et al., 2014) were used for histological researches. For this purpose, expeditions were organized to various areas. Appropriate methodological methods were used to conduct histological analysis of slides from different organs of lizards. Incisions from the muscles tissue of lizards were sampled and fixed in 5-10% formalin. In preparation of muscle tissue slides histological incisions were made both longitudinally and transversely. Paraffin-impregnated blocks were prepared from these samples, and 5-10 μm thick incisions were made using a digital microtome and transferred to the glass. Subsequent histological and cytological studies were performed in the laboratory. Initially deparaffinized samples of muscles were stained with Giemsa stain, hematoxylin and eosin stain. After the preparation the slide observed under the microscope.

RESULTS AND DISCUSSION

Cells of muscle tissue of lizard are rich in microfilaments, which provide muscle contraction. Muscle tissues of lizards, according to their morphofunctional structure, divided into skeletal, cardiac and smooth muscles. Skeletal muscles located on the bones or attached to the skeleton of lizard. An example of smooth muscles are muscles of walls of various internal organs, blood and lymphatic vessels. Cardiomyocytes are observed in the myocardium.

In the embryonic period of lizard, myoblast cells are formed from the myotomes of the paraxial mesoderm in the somites (Najafov, 2007). These cells are proliferatively active. Migration of myoblasts are essential to generate the skeletal muscles. It has been discovered by us that, when myoblasts reach their target area, they first form the muscle plate. The existence of this structure in lizard takes a short time. The number of nuclei in the muscle plate is small and their length is short. Muscle plates are fused with myoblasts to form a large syncytia for producing myosymplast. During the further development of the embryo, related parts of membranes of myoblasts disintegrate and become the sarcoplasmic reticulum. As a result, a long myotube is formed. Each myotube contains many nucleises, which are initially located in the center of the tube. New myofibrils formed in the periphery of the myotube. After the growing myofibrils in the myotube push the nucleies into the cytoplasmic membrane to perform their functions well. If the nucleus retains in the center, it impedes contraction of skeletal muscle fibers and slows down the differentiation of muscle fibers. Thin (actin) and thick (myosin) filaments can be seen under a microscope in the myotube of the lizard (Fig. 1).

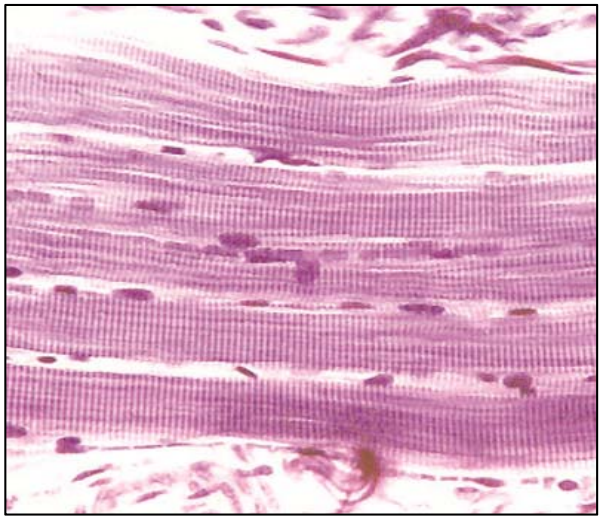

Fig. 1. Microscopic view of thin and thick filaments in the myotube of skeletal muscle.

Some myoblasts do not involve in the formation of myotubes. These cells are located outside of the sarcolemma and beneath the

surrounding basal lamina to form myosatellite cells. These satellite cells are observed in the muscle fibers in the tail of the lizard more. When lizard's tail is cut off for any reasons, these myosattelites are involved in muscle regeneration actively. On the other hand, along with myosatellite, some red bone marrow cells also divide and participate in the restoration of damaged skeletal muscle tissues (Najafov et al., 2014). It is not easy to see myosatellite by light microscope staining techniques. These cells have branched elongated cytoplasmic processes.

Completely formed myotube and satellite cells are surrounded by basal lamina and form the basis of the muscle fiber. Inside of the myosimplast, the nuclei divide and multiply by mitosis. Axon and myotube membrane are closer together significantly on the sarcolemma (Garland et al., 1994). This area is myoneural junction consisting of the axon terminal, the synaptic interval and the plasmalemma of the myotube.

We have identified three different types of muscle fibers in the skeletal muscles of lizard by their color. Red fibers of lizards contain a large amount of myoglobin and lower amounts of myoglobin. Through these fibers have rich capillary network. They are slow but can to do repetitive contraction over long periods of time. White colored fibres fatigue more easily but can sustain more intense activity. These fibres have more myofibrils and a large diameter. Intermediate fibers, as it may be seen by their names, are intermediate in their colour and function. All three of these fibers are found in every skeletal muscle of lizard. When we examine skeletal muscles (*Triceps humeralis*) of same type of forelimb (left), red fibers are more frequent in the Caspian green lizard than in the other two lizards. In Caspian bent-toed gecko light-colored fibers are more frequent in the same muscle. This allows the gecko to move very quickly over vertical substrates in short time. Intermediate fibers in the left forelimb of snake-eyed lizard dominate over than other two lizards. The reasons for these differences in skeletal muscle fiber types are: capillary density, oxidative capacity, myoglobin content, glycolytic capacity and ATPase levels (Nelson et al., 2001). The diameter of the muscle fiber varies depending on species of lizard, age, size of the animal and the functional state of the muscle. The diameter of the muscle fiber varies in different parts of each lizard. Sarcomers of skeletal muscle fibers are seen in lizards quite well. Muscle fibers structural characteristics can be modulated by environmental factors independently. Skeletal muscle of lizard consists of 88% muscle tissue and 12% of connective, vascular, nervous and fat tissues. The connective tissue in skeletal muscle is divided into endomysium, perimysium and epimysium. Near the tendon of lizard the connective tissue are all thicker than in other regions of the muscle.

Each skeletal muscle fiber is surrounded with an endomysium. It separate fibers from each other (Higham et al., 2010). Within the endomysium are present capillaries and neuronal branches. When we examined the cross section of muscles, we saw that endomysium were connected to each other. İt also connected to the perimysium by intermittent perimysial junctional plates. Perimysium is connective tissue partition that is thicker than endomysium. İt surrounds a group of fibers to form a fascicle. Blood vessels and nerves also located in the perimysium (Vitt et al., 2003). Endomysial and perimysial network acts to transmit muscle force. Epimysium surrounds collection of fascicles that constitutes the muscle organ. İt consists of dense connective tissue and connects to perimisium. Near the articular capsule and disc, endomysium, perimysium, and epimysium were all thicker than in other regions of the muscle. We noticed that the thickness of endomysium, perimysium and epimysium varies depending on the type of muscle, the age of animal and the environmental conditions in which it lives.

Lizard cardiogenic area are located in the mesodermal component of the splanchnopleural layer of the plate. Cardiomyocytes are differentiated from cardiac mesodermal primordia. Some cardiomyoblast cells multiply and mature into cardiomyocytes, all other cardiomyoblasts do apoptosis. All cardiomyocyte cells of adult lizard arrested at G_0 cell cycle. It means, they never divide. However, we have observed a large number of polyploid chromatin fibers in their nucleus. Some cardiomyocytes may have 2 or 3 nucleuses. The nucleus is located in the center of the cytoplasm of the cardiomyocyte cell and is oval in shape. In the cytoplasm of cardiomyocytes with the proteins provide contraction accumulate myoglobin, fat drops and glycogen. Myofibrils are

the protein complex that provides contraction. In lizards, the percentage of cell volume that myofibrils occupy in cardiomyocytes is being approximately half of the total cytoplasm. These cells are highly resistant to fatigue. Cardiac muscle cells are branched and combine with neighboring cells to form a muscle network (Fig. 2).

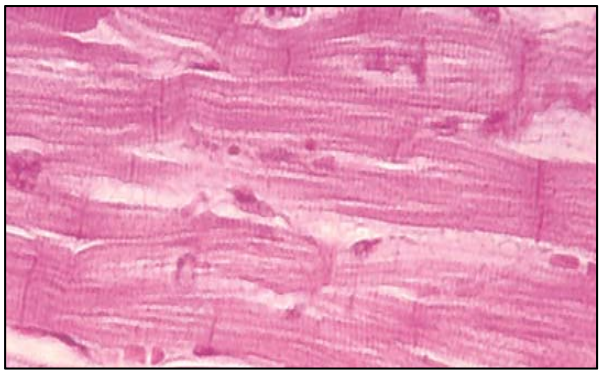

Fig. 2. Cardiac muscle cells form long, branching muscle fibers.

Dark layers appear in the areas where these cells touch each other. Sarcolemma does not disappear in areas where there is a contact between cardiomyocytes cells. The intercalated discs are formed on the contacting areas. The surface of cardiomyocytes is covered by basal lamina. There are no satellite cells under the basal membrane. The endomysium is present on cardiac muscle cells. Elements of connective tissue, rich in capillaries are identified.

We have seen during our observations that cardiomyocyte sarcomeres are differs from skeletal muscle sarcomeres. The sarcomere lies between two Z-lines, A and I bands are not clearly defined (Curtin et al., 2005). There are dyads instead of triads. Cardiomyocyte sarcoplasmic reticulum is better developed than the fibers of skeletal muscles.

During the embryonic development of lizards, mesenchyme gives rise to connective tissue, bone, cartilage, circulatory and lymphatic systems and smooth muscles. Smooth muscle cells are elongated and spindle-shaped. They are tapered at both ends and round at the center. The nuclei are located closer to one lateral membrane of the cell along the midline of myositis. Smooth muscle cells of the lizard retain ability to reproduce throughout their life and have capacity to regenerate. By morphofunctional properties smooth muscles of lizard is divided into two subgroups: single-unit and multi-unit smooth muscles. Each multi-unit smooth muscle cell innervated by a neuron and receive synaptic input. Their contraction does not spread from one cell to the another. These types of smooth muscle is found in the large blood vessels and in the respiratory airways of lizard. İn the walls of all visceral organs single-unit smooth muscle fibers are connected via gap junctions. Few of single-unit smooth muscle cells are innervated by neuron. The smooth muscle cells of lizard are much smaller than that skeletal muscle cells. They have in the cytoplasm actin and myosin filaments but do not have sarcomeres (Fig. 3). During lizard's myositis contraction, it can remain 20% of its volume comparing in the quiet situation. During contraction, the nucleus also alters its shape considerably. İf we compare with the skeletal muscles, smooth muscles contraction are weak.

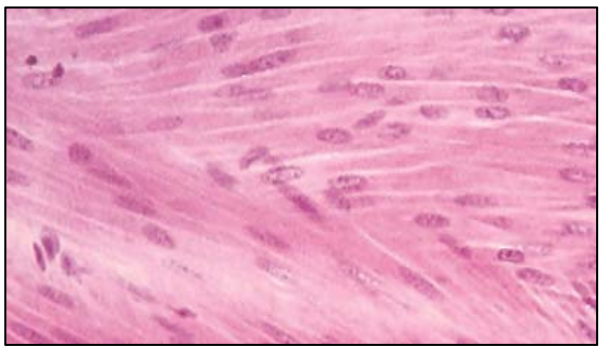

Fig. 3. Location of myocytes in the smooth muscle tissue.

Smooth muscles can remain contraction position for a long time. But contraction strength of smooth muscle observed in lizards is greater than that of skeletal muscle. The reason of this is the transverse bridges from the myosin that can form a strong bond with the actin (Garland et al., 2005). These compounds separate from each other hard. Smooth muscle cells are innervated autonomic nervous system. Another feature of the lizard's smooth muscle is that it does not lose its former strength in spite of being in a stretched or shortened position for a long time.

CONCLUSION

In the embryonic period of lizard, myoblast cells are formed from the myotomes of the paraxial mesoderm in the somites. Migration of these cells are essential to generate the skeletal muscles. When myoblasts reach their target area, they first form the muscle plate. The existence of this structure in lizard takes a short time. The number of nuclei in the muscle plate is small and their length is short. Muscle plates are fused with myoblasts to form large syncytia for producing myosymplast. During the further development of the embryo, myotubes are formed. Three types of muscle fibers in the skeletal muscles of the lizard were determined in the basis of their colours. When we examine the skeletal muscles of the same type locating in the forelimb, red fibers are more observed in the Caspian green lizard more commonly than in the other two lizards. The intermediate fibers are more commonly in the snake-eyed lizard in the same muscles. The light fibers of skeletal muscle of limbs of Caspian bent-toed gecko are dominant over the other two lizards. Species, age and size of lizard affects to diameter of the muscle fiber. The thickness of endomysium, perimysium and epimysium observed in the same lizard alters depending on kind of muscle, age of the animal, its weight, and the environmental conditions in which it lives. Cardiomyocytes contain up to half of the total amount of contractile proteins. On the cardiomyocyte endomysium is present. The sarcomere areas of cardiomyocytes under the microscope are almost indistinguishable from the sarcomeres of the skeletal muscles. If we compare to the skeletal muscles, the smooth muscles contraction more slowly. These muscles can stay in a contraction position for a long time. But the erection strength of smooth muscles of lizards are greater than that of skeletal muscles.

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Kərtənkələlərdə (*Reptilia, Squamata*) əzələlərin histoloji və sitoloji analizi

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Kərtənkələlərin bütün hüceyrələrinin tərkibində mikrofilamentlər mövcuddur. Amma əzələ toxumasının hüceyrələrində mikrofilamentlər çoxdur və bu hüceyrələr təqəllüsü təmin edir. Biz tədqiqatlarımızda üç növ kərtənkələdən istifadə etmişik (*Ophisops elegans* (Menetries, 1832), *Lacerta strigata* (Eichwald, 1831), *Tenuidactylus caspius* (Eichwald, 1831)). Kərtənkələnin embrional inkişafında somatik əzələ toxuması miotomlardan yaranır. Mioblastların miqrasiyası əzələlərin formalaşması üçün əhəmiyyətlidir. Mioblastlar hədəflədikləri nahiyəyə gəldikləri zaman ilk əvvəl əzələ plastinkası əmələ gətirirlər. Bu quruluşun mövcudluğu kərtənkələdə çox qısa vaxt çəkir. Əzələ plastinkasında nüvələrin sayı az olur və ölçüsü qısadır. Daha sonradan mioblastların plastinkaya birləşməsi yolu ilə miosimplast formalaşmış olur. Embrionun sonrakı inkişafının nəticəsində miotubul yaranır. Kərtənkələnin skelet əzələlərində rənginə görə üç tipdə olan əzələ lifləri müəyyən edilmişdir. Ətraflarda yerləşən eyni növ skelet əzələlərini araştırdığımızda, zolaqlı yaşılkərtənkələdə qırmızı liflər digər iki kərtənkələyə nisbətən çox müşahidə olunur. Eyni əzələlərdə xəzər nazikbarmaq gekkonunda açıq rəngli liflər daha çox müşahidə olunur. Bu da gekkonun şaquli vəziyyətdə olan substratlar üzərində qısa zaman ərzində çox cəld hərəkət etməsinə səbəb olur. Biçimli ilanbaşda ətraflarda aralıq liflər digər iki kərtənkələyə nisbətən üstünlük təşkil edir. Miosimplastın diyametri kərtənkələnin növündən, yaşından, heyvanın ölçüsündən və əzələnin növündən asılıdır. Eyni bir kərtənkələdə müşahidə olunan endomiz, perimiz və epimizin qalınlığı da əzələnin növündən, heyvanın yaşından, onun kütləsindən və yaşadığı ekoloji şəraitdən asılı olaraq dəyişir. Kərtənkələlərdə miofibrillər kardiomiosit hüceyrələrinin sitoplazmasının ümumi həcminin yarısına qədər olan hissəsini tutur. Kərtənkələdə müşahidə etdiyimiz saya əzələlərin yığılma gücü eninəzolaqlı əzələlərin gücünə nisbətən daha çoxdur.

Açar sözlər: *Kərtənkələ, biçimli ilanbaş, zolaqlı yaşılkərtənkələ, Xəzər nazikbarmaq gekkonu, histoloji quruluş, əzələ, mioblast, miotub, miosit*

Гистологический и цитологический анализ мышц у ящериц (Reptilia, Squamata)

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Во всех клетках ящериц содержатся микрофиламенты. Однако в клетках мышечной ткани, обеспечивающей сокращение, микрофиламентов много. В нашей работе мы исследовали три вида ящериц (*Ophisops elegans* (Menetries, 1832), *Lacerta strigata* (Eichwald, 1831) и *Tenuidactylus caspius* (Eichwald, 1831)). В эмбриональном развитии ящериц соматическая мышечная ткань формируется из миотомов. Миграция миобластов необходима для формирования скелетных мышц. Когда миобласты достигают необходимой области, они вначале формируют мышечную пластину. Мышечная пластинка у ящериц существует очень короткий промежуток времени, количество ядер в ней немногочисленно и она достаточно короткая. Мышечная пластинка сливается с миобластами, образуя миосимпласты. В процессе дальнейшего развития эмбриона образуется мышечная трубка. В зависимости от цвета в скелетных мышцах ящериц различают три типа мышечных волокон. Когда

мы исследовали скелетные мышцы, расположенные в задних конечностях, красные миофибриллы чаще наблюдались у *Lacerta strigata* (Eichwald, 1831), чем у двух других видов ящериц. Светлые миофибриллы чаще встречались у каспийского тонкопалого геккона. Эта особенность позволяет геккону очень быстро перемещаться по вертикальным поверхностям. У *Ophisops elegans* (Menetries, 1832) больше промежуточных миофибрилл, чем у двух других ящериц. Диаметр миосимпласта зависит от типа ящерицы, ее возраста, размера животного и типа мышц. Толщина эндомизия, перимизия и эпимизия у одной и той же ящерицы также варьирует в зависимости от типа мышц, возраста животного, его веса и экологического состояния окружающей среды. В клетках кардиомиоцитов миофибриллы занимают до половины общего объема цитоплазмы. У ящериц сократительная сила гладких мышц выше, чем скелетных.

Ключевые слова: *Мышцы, миофибриллы, гистологическое строение, форма клеток, Ophisops elegans*, *Lacerta strigata*, *Tenuidactylus caspius*

Journal of Life Sciences & Biomedicine, vol. 3(76), No 1, p. 45-51 (2021)

Some biomorphological features of the *Stevia rebaudiana* Bertoni and its *in vitro* cultivation

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Received: October 30, 2020; Received in revised form: March 21, 2021; Accepted: May 03, 2021

Stevia rebaudiana **Bertoni was reproduced vegetatively under greenhouse conditions and its different developmental stages were studied. Seeds were obtained from Absheron cultivated plants. In order to obtain cell culture under *in vitro* conditions, various plant organs were isolated and transferred to nutrient environments that differed in mineral and phytohormonal composition. The reaction of plant tissue culture to the *in vitro* conditions was studied.**

Keywords: *Stevia rebaudiana* Bertoni*, in vitro, morphogenesis, developmental stage, explant, sterilization mode, kallusogenesis*

INTRODUCTION

Stevia Rebaudiana Bertoni (*Asteraceae*) is considered as one of the most promising drugs and nutritional plants. This subtropical perennial herb lasts 8 to 11 years while its aerial part dries every year. The natural habitat of this plant is located in South America (Paraguay, Brazil) and it grows in bush, riverbeds and swamps. It produces kurtin via both seeds and vegetative propagation (Sumida, 1980; Verzilina, 2005). At the same time, the *Stevia* is also known as honey plant, sweet leaf, honey leaf, sweet grass, etc (Lyakhovkin et al, 1990; Pototsky, Pokrovsky, 2004; Ozerova, 2005; Carakostas et al, 2008). The main feature that distinguishes *Stevia* from other crops is that its leaves contain a complex of 8 diterpene glycosides (Tanaka, 1980; Semenova, 2004; Podporinova, 2005, 2007; Amin et al, 2017), which sweetness is 200-350 times higher than sugar's: stevioside, rebaudioside A, B, C, D, E, steviolbioside, duclucid (Zubenko, 1990; Sokolov, 2004; Abou-Arab et al, 2010). Nowadays, stevioside and rebaudioside A are used as natural sweeteners in many countries. European countries, Canada, Australia, New Zealand, Japan, Korea, Great Britain, Russia and China have already started to use *Stevia* plants for nutritional purposes.

Stevioglycosides were first isolated from the leaves of *Stevia rebaudiana* in 1931. Glycosides in 1 kg of dry leaves of the plant completely substitute 30 kg of sucrose and unlike sucrose do not increase the nutritional value, because there are only 18 kcal per 100 gram (Gregersen, 2004; Massoud, 2005; Brahmachari, 2011). Substitution of sucrose with *stevia* products in food and sweet drinks reduces the amount of glucose and insulin in the blood without increasing the energy intake of the diet, thus having a positive effect on the human body (Tsapko et al, 1990; Smolyar, 1990; Gorbatenko, 2003; Kuznetsova, 2014). It should be noted that the daily maximum dosage of steviosides is 4 mg/kg (depending on body weight) (AO, WHO, 1985), which is much less than of sucrose's. In addition, the sweetener extract from *Stevia* is completely natural and its glycemic index is zero (Youssef, 2007).

http://dx.doi.org/10.29228/proc.76
Available online 30 June 2021

In economic terms, E 960 sweetener obtained from *Stevia rebaudiana* costs 3 times cheaper. Addition of dried and cut plant leaves to livestock feed increases meat and wool production (Surkova, 2007; Rastovarov, 2009; Lavrenova, 2018). Furthermore, as recent research shows *stevia* glycosides and the extract obtained from the plant have a therapeutic effect and can be used in the treatment of various tumors (Limarenko, 1995; Massoud et al, 2005; Ghoshet al, 2008).

Extensive use of *Stevia rebaudiana* for various needs (preparation of medicines, food, as well as use as feed additives) attracted attention of scientists to this plant and made it a research target. Unfortunately, this culture isn't still widely known in Azerbaijan, even though the country has favorable soil and climatic conditions for its cultivation. In order to put in place cultivation of *Stevia* in the country first of all it is necessary to study its bioecological and biomorphological features, develop appropriate agricultural methods, conduct breeding work using biotechnological methods, which lets possible the study of the plant response *in vivo* and *in vitro* conditions. Considering the above, the study of *Stevia rebaudiana* in closed ground and *in vitro* conditions has begun for the first time in Azerbaijan.

MATERIALS AND METHODS

Planting material of Stevia rebaudiana brought from Turkey was planted in flower pots with a substrate consisting of soil, sand, humus and rotted manure (2:2:1:0.5).

The plants were cultivated in the greenhouse of the Central Botanical Garden of the Azerbaijan National Academy of Sciences. Stevia *rebaudiana* cuttings were used as the study object, and their various parts were isolated for cultivation under *in vitro* conditions. Leaf blades of large, medium, small leaves, primordial leaves, petioles, parts of the stem and inflorescences were used as explants (Figure 1). The following sterilization options were used to introduce the explants *in vitro*:

1) 1 min in 70% ethanol + 10 min in 5% sodium hypochlorite solution;
2) 1 min in 70% ethanol + 12 min in 5% sodium hypochlorite solution;
3) 1 min in 70% ethanol + 15 min in 5% sodium hypochlorite solution;

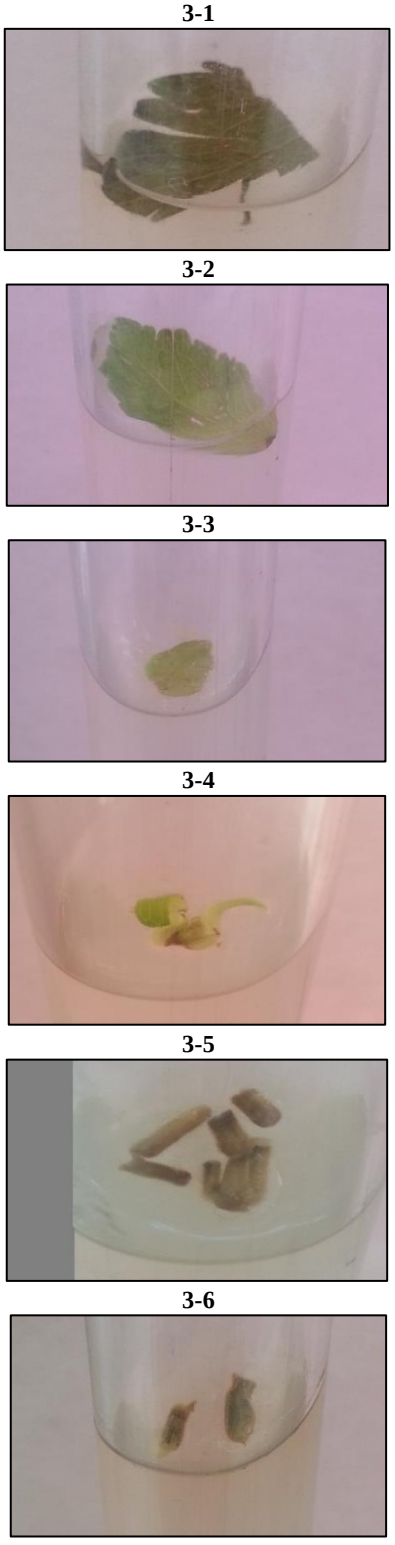

Fig. 1. Explants introduced into in vitro culture: Large (3-1), medium (3-2) and small (3-3) leaves; growth points with primordial leaves (3-4); parts of the stem (3-5); inflorescences (3-6).

4) 1 min in 70% ethanol + 18 min in 5% sodium hypochlorite solution;
5) 1 min in 70% ethanol + 20 min in 5% sodium hypochlorite solution;
6) 3 min in 70% ethanol + 15 min in 5% sodium hypochlorite solution;
7) 3 min in 70% ethanol + 18 min in 5% sodium hypochlorite solution;
8) 5 min in 70% ethanol + 15 min in 5% sodium hypochlorite solution;
9) 5 min in 70% ethanol + 18 min in 5% sodium hypochlorite solution.

After sterilization the seedlings were planted in two nutrient media with different mineral composition: Gamborg (B_5) and Murashige-Skoog (M-S). In order to obtain callus tissue, phytohormones of different concentrations were added to medium where the transplants were planted: 2,4-dichlorophenoxyacetic acid (2,4-D) - 5 mg/l and 8 mg/l; kinetin (KIN) - 5 mg/l and 8 mg/l; α-naphthylic acid (NST) - 0.5 mg/l.

RESULTS AND DISCUSSION

Observations showed that since March plants began to actively vegetate (Fig. 2). In August and early September, buds started to form and by the end of September, flowering was observed. Mass flowering (Fig. 3) continued until the end of October. Fruit ripening and seed formation began in late October.

Based on experimental data obtained in *in vitro* conditions, it can be stated that during the induction of callusogenesis no significant differences were observed on nutrient media with the same phytohormonal but different mineral composition (M-S and B_5). The lack of differences can be explained by a very high level of explant infection and the associated reduction in the number of repeatability. In general, callus formation is not induced when transferring differentiated plant tissues to hormone-free media regardless of the mineral composition of the nutrient medium. Callus and morphogenesis are obtained only from the vegetative organs of plants with active meristematic cells (Sidorov, 1990).

Despite this, each plant is characterized by its endogenous phytohormonal balance and the need for mineral elements. Therefore, for each type of plant and even for each genotype customised regeneration protocol is developed (Kolesnikova, Zhuzhzhalova, 2012). Thus, during induction of callusogenesis for flax both B_5 and M-S media were equally favorable, whereas for alfalfa and cockle the favorable ones were B_5 and M-S respectively. Whereby, various combinations and concentrations of phytohormones were required in order to obtain a cell culture of each of these plant species. It should be noted that in the cockle, a poisonous weed, morphogenic calli formed more intensively on hormone-free M-S medium (Litvinova and Gladkov, 2012), which let us classify this plant as a high auxin type.

Fig. 2. General view of *Stevia rebaudiana.*

Fig. 3. Inflorescences of *Stevia rebaudiana.*

In literary sources, information on the introduction of *Stevia rebaudiana* into an *in vitro* culture is scarce. But the results of some experiments shows that without the use of phytohormones, a *Stevia* cell culture cannot be obtained, despite the fact that this culture belongs to the high auxin type of plants.

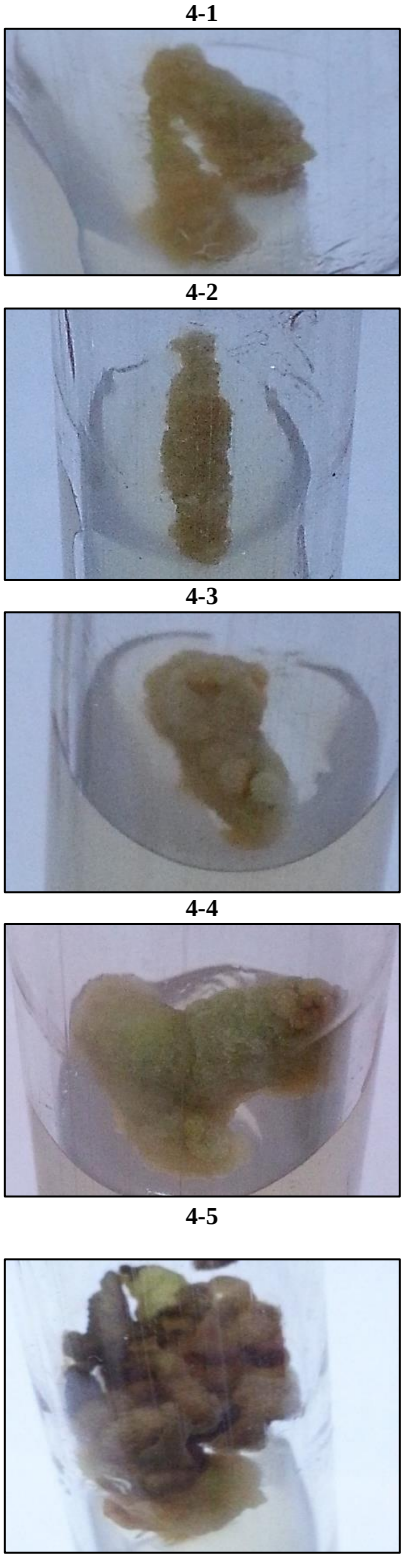

Fig. 4. Callusogenesis on *Stevia rebaudiana* explants: primordial leaves (4-1), stem parts (4-2), small leaf (4-3), middle leaf (4-4), large old leaf (4-5).

Callus and morphogenesis could be obtained from leaf and stem explants only by using auxins (Kolesnikova, Zhuzhzhalova, 2012; Kononova, 2015). Both 2,4-D and cytokinin were used in our experiment, and callus cultures were obtained in each experiment (Figure 4). It can be seen from the pictures that calli are composed of dense greenish, somewhat loose yellowish and hydrated white cells. Due to the fact that numerous explants were infected, many of them were withdrawn from the experiment, so the number of repeatability decreased. Therefore, it was not possible to carry out statistical processing confirming the advantage of one explant over another. However, we can state that in the medium with 5 mg/l 2,4-D and 5 mg/l KIN, the process of callus proliferation was more intensive and there were significantly less white hydrated cells. In experiments of other authors, morphogenic calli were obtained when 0.5 mq/l 2,4-D was introduced into the nutrient medium. In this case, the calli were of medium density and had a light yellow color, while separate meristematic foci were observed (Kolesnikova, Zhuzhzhalova 2012). Undoubtedly, an optimal balance must be maintained between endogenous phytohormones of the explant and exogenous hormones added to the nutrient medium in order to obtain a cell culture *in vitro* and induce morphogenesis. It depends on both the type of implant and the characteristics of the genotype. In our experiment, depending on the type of explant, the structure and shape of the calli was different (Fig. 4). For example, dense, knotty dark-colored calli formed on large old leaves (Fig. 4-5).

This may be due to the accumulation of phenols during explant aging. Despite this, the mass of calli increased significantly after 4 weeks, and calli for the induction of morphogenesis were transferred to a nutrient medium containing 0.2 mg/l BAP.

Stevia seeds obtained from plants grown in greenhouses were also planted on nutrient media. However, during sterilization the seeds were very wet and within 1.5 months cultivation did not germinated (Fig. 5). This may be due to defects in the seeds themselves or destruction processes that occur in the shell of the seeds during sterilization.

It is known that *Stevia rebaudiana* is a self-incompatible plant and is characterized by a very weak germination of seeds and its germination ability is quickly lost (Bodrug, 1995; Verzilina,

2005; Bozhimirov, 2011). However, the lack of germination and wettability of seeds in our experiment is probably associated with adverse greenhouse conditions — whiteflies, which are carriers of the sooty fungus, were seen on plants (Fig. 6).

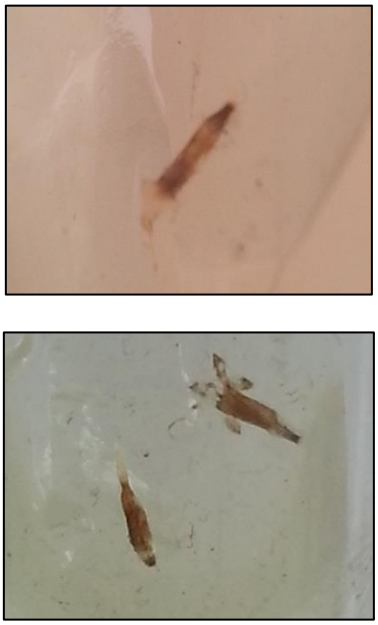

Fig. 5. Seeds of *Stevia rebaudiana* obtained in a greenhouse and planted on an artificial nutrient medium.

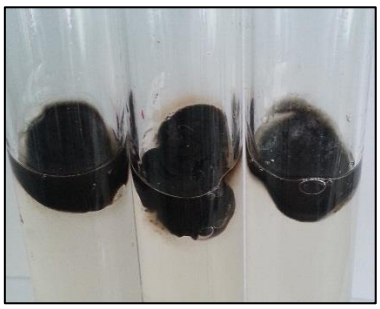

Fig. 6. *Stevia rebaudiana* explants infected with a black fungus.

A similar type of infection was observed in explants isolated, sterilized and transferred to artificial nutrient media. And this indicates the presence of an internal infection in the plants from which the seeds were obtained. Probably, formation of defective seeds is the consequence of internal infection which weaken the plants.

Despite this, as a result of research it can be stated that *Stevia rebaudiana* propagated under the greenhouse conditions exhibited competence for *in vitro* cultivation. The calli obtained as a result of the experiment had a dense structure and numerous meristematic foci. Exactly such calli demonstrated successful morphogenesis (Kolesnikova, Zhuzhzhalova, 2012). This fact indicates the possibility of selection in *in vitro* conditions.

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Stevia rebaudiana Bertoni bitkisinin bəzi biomorfoloji xüsusiyyətləri və onun *in vitro* kulturaya daxil edilməsi

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Stevia rebaudiana Bertoni vegetativ yolla (qələmlə ilə) çoxaldılmış və müxtəlif inkişaf fazaları öyrənilmişdir. Abşeronda örtülü şəraitdə becərilən bitkilərdən toxumlar alınmışdır. *İn vitro* şəraitində hüceyrə kulturasının alınması məqsədi ilə bitkinin müxtəlif orqanları təcrid edilmiş, mineral və fitohormonal tərkibinə görə fərqlənən süni qida mühitlərinə köçürülmüşdür. Bitkinin toxuma kulturasının *in vitro* şəraitinə cavab reaksiyası öyrənilmişdir.

Açar sözlər: *Stevia rebaudiana* Bertoni, *in vitro, morfogenez, inkişaf mərhələləri, eksplant, sterilizasiya rejimi, kallusogenez*

Некоторые биоморфологические особенности растения *Stevia rebaudiana* Bertoni и введение ее в культуру *in vitro*

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Изучались фазы развития растения *Stevia rebaudiana* Bertoni, размноженного вегетативным путем (черенкованием). У растений, выращенных на Апшероне в условиях закрытого грунта, получены семена. С целью получения клеточной культуры изолированные органы растений культивировались на искусственных питательных средах с различным минеральным и фитогормональным составами. Изучена ответная реакция растений на культивирование в условиях *in vitro.*

Ключевые слова: *Stevia rebaudiana* Bertoni, *in vitro, морфогенез, стадии развития, эксплант, режим стерилизации, каллусогенез*

Journal of Life Sciences & Biomedicine, vol. 3(76), No 1, p. 52-59 (2021)

The role of conservative treatment in morbid obesity (minireview)

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Received: October 30, 2020; Received in revised form: March 21, 2021; Accepted: May 03, 2021

The article analyzes the treatment of long-term weight increment, morbid obesity and non-alcoholic steatohepatosis of the liver through medication, diet, lifestyle changes, and physical activity. An analysis of the literary data suggests that at morbid obesity, lifestyle changes, increased physical activity, different diets, or medications do not provide a basis for complete cure of the disease. After stopping taking both drugs and cures to reduce body weight, it begins to increase again.

Keywords: *Body weight, morbid obesity, diet, drug treatment*

Obesity and type-2 diabetes are interrelated and are considered to be the epidemic of the XXI century. Approximately ¼ part of the population in economically developed countries suffers from this disease. According to the World Health Organization, by 2025, 2.3 of the world's elderly overweight and morbidly obese (Jensen et al., 2014; Younossi et al., 2016).

Visceral obesity plays an important role in the development of insulin resistance, type-2 diabetes, non-alcoholic steatohepatosis of the liver, cardiovascular diseases. Therefore, the treatment of this disease is in the focus of attention of the medical community, both in the past and in the present century.

Many researchers are devoting their research to developing different methods to prolong the lives of those with the disease, and are working to prevent the association of morbid obesity with other diseases and to improve the quality of life and psychological status of patients. Decreasing of the body weight, blood sugar and blood pressure were considered as the main criteria for treatment.

Recently, some changes have been made in the main criteria for the treatment of morbid obesity. If in the past the attending physician tried to reduce body weight suddenly, now it is recommended to perform this procedure gradually. L.V.Savelieva shows that the gradual reduction of body weight (0.5-1.0 kg per week) in patients with morbid obesity, as well as non-alcoholic fatty liver is more effective in terms of compensation of metabolic syndrome (Savelieva, 2011**)**.

S.A.Buturova considers that if treatment reduces body weight by 10% before treatment, it should be considered an effective treatment (Buturova 2004).

From the first years of the XXI century, doctors have tried to treat this disease with medication, lifestyle changes and various diets (Promrat et al., 2010; Marra et al., 2013; Eckard et al., 2013). Thanks to research in this area, a new strategy for morbid obesity, as well as non-alcoholic obesity of the liver has been developed. According to this strategy, treatment consists of gradually reducing body weight by changing lifestyle. For this purpose, the treatment period is divided into the following stages:

1. The period of weight loss - 3-6 months;
2. Stabilization of body weight - 6-12 months:

Malnutrition in the pathogenesis of morbid obesity has led researchers to use diets to treat it. In this regard, diet has been widely used in the treatment of morbid obesity since the end of the last century, including the first decade of modern times (Rytting et al., 1995; Astrup, 2000; Lichtenstein, 2006). However, the diet is not based on a single system. Some authors prefer to eliminate high-calorie foods from the diet, while others prefer to limit fats and carbohydrates without compromising total calories.

https://doi.org/10.29228/jlsb.7
Available online 30 June 2021

L.V.Savelieva took a more realistic approach to dieting (Savelieva, 2011). According to her, before determining the composition of the diet, it is recommended to take into account the patient's diet and what type of food he prefers when eating. According to him, the patient should keep a daily weight record to control his/her weight while eating according to the prescribed diet.

On the basis of this registration, the nutritionist should monitor the changes in the patient's condition and, if necessary, make some changes in the diet.

At present, nutritionists prefer the WHO-approved diet. According to the WHO, in addition to reducing calories from the diet, it is necessary to limit the taking of fats.

Thus, the diet should consist of 10-15% protein, 55-60% carbohydrates, 25-30% fat (provided that the fats of animal origin do not exceed ⅓). When calculating calories, the patient's age, weight, sexual and physical activity should be taken into account. In addition, the diet should be adjusted to the changes in the patient's blood. If excess body weight has caused dyslipidemia in the blood, it is necessary to limit the entry of nutrients and cholesterol into the body to 250-300 g.

Also, the daily intake of salt in the diet of people with high blood pressure should be reduced to 5 grams.

Thus, by the first decade of the 21st century, the increase in diet and physical activity in the treatment of morbid obesity was more widespread.

The authors of this idea (Savelieva, 2011; Popov et al., 2015; Lazebnik et al., 2017; Zhirkov et al., 2019; Wong et al., 2013 etc.) believe that the establishment of this aspect of treatment in patients with morbid obesity and non-alcoholic fatty liver based on it can reduce elevated levels of liver enzymes in the blood without the use of pharmacological drugs is successful. According to their data, the proposed diet has led to a decrease in adipose tissue in the liver and an improvement in the histological structure of the liver.

However, this idea has not been unequivocally accepted in the scientific literature.

Promart et al. believe that the treatment of non-alcoholic liver obesity cannot be achieved by diet alone (Promart et al., 2010). According to them, the diet should be accompanied by a variety of physical activities, including track and field athletics, as well as regular brisk fast movement. Summarizing the results of their clinical observations, they concluded that the combined effect of diet and physical activity, along with a decrease in body weight, improves the histological picture of non-alcoholic steatohepatosis of the liver in 3-5% of cases. However, it is unrealistic for Promart and his colleagues to reach such a conclusion without performing a liver biopsy.

F.Maagkos (2010), V.Heijden et al. (2010) note that a diet combined with increased physical activity improves patients with non-alcoholic hepatic obesity. The authors came to this conclusion based on a positive change in the concentration of markers in the blood of patients, including aminotransferase, which reflect the functional state of the liver.

Although dietary treatment of morbid obesity, as well as non-alcoholic liver obesity has been developed, no standard diet has been developed for the treatment of this disease. Most supporters of diet therapy have limited themselves to the proposal to exclude fructose and trans-fats from the diet (Petta et al., 2013; Simopoulos, 2013).

However, clinical observations and experimental results accumulated over time have shown that non-drug therapy is not an effective treatment for morbid obesity.

E.G.Starostina (2011) rightly points out that a special diet and increased physical activity create certain problems in the treatment of morbid obesity, especially in the quality of life of patients. It is known that dieting of overweight people faces the problem of hunger. In addition to having a psychological effect on a person, starvation also results in a violation of the balance of a number of metabolic processes in the body (Timofeeva et al., 2008; Askerov, 2018).

In addition, as noted by Ye.Q.Starostina (2011), diet used as "Treatment starvation" often leads to fatal arrhythmias. In this regard, new, more convenient methods were sought. As a result of these studies, in addition to diet therapy, physical therapy, physiotherapy and psychotherapy are widely used in modern clinical practice in the treatment of morbid obesity. Barte et al. (Barte et al., 2010) note that the application of these methods significantly reduces body weight, lowers blood pressure, stabilizes blood sugar, relieves metabolic

syndrome. As the functional state of the liver improves, the concentration of markers in the blood decreases, and even in 10-15% of patients, the increased concentration of enzymes falls to normal. However, this positive dynamic is not sustainable. After 1-2 years, body weight begins to increase again, and the positive results obtained change in a negative direction.

In the treatment of morbid obesity, the program developed by L.V.Savelieva (Savelieva, 2011) in 1998 at the Scientific Center of Endocrinology of the Russian Federation (Program of therapeutic training and treatment of obese patients) is more advanced method of diet therapy.

The program contains information for patients to get used to diet therapy and physical training, and shows the rules of their conduct. The program was used by 2,000 patients, and the author of the program analyzed the obtained results and concluded that the patients who were difficult to treat, were patients with morbid obesity caused by eating disorders. They not only struggle to follow the diet, but they also die as a result of a number of dietary complications, especially arrhythmias.

Along with all this, a number of researchers have considered the use of drugs in the treatment of morbid obesity and non-alcoholic obesity of the liver, and extensive research has been conducted in this direction (Popov et al., 2015; Ryan et al., 2010; James et al., 2010).

Drugs used for this purpose are divided into 2 groups:

1. Drugs that reduce the need for food.

2. Drugs that reduce the absorption of nutrients.

Although the first drug to reduce the need for food was synthesized in 1893, each drug synthesized was soon withdrawn due to its side effects. About a century later, in 1980-1990, a drug called "Phen-Phen" was synthesized from Phenteramine and Phenfluramine and began to be widely used in medical practice for a long time (Tishkovskiy et al., 2015).

This drug has a stimulating effect on serotonin metabolism through phenfluramine, sympathetic nerve through phentramine. However, over time, the scientific literature began to collect information about its undesirable effects on the body. These data include changes in the heart's valve apparatus, a sharp rise in blood pressure, an increase in the number of heartbeats above normal, and so on, which also restricted the use of this drug (Michka et al., 2010).

Acompila, manufactured by the French company Sanofi-Aventus in Europe since 2006, has entered medical practice and is considered a pathogenetic drug for morbid obesity. This is because the drug drastically reduces the need for food by selectively inhibiting the type 1 receptors of Cannabinoid. However, this drug, which has been popular among gastroenterologists for only 3 years, has been banned from clinical use due to its undesirable side effects on the central nervous system. However, the synthesis of new drugs to inhibit Cannabinoid type 1 receptors has not escaped the interest of researchers. In 2010, OOO Company, "NPF", "Materials Medical Holding" registered a relatively mild drug called Dietress. However, it did not take long for this drug to be as successful as the others.

Although the failure of physicians to treat morbid obesity conservatively led to the abandonment of pharmacological drugs, individual researchers continued to search for new pharmacological drugs. At this stage, the search for drugs is aimed at creating a feeling of satiety. For this purpose, Gelesis Inc. Company has produced the drug in tablet form. This drug dissolves in the stomach and prevents the intake of excess food by creating a feeling of satiety (Nerobeev, 2014).

Thus, many drugs corresponding to the pathophysiological mechanism of morbid obesity and developed from it of non-alcoholic liver obesity (pentoxifylline, rosiglitazone, orlistat pioglitazone, metformin glucagon-like peptide 1 (GLP-1), vitamin E, angiotensin receptor blockers, probiotics, synobiotics) was synthesized and widely used in medical practice. The drugs synthesized should reduce insulin resistance, eliminate inflammation and, most importantly, reduce body weight.

One of such drugs is Orlistat, synthesized in 1998 in Belarus by Lekfarma Production Association. The proponent of this drug, S.A.Harriston and colleagues (2009) claim that patients with morbid obesity, as well as non-alcoholic hepatic obesity, had positive results from Orlistat by activating physical activity. According to the author, this drug, along with increasing physical activity significantly reduces body weight and improves the functional state of the liver.

We consider unfounded the arguments for the positive effect of the Orlistat given by S.A.Harriston and his colleagues in the article "Orlistat for patients with overweight and non-alcoholic steatohepatitis: randomized prospective study" published in 2009. First, the research was not divided into groups. As a rule, only Orlistat should be given to 1 group of patients. Without conducting such a study, it is incorrect for the authors to explain the positive result obtained under the influence of Orlistat. This is because by increasing physical activity during the day, for example, walking at a distance of 5-10 km at a brisk pace can lower body weight even without taking medication.

Clarification of the mechanism of pharmacological action of the drug is also not in its favor.

H.K.Lee has shown that this drug absorbs into the stomach and blocks the lipase molecule, which in turn prevents enzymes from breaking down fat (Lee et al., 2009). For this reason, about 30% of triglycerides are not absorbed by the body.

On the other hand, Riyan et al. (Ryan et al., 2010), James et al. (James et al., 2010) and others note that Orlistat is a contraindication for diseases of the cardiovascular system. In this regard, the use of Orlistat in morbid obesity is not recommended. Because, morbid obesity is the most associated disease of the cardiovascular system.

C.Zein et al. noted that pentoxifylline not only reduces body weight to some extent, but also has an emollient effect on non-alcoholic steatohepatitis (Zein et al., 2011). However, the results obtained cannot be considered reliable, as the drug was administered to 9 patients. On the other hand, the authors did not provide information in the article about the long-term consequences of taking the drug.

V.Ratziu et al. have found that rosliglitazone improves steatohepatosis level and significantly lowers aminotransferase level in blood (Ratziu et al., 2008). It is known that aminotransferase is a marker that reflects the functional state of the liver. In this regard, rosliglitazone can be considered a useful drug for the treatment of non-alcoholic steatohepatosis of the liver. However, Yu. Takahashi and colleagues have shown that rosliglitazone is a high risk factor for coronary heart disease (Takahashi et al., 2015). Taking into account that high body weight is a factor that accelerates atherosclerosis and creates real conditions for ischemia of the heart, it can be assumed that the use of rosliglitazone has a greater risk of developing myocardial pathology than the therapeutic effect.

In addition, the sale of this drug is currently completely banned in Europe, although it is partially banned in the United States.

Pioglitazone has been shown to significantly reduce elevated aminotransferase concentrations in the blood. Signs of steatosis and inflammation in the liver samples of these individuals showed a significant change in the positive direction (Sanyal et al., 2010).

However, pioglitazone is also banned in European countries because it is a trigger for bladder cancer.

Positive histological changes in the structure of the liver also occur after the administration of Liraglutide, a glycogen-like peptide-1 (GLP-1) analogue (Armstrong et al., 2016). However, although this study was a randomized study, no long-term results of the drug have been reported. The authors described only 48 hours of changes. In our opinion, the fact that the histological profile of the liver changes in a positive direction within 48 hours of any drug is not very convincing.

There are also various reports in the scientific literature on the consequences of taking Vitamin E in patients with non-alcoholic hepatic steatohepatosis with diabetes mellitus (Sanyal et al., 2010).

These data suggest that Vitamin E may have a positive effect on steatosis due to its antioxidant properties. However, numerous studies (Yoneda et al., 2010; Klein et al., 2011; Takeshita et al., 2014) have unequivocally shown that long-term intake of Vitamin E increases the risk of prostate cancer. Therefore, we believe that the use of Vitamin E in non-alcoholic steatohepatosis of the liver is unacceptable.

Sibturamine has also been widely used in the treatment of morbid obesity.

Sibturamine selectively captures serotonin and norepinephrine on the back side and stores them in brain neurons. This affects both parts of the energy balance. Therefore, unlike other drugs, sibturamine is a centrally acting drug that affects the donaminergic system (Florentin et al., 2008). In the Russian Federation, sibturamine is used under the name Reduksin. According to opinion of the researchers, sibturamine has a strong effect on 79% of patients, reducing their body weight and helping

to maintain that level for a long time (Vlasova et al., 2012; Romantsova et al., 2012; Ametov, 2013). At the same time, Sibutramine has attracted attention with its stimulating effect on carbohydrate metabolism, blood lipid profile and blood pressure.

An analysis of the literary data suggests that at morbid obesity lifestyle changes, increased physical activity, different diets, or medications does not provide a basis for complete cure of the disease. Summerbell et al. rightly point out that after stopping taking both drugs and drugs to reduce body weight, body weight begins to increase again (Summerbell et al., 2008).

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Morbid piylənmədə konservativ müalicənin rolu

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Məqalədə uzun bir dövr ərzində bədən kütləsinin artmasının, morbid piylənmənin və qaraciyərin qeyri-alkoqol mənşəli steatihepatozunun dərman, diyeta, həyat tərzinin dəyişdirilməsi, fiziki aktivlik vasitəsilə müalicəsi üsulları təhlil edilmişdir. Ədəbiyyat məlumatlarının təhlilindən belə bir qənaətə gəlinmişdir ki, morbid piylənmə zamanı istər həyat tərzinin dəyişdirilməsi, fiziki fəallığın artırılması, müxtəlif diyetalardan istifadə edilməsi və eləcədə dərman preparatlarının qəbulu xəstəliyin tam müalicəsinə zəmin yaratmır. İstər dərman preparatlarının və istərsə də bədən kütləsinin aşağı salınmasına yönəlmiş vasitələrin qəbulu dayandırıldıqdan sonra bədən kütləsi yenidən artmağa başlayır.

Açar sözlər: *Bədən kütləsi, morbid piylənmə, diyeta, dərman müalicəsi*

Роль консервативного лечения при патологическом ожирении

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В статье анализируются методы лечения в течение длительного периода увеличения веса, патологического ожирения и неалкогольного стеатогепатоза печени с помощью медикаментов, диеты, изменения образа жизни, физической активности. Анализ литературы показывает, что при патологическом ожирении изменение образа жизни, повышение физической активности, использование различных диет, а также лекарственных препаратов не обеспечивают полное излечение заболевания. После прекращения приема как лекарственных препаратов, так и средств для похудения, вес тела снова начинает расти.

Ключевые слова: *Масса тела, патологическое ожирение, диета, медикаментозное лечение*

Journal of Life Sciences & Biomedicine, vol. 3(76), No 1, p. 60-66 (2021)

In silico analysis of Dreb transcription factor genes in bread wheat

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Received: March 09, 2021; Received in revised form: April 09, 2021; Accepted: May 09, 2021

The dehydration responsive element binding (Dreb) genes which are representatives of the AP2 / ERF transcription factor family and playing a key role in drought-induced transcriptome in wheat were studied using *in silico* methods. For this purpose, information on relevant genes (Accession Nr. AF303376.1, AB193608.1, KM520370.1, DQ195068.1) was obtained from the NCBI. FASTA data of proteins corresponding to each gene were analyzed comparatively by the MAFFT CLUSTAL format alignment software, and the main conservative areas were identified. Two conservative functional amino acids specific for AP2 domain - valine and glutamine - were identified in positions 14 and 19 in all studied genes. Specific amino acid substitutions have been identified in the protein (DQ195068.1) that binds to the dehydration element specific to the D genome in the areas involved in the formation of the nuclear localization signal (NLS) and the α-helix structure. The results obtained could be a scientific basis for future laboratory studies of Dreb genes in wheat.

Keywords: *Dreb, AP2 domen, nuclear localization signal (NLS), α-helix, in silico analysis*

INTRODUCTION

Although the advances in genomics contributed to the improvement of some agriculturally important crops, similar efforts in wheat (Triticum spp.) were more challenging. This is attributed to the size and complexity of the wheat genome, and the lack of genome-assembly data for multiple wheat lines (Walkowiak et al., 2020, Alotaibi et al., 2021). The current knowledge of wheat biology and the molecular basis of central agronomic traits are not sufficient for wheat breeding. There is an urgent need for wheat research and breeding to accelerate genetic gain as well as to increase and protect wheat yield and quality traits for meeting the demands of human population growth (Zhu et al., 2021). Clarification of gene functions and availability of wheat genome sequence information as well as genome editing methods will open up new opportunities for improving crops under stress conditions (Rathan, 2021).

Some candidate genes involved in the adaptive responses to abiotic stress have been determined in cereals. Transcription regulators have been found to play an important role in the adaptation of plants against changing environmental conditions. According to large-scale transcriptome analyses, protective proteins and regulatory proteins are the main types of molecular stress responses (Shahzad et al., 2021). Proteins such as chaperones, osmotin, antifreeze proteins, mRNA-binding proteins protect cells against stress conditions. Regulatory proteins such as transcription factors, including myeloblastosis oncogene (MYB), basic leucine zipper (bZIP), NAM, ATAF, CUC (NAC), and dehydration responsive element binding (DREB) proteins rearrange the gene expressions to protect the plant from stresses. The interaction between transcription factors and cis-elements of target gene promoters is very important for the regulation of stress-related gene expression.

Dehydration responsive element-binding proteins are essential transcription factors that stimulate stress-related genes (Niu et al., 2020). Two types of DREB transcription factors were

https://doi.org/10.29228/jlsb.8
Available online 30 June 2021

observed: DREB1 and DREB2. They are contained in different signal transduction pathways under low temperature and dehydration, respectively. The C-repeat (CRT) and low-temperature-responsive element (LTRE) known as cis-acting elements both contain an A/GCCGAC motif similar to the core of the DRE sequence regulating cold-inducible gene expression. DREB1/DREB2 homologous genes were identified in several grasses, including wheat, rice, barley, sorghum, maize, oat, rye, and perennial ryegrass. Dreb1/CBF genes are stimulated by cold, whereas the Dreb2 genes are generally stimulated by dehydration, high salinity, and heat (Hassan et al., 2021). DREB family proteins belonging to the AP2/ERF transcription factor family contain one AP2/ERF DNA-binding domain.

We aimed at the detailed analyses of Dreb genes in the bread wheat genome. For this purpose, we performed *in silico* comprehensive analysis of four Dreb genes using the available nucleotide and protein sequences from the current databases.

MATERIALS AND METHODS

Sequence Sources

The complete cDNA and corresponding protein sequences of DREB in wheat (*Triticum aestivum* L.) were retrieved from the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/).

Multiple Sequence Alignment

Multiple Sequence Alignment is generally the alignment of three or more biological sequences (protein or nucleic acid) of similar length. Based on the results, homology can be assessed and the evolutionary relationships between the sequences studied. MAFFT v7.427 (https://mafft.cbrc.jp/alignment/server/)

CLUSTAL format alignment program was used for sequencing. MAFFT (Multiple Alignment using Fast Fourier Transform) is a high-speed multiple sequence alignment program.

Annotation of Functional Motifs

For searching functional motives in the selected Dreb genes, the relevant functions of Softberry, Inc. software (http://www.softberry.com/) intended for annotation of plant genomes was used.

Softberry, Inc. is known as a leading developer of software tools for genomic research focused on computational methods of high throughput biomedical data analysis, including software to support next-generation sequencing technologies, transcriptome analysis (with RNASeq data), SNP detection and selection of disease-specific SNP subsets.

NSITE-PL service was used to search for promoter/functional motives (http://www.softberry.com/berry.phtml?topic=nsitep&group=programs&subgroup=promoter) (Shahmuradov and Solovyev, 2015; Solovyev et al., 2010)

Annotation of Sub-Cellular Localization

ProtComp v. 9.0 service was used to study the localization of the studied gene products throughout cell compartments.

RESULTS AND DISCUSSION

Despite both the fundamental knowledge gained from relevant studies concerning the wheat genome and the importance of the crop, a comprehensive genome-wide analysis of gene content was not conducted until recently (Dabab Nahas et al., 2019). This was because of the large size, repeat content, and polyploid complexity of the genome. However, assembly of the 17-Gb allohexaploid genome of *Triticum aestivum* faced major difficulties, because it is composed of three large, repetitive, and closely related genomes. In the current study, the Dreb genes of bread wheat were analyzed *in silico*. For this purpose, four genes encoding the Dreb genes were selected from the GenBank database, and information about them was obtained. The first information we analyzed was Access Nr. JQ004969.1, the beta isoform of the DREB AP2 binding factor, labeled "*Triticum aestivum* DREB AP2 binding factor beta isoform mRNA, complete cds". The length of this mRNT is 1286 bp. The protein-encoding area is shown to be smaller, in other words, it is located in the area between 64-264 nucleotides. The id of the protein corresponding to this gene: "AEX59145.1". This gene is translated to the amino acid sequence as follows:

"MTVDRKHAEAAAAAPFEIPALQPGRTCGAEESTRSHVLVKPIGKSDLGDHVMGLIQSLKRSGDGKK".

The second researched information is an AP2-containing protein-encoding gene labeled "*Triticum aestivum* AP2-containing protein (Dreb1) mRNA, complete cds" with Access Nr. AF303376.1. The length of this locus is 1292 bp. The region encoding the protein covers an area between 252-1088 nucleotides. The id of the protein corresponding to this gene in GenBank is: "AAL01124.1". The translation of the gene into an amino acid sequence was as follows: "METGGSKREGDCPGQERKKKVRRRSTGPD SVAETIKKWKEENQKLQQENGSRKAPAKGS KKGCMAGKGGPENSNCAYRGVRQRTWGK WVAEIREPNRGNRLWLGSFPTAVEAARAYD DAARAMYGAKARVNFSEQSPDANSGCTLA PPLPMSNGATAASHPSDGKDESESPPSLISNA PTAALHRSDAKDESESAGTVARKVKKEVSN DLRSTHEEHKTLEVSQPKGKALHKAANVSY DYFNVEEVLDMIIVELSADVKMEAHEEYQD GDDGFSLFSY".

The Dreb gene searched in NCBI under the name "*Triticum aestivum* Wdreb2 mRNA for EREBP / AP2 type transcription factor, complete cds" is information available under Accession Nr. AB193608.1. The length of the current locus was 1456 bp. The region encoding the protein covers the area between 123-1157 nucleotides. The amino acid sequence of the EREBP / AP2 type transcription factor protein corresponding to this gene is shown as follows:

```
CLUSTAL format alignment by MAFFT (v7.481)

AAL01124.1      METGGSKREGDC----------PGQERKKKVRRRSTGPDSVAETIKKWKEENQKLQQE--
ABA08424.1      METGGSKREGDC----------PGQERKKKVRRRSTGPDSVAETIKKWKEENQKLQQE--
BAD97369.1      MTVDRKHAEAAAAAPFEIPALQPG--RKKRPRRSRDGPNSVSETIRRWKEVNQQLEHDPQ
                * .. .: *. .          **  ***: **   **:**:***::*** **:*:::

AAL01124.1      --NGSRKAPAKGSKKGCMAGKGGPENSNCAYRGVRQRTWGKWVAEIREPNRGNRLWLGSF
ABA8424.1       --NGSRKAPAKGSKKGCMAGKGGPENSNCAYRGVRQRTWGKWVAEIREPNRGNRLWLGSF
BD97369.1       GAKRARKPPAKGSKKGCMLGKGGPENTQCGFRGVRQRTWGKWVAEIREPNRVSRLWLGTF
                 : :**.********** *******::*.:******************** .*****:*

AAL01124.1      PTAVEAARAYDDAARAMYGAKARVNFSEQSPDANSGCTLAPPLPMSNGATAASHP-SDGK
BA0424.1        PTAVEAARAYDDAARAMYGAKARVNFSEQSPDANSGCTLAPPLPMSNGATAASHP-SDGK
BAD97369.1      PTAEDAARAYDEAARAMYGALARTNFPVHPAQAPAVAVPAAIEGVVRGASASCESTTTST
                *** :******:******** **.**. :..:* : .. *.   : .**:*:... : ..
AAL01124.1      DESESPPSLISNAPTAALH-RSDAKDESESAGTVARKVKKEVSNDL---------RSTHE
AB08424.1       DESESPPSLISNAPTAALH-RSDAKDESESAGTVARKVKKEVSNDL---------RSTHE
AD97369.1       NHSDVASSLPRQAQAPEIYSQPDALESTES--VVLESVEHYSHQDTVPDAGSSISRSTSE
                :.*: ..**  :* :. :: :.** :.:**  .* ..*::   :*          *** *

AAL01124.1      EH--KTLE--VSQPKGKALHKAANVSYDYFNVEEVL-----DMIIVEL----------SA
AA08424.1       EH--KTLE--VSQPKGKALHKAANVSYDYFNVEEVL-----DMIIVEL----------SA
BAD97369.1      EDVFEPLEPISSLPDGEA---------DGFDIEELLRLMEADPIEVELVTGGSWNGGANT
               *.  :.**   * *.*:*         * *::**:*     * * ***          .:

AAL01124.1      DVKMEAHEE-YQDGDDGFSL-------FSY--------------------------
ABA08424.1      DVKMEAHEE-YQDGDDGFSL-------FSY--------------------------
BAD97369.1      GVEMGQQEPLYLDGLDQGMLEGMLQSDYPYPMWISEDRAMHNSAFHDAEMSEFFEGL
                .*:*  :*  * ** *   *       :.*
```

Fig. 1. CLUSTAL format alignment by MAFFT. Specific nuclear localization signal (NLS) area for the Dreb genes is highlighted in green, the amino acid substitutions in this area are highlighted in red. The valine 14 and glutamic acid 19 amino acids of the AP2 domain are yellow. The specific sequence for α-helix is pink, and the amino acid changes observed in this region are highlighted in blue.

"MTVDRKHAEAAAAAPFEIPALQPGRKKRP
RRSRDGPNSVSETIRRWKEVNQQLEHDPQG
AKRARKPPAKGSKKGCMLGKGGPENTQCG
FRGVRQRTWGKWVAIREPNRVSRLWLGTFP
TAEDAARAYDEAARAMYGALARTNFPVHP
AQAPAVAVPAAIEGVVRGASASCESTTTSTN
HSDVASSLPRQAQAPEIYSQPDALESTESVV
LESVEHYSHQDTVPDAGSSISRSTSEEDVFEP
LEPISSLPDGEADGFDIEELLRLMEADPIEVE
LVTGGSWNGGANTGVEMGQQEPLYLDGLD
QGMLEGMLQSDYPYPMWISEDRAMHNSAF
HDAEMSEFFEGL". The protein corresponding to this gene is placed in GenBank with the id "BAD97369.1".

Finally, the last gene we studied is information placed under the name "*Triticum aestivum* genome D dehydration-responsive element-binding protein (Dreb1) gene, complete cds" with Accession Nr. DQ195068.1. This locus is longer and amounts to 1748 bp. The region encoding the protein consists of areas between nucleotides 20-69 and 771-1557, and covers two exons. The id of this protein, which combines the element responsible for dehydration, is "ABA08424.1" in GenBank. The translation of a nucleotide sequence into an amino acid sequence was as follows: "METGGSKREGDCPGQERKKKVRRRSTGPD
SVAETIKKWKEENQKLQQENGSRKAPAKGS
KKGCMAGKGGPENSNCAYRGVRQRTWGK
WVAEIREPNRGNRLWLGSFPTAVEAARAYD
DAARAMYGAKARVNFSEQSPDANSGCTLA
PPLPMSNGATAASHPSDGKDESESPPSLISNA
PTAALHRSDAKDESESAGTVARKVKKEVSN
DLRSTHEEHKTLEVSQPKGKALHKAANVSY
DYFNVEEVLDMIIVELSADVKMEAHEEYQD
GDDGFSLF"

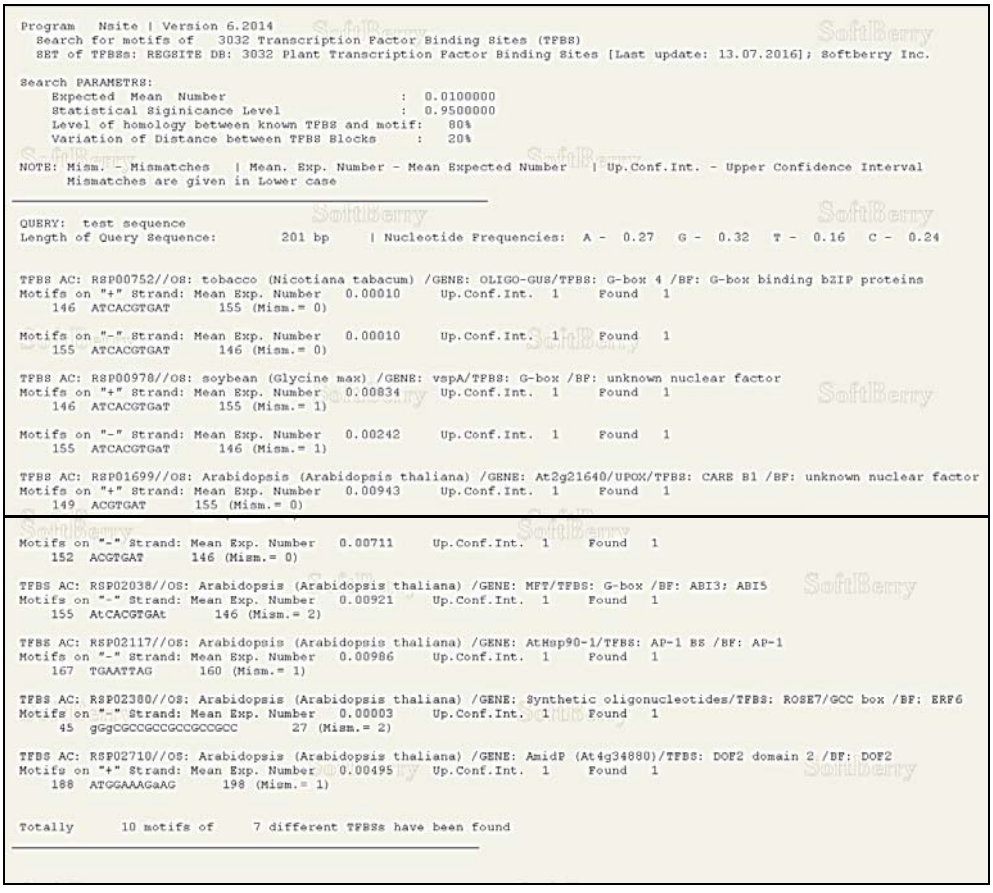

Fig. 2. Annotation of functional motifs for *Triticum aestivum* DREB AP2 binding factor gen (Accession Nr. JQ004969.1) using NSITE-PL (http://www.softberry.com).

```
5724 multiple located sequences are accepted
ProtComp Version 9.0. Identifying sub-cellular location (Plant)
Seq name:  test sequence, Length=278
Significant similarity in Location DB - Nuclear
Database sequence: AC=Q0JQF7 Location:Nuclear  DE  Dehydration-responsive element-
Score=98, Sequence length=275, Alignment length=270
Predicted by Neural Nets - Extracellular (Secreted) with score    1.0
Integral Prediction of protein location: Nuclear with score    8.9
Location weights:     LocDB / PotLocDB / Neural Nets / Pentamers / Integral
 Nuclear              10.0 /      3.0 /        0.00 /      0.06 /     8.93
 Plasma membrane       0.0 /      0.0 /        0.97 /      0.03 /     0.71
 Extracellular         0.0 /      0.0 /        0.97 /      1.02 /     0.00
 Cytoplasmic           0.0 /      0.0 /        0.00 /      2.14 /     0.00
 Mitochondrial         0.0 /      0.0 /        0.00 /      1.43 /     0.00
 Endoplasm. retic.     0.0 /      0.0 /        0.00 /      0.09 /     0.00
 Peroxisomal           0.0 /      0.0 /        0.97 /      0.00 /     0.10
 Golgi                 0.0 /      0.0 /        0.10 /      0.31 /     0.00
 Chloroplast           0.0 /      0.0 /        0.00 /      0.21 /     0.01
 Vacuolar              0.0 /      0.0 /        0.00 /      0.00 /     0.26
```

Fig. 3. Annotation of sub-cellular localization for *Triticum aestivum* genome D dehydration-responsive element-binding protein (protein_id "BAD97369.1") using ProtComp 9.0 (http://www.softberry.com).

CLUSTAL format alignment by MAFFT (v7.481) revealed significantly conservative areas in the amino acid sequences of these genes. The beta isoform (protein_id: "AEX59145.1") of the DREB AP2 binding factor, with Accession Nr. JQ004969.1 in GenBank has been excluded due to inconsistencies in this alignment. Homology is more pronounced between the Dreb1 gene with Accession Nr. AF303376.1 (protein_id. "AAL01124.1") and Wdreb2 genes with Accession Nr. AB193608.1 in GenBank. In the amino acid sequences of both proteins, a specific nuclear localization signal (NLS) for the Dreb genes is observed in the peptide sequence (RKKKVR) (highlighted in green in Figure 1). In the amino acid sequence encoded by the dehydration-responsive element-binding protein (Dreb1) gene (Accession Nr. DQ195068.1) of the *Triticum aestivum* genome D, in the 4th position of the signal peptide sequence (RKKRPR), arginine is located instead of lysine and in the 5th position, proline amino acid is located instead of valine (substituted amino acids in Figure 1 are highlighted red).

Besides, alignment of amino acid sequences corresponding to the Dreb gene by MAFFT revealed the AP2 domain with the two conserved functional amino acids (valine (V) and glutamic acid (E)) at the 14th and 19th residues which play crucial roles in recognition of the DNA - binding sequence (Fig. 1). However, according to the results of further research, E19 might not be as necessary as V14 for this case (Sakuma et al. 2002). Nevertheless, both of these amino acids are found in the three proteins we studied (highlighted yellow in the figure).

The characteristic sequence for the α-helix (VEAARAYDDAARAMYG) was also identified in our analysis *in silico*. Interestingly, BAD97369.1 protein contains amino acid substitutions also in this area. Valine, the primary amino acid involved in the formation of α-helix, was replaced by glutamine in this protein, and the second glutamine was replaced by the amino acid aspartate. In the ninth position of this domain, on the contrary, aspartate was replaced by glutamine. It should be noted that the gene of this protein, which changes both in the NLS sequence and in the α-helix region, is a Dreb gene specific to the D genome. For the first time, Pandey et al. (2014) built the tertiary structure of DREB2 protein from wheat by homology modeling based on the crystal structure of GCC-box binding domain of Arabidopsis thaliana, which contributed to understanding the structure-function relationships. Protein docking with the DNA containing GCC-box revealed more similarities between AP2/EREBP protein of A. thaliana and T. aestivum. A protein was found to interact through their β-sheet, with the major DNA groove by hydrogen and hydrophobic bond, which provides structural stability to the molecule. This model comprises a three-stranded antiparallel β-

heet followed by α-helix and relatively unstructured C'-terminal.

To search for functional motives in the selected DREB genes, NSITE-PL service for annotation of plant genomes of the Softberry, Inc. (http://www.softberry.com/) software was used (Shahmuradov and Solovyev, 2015). Ten motifs for seven different transcription factor-binding sites (TFBS) were found in *Triticum aestivum* DREB AP2 binding factor beta isoform with Accession Nr. JQ004969.1 (Figure 2). Seventeen motifs for 15 different TFBS were found in *Triticum aestivum* AP2-containing protein (Dreb1) with Accession Nr. AF303376.1. Nine motifs for eight different TFBS in *Triticum aestivum* Wdreb2 mRNA for EREBP/AP2 type transcription factor (Accession Nr. AB193608.1) and seventeen motifs for fifteen different TFBS in *Triticum aestivum* genome D dehydration-responsive element-binding protein (Dreb1) gene (Accession Nr. DQ195068.1) were found.

Annotation of sub-cellular localization was performed by the ProtComp v. 9.0 service of Softberry for the studied products. Nuclear location was determined for three of the transcription factors studied (Figure 3), and extracellular localization was found only for the DREB AP2 binding factor beta isoform. This result requires more in-depth research.

In silico identification and characterization of the genes in various organisms under different conditions got importance due to growing data in the data bases (Dabab Nahas et al., 2019). Our analyses could be a scientific base to understand Dreb genes and proteins to further wet lab studies in wheat plants.

ACKNOWLEDGMENT

This work was supported by the Science Development Foundation under the President of the Republic of Azerbaijan – Grant № EIF-ETL-2020-2(36)-16/15/3-M-15).

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Yumşaq buğdada Dreb transkripsiya faktoru genlərinin *in silico* analizi

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Buğdada quraqlıqla induksiya olunan transkriptomda əsas yer tutan AP2/ERF transkripsiya faktorları ailəsinin üzvlərindən dehidratasiyaya cavabdeh element birləşdirən (Dreb) genlər *in silico* analiz edilmişdir. Bu məqsədlə NCBI məlumat bazasından genlər (qeydiyyat nömrələri: AF303376.1, AB193608.1, KM520370.1, DQ195068.1) haqqında məlumatlar əldə edilmişdir. Hər bir genə uyğun proteinlərin FASTA məlumatları MAFFT CLUSTAL format düzlənmə proqramı ilə müqayisəli analiz edilərək əsas konservativ sahələr müəyyən edilmişdir. Tədqiq olunan genlərin hamısında AP2 domen üçün spesifik iki konservativ funksional amin turşusu - valin və qlutamin 14-cü və 19-cu vəziyyətlərdə müəyyən edilmişdir. D genomu üçün spesifik olan dehidratasiyaya cavabdeh elementi birləşdirən proteində (DQ195068.1) nüvədə lokalizasiya siqnalının (NLS) və fəza quruluşunda α-spiral quruluşun yaranmasında iştirak edən sahələrdə spesifik amin turşu əvəzlənmələri müəyyən edilmişdir. Əldə olunan nəticələr buğda bitkisində Dreb genlərin gələcək laboratoriya tədqiqatları üçün elmi əsas ola bilər.

Açar sözlər: *Dreb, AP2 domen, nüvədə lokalizasiya siqnalı (NLS), α-spiral quruluş, in silico analiz*

In silico анализ генов факторов транскрипции Dreb в мягкой пшенице

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С использованием методов *in silico* были изучены относящиеся к представителям семейства факторов транскрипции AP2/ERF и играющие ключевую роль в индуцированной засухой транскриптоме пшеницы гены, связывающие элемент, ответственный за дегидратацию (Dreb). С этой целью информация о соответствующих генах (номер доступа AF303376.1, AB193608.1, KM520370.1, DQ195068.1) была получена из NCBI. Данные FASTA белков, соответствующих каждому гену, были сравнительно проанализированы с помощью программного обеспечения для выравнивания MAFFT CLUSTAL, идентифицированы основные консервативные области чтения. Две консервативные функциональные аминокислоты, специфичные для домена AP2 - валин и глутамин - идентифицированы в положениях 14 и 19 во всех изученных генах. Определенные аминокислотные замены были идентифицированы в белке (DQ195068.1), который связывается с элементом дегидратации, специфичным для генома D, в областях, участвующих в формировании сигнала ядерной локализации (NLS) и структуры α-спирали. Полученные результаты могут стать научной основой для будущих лабораторных исследований генов Dreb у пшеницы.

Ключевые слова: *Dreb, домен AP2, сигнал ядерной локализации (NLS), α-спираль, in silico анализ*

Journal of Life Sciences & Biomedicine, vol. 3(76), No 1, p. 67-76 (2021)

Raman spectroscopy of complex defined media: Biopharmaceutical applications

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Received: May 14, 2021; Received in revised form: June 12, 2021; Accepted: June 19, 2021

Hamster ovary cells were grown in a batch culture flask for 10 days in an incubator maintained with 5% CO_2 and 37 °C temperature. The 2 ml supernatant was collected each day, in duplicate, and was stored in a freezer at -20 °C. Concentrations of glucose and lactate were estimated, in the supernatant, on an HPLC machine, and the same sample was used to immediately obtain the results of Raman measurements. We demonstrate the detection of glucose and lactate concentrations with high accuracy in the supernatants of hamster ovary (HO) cell culture, grown in shake flasks in batch fermentation mode, using Raman spectroscopy and explicit model-based classical least squares (CLS) algorithm. A deterministic Raman spectral library of pure components was created by acquiring Raman spectra from credible nutrient media constituents and HO cell culture metabolites. Only analyzers present with concentrations above the instrument's detection limit were included in this library. Residuals obtained after CLS analyses were used to identify missing components and to generate a revised library. An algorithmic sieve was thus, construed to obtain an appropriate Raman spectral library from a complex chemical mixture that is well-defined but an industrial secret. High performance liquid chromatography (HPLC) was used to provide reference glucose and lactate concentrations. We demonstrate the detection of glucose and lactate concentrations in the supernatants of HO cell culture using Raman spectroscopy and explicit model-based CLS analysis.

Keywords: *Raman spectroscopy, bioreactors, classical least squares, glucose detection, lactate detection*

INTRODUCTION

Real time monitoring and control of bioprocesses are essential in improving their efficiency and reducing the cost of the final product. With the advancement of biotechnology, bypasses are no longer restricted to the production of alcohols, organic and amino acids, and small biomolecules such as insulin, enzymes, and some antibiotics. Now, they are also a method of choice for using modified cell culture expression systems and the production of large biomolecules such as monoclonal antibodies that include some vaccines for human use (Zhu, 2012).

Among the mammalian cell culture expression systems, the relatively high robustness of hamster ovary (HO) cells in different bioreactor systems is well established. Furthermore, HO cells are very efficient in posttranslational glycosylation of proteins, making them closely resemble human glycosylation patterns in order to avoid or to reduce the immune responses after drug administration. Some examples of such proteins are follicle stimulating (Yamamoto et al., 2011; Whelan et al., 2012).

To enhance the technologies available for monitoring and control of environmental parameters in bioreactors, several optical and spectroscopic modalities have been investigated as they are non-invasive and provide information in real time. Raman spectroscopy is one of the most promising techniques in this regard. It is based on the inelastic scattering of light and provides a biochemical fingerprint for the sample under investigation.

https://doi.org/10.29228/jlsb.9
Available online 30 June 2021

High chemical specificity of Raman spectroscopy is the result of its measurement of vibrational energies in chemical bonds. Near infrared (NIR) laser excitation helps to minimize luminescence background generated either from the substrate or from the biological sample being probed. It also has a higher penetration depth of biological materials and less tissue damage at a higher excitation power compared with visible excitation. Miniaturization and integration of Raman technology are highly desirable, and in recent years, there has been an increase in efforts in this direction (Abu-Absi et al., 2011; Li et al. 2010).

Raman spectroscopy coupled with Chemometrics has recently been used for real time monitoring and simultaneous prediction of multiple culture parameters including glutamine, glutamate, glucose, lactate, ammonium, viable cell density, and total cell density in HO cell culture bioreactors. It has also been used for the rapid identification, characterization, and quality assessment of complex cell culture media components used for industrial mammalian cell culture. But, all of the studies have used implicit models, where reference measurements on a training sample set are performed to acquire information about the system to be investigated. A model is thus constructed, based on the training set, which implicitly accounts for any physical effects that influence the measured Raman spectra, allowing concentration estimation of unprocessed samples. However, the training samples are system specific and only model the particular system under investigation.

They are not generally applicable to other systems. Implicit models do have utility and validity in bioprocess supervision applications, where the same bioreaction is to be repeatedly monitored for production purposes, but they have limited utility in bioprocess development applications where variations in operating conditions, growth rates, and medium composition are a necessary requirement.

Explicit methods, based on physical modeling the system to be analyzed, are preferred for bioprocess development applications. 161 Explicit models have previously been used to estimate the ethanol concentration in Baker's yeast fermentation and classical least-squares (CLS) fitting was used to estimate glucose and spiked quantities of glutamine, lactate, and ammonia, in filtered samples of a bioreactor (Lee et al., 2004; Shope et al., 1987).

The industrial mammalian cell culture requires complex nutrient media components. Chemically defined media components are preferred to avoid unknown parameters and subsequently facilitate the regulatory approval of the product. The exact composition of the nutrient media in most cases is kept an industrial secret. Hence, it is not straightforward to use explicit methods such as CLS analysis for the prediction of metabolite concentrations.

In this article, we have shown that explicit model-based CLS algorithm can be used to detect mammalian (HO) cell culture metabolites (glucose and lactate) with high accuracy using NIR Raman spectroscopy. An algorithmic sieve was construed and an appropriate Raman spectral library obtained from a complex chemical mixture that is well-defined but kept confidential by respective pharmaceutical industries. High performance liquid chromatography (HPLQ was used to obtain reference concentrations of glucose and lactate.

MATERIALS AND METHODS

Mammalian cells. Hamster ovary cells were grown in a batch culture flask for 10 days in an incubator maintained with 5% CO_2 and 37 °C temperature. Similarly, HO cells were batch cultured for 13 days. The 2 ml supernatant was collected each day, in duplicate, and was stored in a freezer at -20°C. Concentrations of glucose and lactate were estimated, in the supernatant, on an HPLC machine, and the same sample was used to obtain Raman measurements immediately.

Confocal near infrared Raman spectroscopy system. Short description of spectroscopy: Raman scattering studies were carried out on a three-dimensional confocal Raman microspectrometer Nanofinder 30 (Tokyo Instr.), Excitation wavelength λ=532 nm. The radius of the cross section of the incident laser beam was approximately 4 μm. The investigations were carried out in the geometry of backscattering. The radiation receiver was a cooled CCD camera (-70◦C) operating in the photon counting mode. All measurements are taken at an exposure time of 1 second and an excitation

Table 1. Comparison of explicit and impilicit calibration models

Whelan et al. Partial least (implicit)	This work Partial least squares (PLS) [leave-one-out cross validation (LOOCV)]					
	R^2	Standard error	R^2	Standard error	R^2	Standard error
Glucose	0.91	2.09	0.99	2.4	0.98	3.5
Lactate	0,99	11.49	0.99	3.1	0.99	5.2

Table 2. List of constituents present in hamster ovary cell culture nutrient media (adapted from Scröder et al. (2004))

Inorganic salts	Molar concentration (mM)	Carbohydrates	Molar concentration (mM)
$CaCL_2$	1.9	d-Clucose	34
$CuSO_4{\cdot}5H_2O$	$4.81x10^{-6}$	I-Amino acids	0.377
$FeSO_4{\cdot}7H_2O$	$1.20x10^{-3}$	I-Alanine	0.879
KNO_3	$9.02x10^{-4}$	I-Arginine ·HCl	0.267
KCl	6.51	I-Asparagine·H_2O	0.31
$MgSO_4$	0.576	I-Cysteine-HCl·H_2O	0.349
NaCl	52	I- Cysteine · 2HCl	0.652
$NaHCO_3$	48.8	I-Glutamic acid	7.2
Na_2HPO_4	0.4	I-Glutamine	0.52
$NaH_2PO_4{\cdot}H_2O$	1.25	Glycine	0.28
$Na_2SeO_3{\cdot}5H_2O$	$9.98x10^{-5}$	I-Histidine ·HCl·H_2O	0.973
$ZnSO_4{\cdot}7H_2O$	$1.20x10^{-3}$	I-Isoleucine	1
		I-Leucine; I-Lysine ·HCl	1.04
Vitamins and miscellaneous compounds			
dl-Pantothenic acid, calcium salt	$1.09x10^{-2}$	I-Methionine	
Choline chloride	$7.44x10^{-2}$	I-Phenylalanine	
Ethanolamine	0.02	I-Proline	
Folic acid	$1.21x10^{-2}$	I-Serine	
Hypoxanthinea	0.1	I-Theronine	
İ-Inositol	$8.79x10^{-2}$	I-Tryptophan	
Linoleic acid	$1.20x10^{-4}$	I-Tyrosine comma>disc	
Lipoic acid	$4.07x10^{-4}$	I-Valine	
Methotrexate (MTX)b			
Nicotinamide	$1x10^{-4}$ to 0.1		
Phenol red	$3.94x10^{-2}$		
Pluronic F-68c	$5.21x10^{-2}$		
Pyridoxal·HCl	$2.36x10^{-2}$	Peptones and proteins	
Pyridoxine·HCl	$1.21x10^{-4}$	Fetuid	
Riboflavin	$1.32x10^{-3}$	İnsulin	
Sodium pyruyate	2.51	Holo-Transferrin	
Thiamine·HCl	$1.46x10^{-2}$	Casein peptone	
Thymidinea	0.016	Soybean flour peptone	
Vitamin B12	$4.13x10^{-4}$	Soybean flour brothe	

CLS analysis. Because the exact composition of HO cell culture nutrient medium is kept confidential by respective pharmaceutical industries, we decided to select nutrient media constituents and HO cell culture metabolites from a reliable literature. For example. Table 1 by Schroder et al., provides information in this regard and is used to develop the Raman spectral library (Schroder et al., 2004). This table is shown in Fig. 1. We initially selected only those constituents from Fig. 1, which were known to generate Raman spectra (for example, the inorganic salts do not show Raman spectra as they dissociate into ions in an aqueous solution) and were present in concentration above the Raman instrument detection limit of ~1 mM. Because several of the amino adds mentioned in Table 1

have concentration reaching 1 mM, we selected the ones with high Raman cross section. Zhu et al. provide in detail the Raman spectra of amino acids in solution (Zhu et al., 2011).

A deterministic Raman spectral library of pure components was thus obtained from credible nutrient media constituents and HO cell culture metabolites and was used as a calibration routine for the CLS analysis. Phosphate buffered saline (PBS) was used to prepare all pure component solutions. Raman spectra were acquired from each day's supernatant. Polynomial fitting was not used to estimate and remove fluorescence background from Raman spectra before using the CLS analysis and performing concentration prediction for glucose and lactate. Cosmic rays were removed from the Raman spectra, and fifth order smoothing using Savitzky-Golay algorithm was performed using MATLAB to suppress noise. The Raman spectra were normalized with respect to the CLS coefficient of water. It was also taken care that the concentration prediction of all basis spectra constituents generates non-negative numbers. A final Raman spectral library was thus conceived. Using CLS calibration routine developed with this Raman spectral library, concentrations of glucose and lactate were predicted. HPLC was used to obtain reference concentrations of glucose and lactate.

Underlined constituents were part of the Roman spectral library used for classical least squares (CLS) calibration routine development (Schroder et al., 2004).

Calculated as the root mean-squared error of predication, using Raman-predicted concentration values and HPLC-generated reference concentration values.

RESULTS AND DISCUSSION

The Raman spectral library, which was obtained after implementing the algorithmic sieve, is shown in Fig. 2. In the process of obtaining this library, some constituents that were mentioned as ~1 mM concentration in Fig. 1 such as L-leucine and L-valine were removed. Their presence made the concentration predictions worse and also the CLS fit to the supernatant Raman spectrum- produced residuals with structure. Removing any of the Raman spectra present in spectral library of Fig. 2 made the concentration predictions worse as did the addition of spectra from additional constituents. Thus, a final Raman spectral library for CLS analysis was conceived.

Raman spectra of L-arginine HCI, glucose, L-threonine, L-valine, sodium pyruvate, sodium bicarbonate, glycine, and sodium lactate were acquired at 20 mM concentration each in PBS. Raman spectra of PBS and water were also acquired and included in the Raman spectral library.

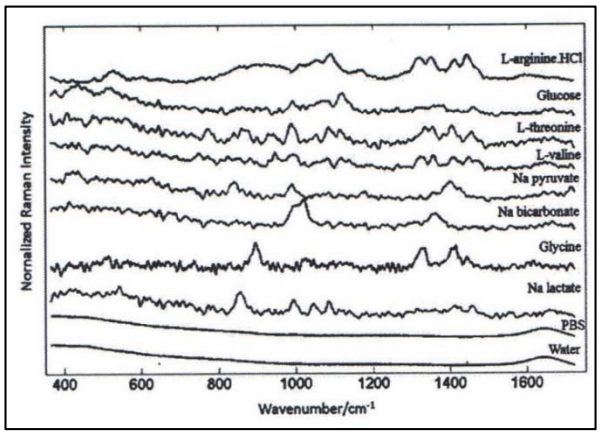

Fig. 1. Raman spectral library consisting of pure components; some selected from hamster ovary cell culture constituents mentioned in Fig. 1. PBS, phosphate buffered saline.

In Fig. 1, the Raman spectrum of water has been subtracted from each of the solute Raman spectrum for better visualization. The main Raman peaks and bands observed in the spectra of pure components mentioned in Fig. 2 are listed in Table 2. A broad Raman peak at 1641 cm^{-1} was observed in the Raman spectrum of water, and a weak Raman peak at 992 cm^{-1} was observed in the Raman spectrum of PBS.

The detection limit of sodium lactate and D-glucose aqueous solutions is shown in Fig. 3, using the NIR Raman spectroscopy system. Flere, we define the detection limit as noise equivalent concentration, i.e. the concentration at which $SNR = 1$. It was found that the detection limit of sodium lactate is better than 3 mM (obtained after extrapolation of curve), while that of D-glucose is better than 2 mM. The data in this figure were an average of three measurements, and the error bars were smallerthan the shown size of data points.

Table 3. Main Raman peaks (Raman shift cm^{-1}) observed in the Raman spectra of pure components mentioned in Fig.1. Water exhibited a broad Raman peak at 1641 cm^{-1} white phosphate buffered saline (PBS) at 992 cm^{-1}

L-arginine HCl	Clucose	L-threonine	L-valine	Na pyruvate	Na bicarbonate	Clycine	Na lactate
-	435	-	-	-	-	-	-
-	516	-	-	-	-	-	-
530	-	-	-	-	-	-	-
-	-	-	750	-	-	-	-
-	-	771	-	-	-	-	-
-	-	-	-	840	-	-	-
-	-	-	-	-	-	-	855
-	-	861	-	-	-	-	-
-	-	-	-	-	-	897	-
919 (broad)	-	-	-	-	-	-	-
-	-	-	946	-	-	-	-
-	990	990	991	991	-	-	992
-	-	-	-	-	1027	-	-
-	-	-	-	-	-	-	1045
-	-	1055	-	-	-	-	-
-	1076	-	-	-	-	-	-
-	-	1085	1084	-	-	-	1085
1091	-	-	-	-	-	-	-
-	-	1112	-	-	-	-	-
-	1121	-	-	-	-	-	-
1172	-	-	-	-	-	-	-
1324	-	-	1324	-	-	-	-
-	-	1335	-	-	-	1333	-
1356	-	-	1358	-	1361	-	-
-	1374	-	-	-	-	-	-
-	-	-	-	1401	-	-	-
1415	-	1409	1412	-	-	1413	-
1453	1460	1456	1455	-	-	-	1456

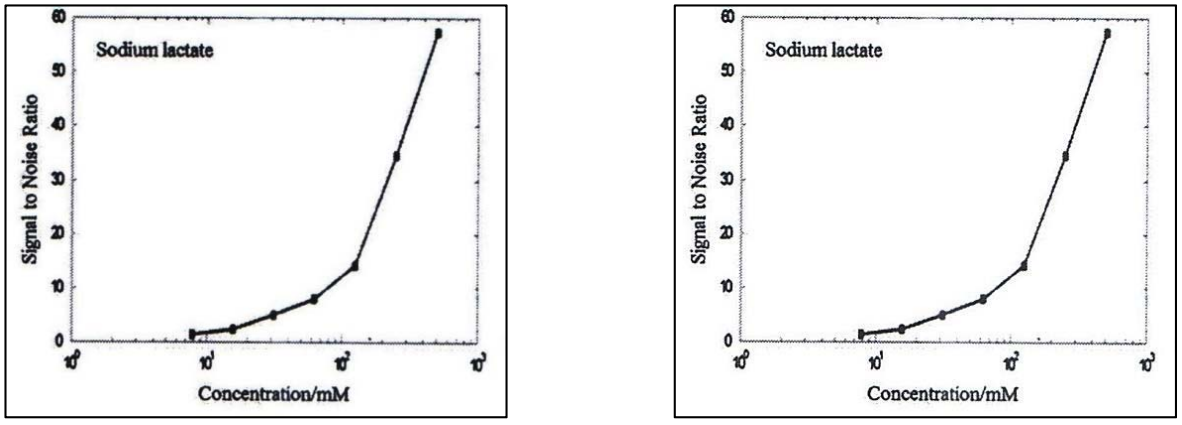

Fig. 2. Detection limit of sodium lactate and D-glucose solutions measured using near infrared (NIR) Raman spectroscopy system. Error bars were smaller than the shown size of data points. For D-glucose, the mean of area under Raman peak at 1121 cm^{-1} was used for signal calculation, while for sodium lactate, the mean of area under Raman peak at 855 cm^{-1} was used.

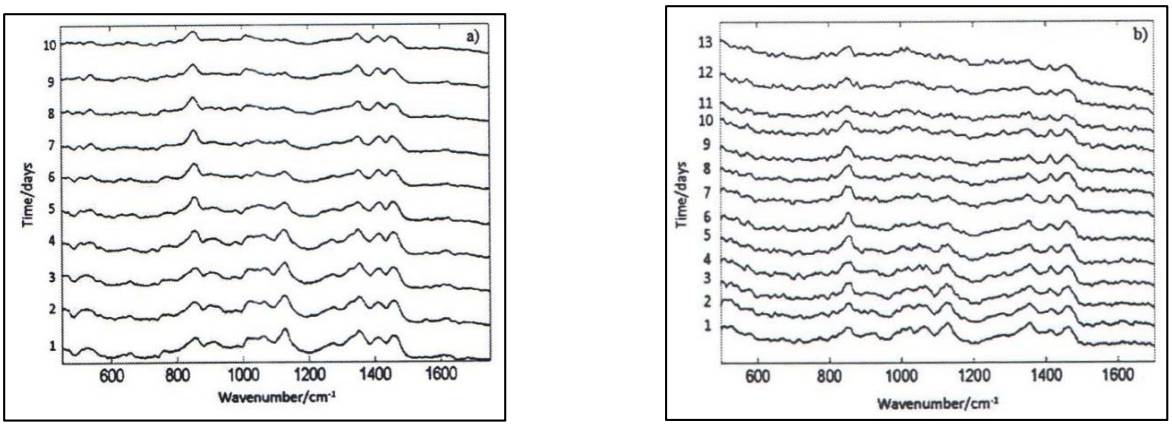

Fig. 3. Raman spectra obtained from hamster ovary cell culture supernatants using (a) Invitrogen cell line and (b) Sanofi cell line during 10 and 13 days of shake flask culture, respectively, in batch mode.

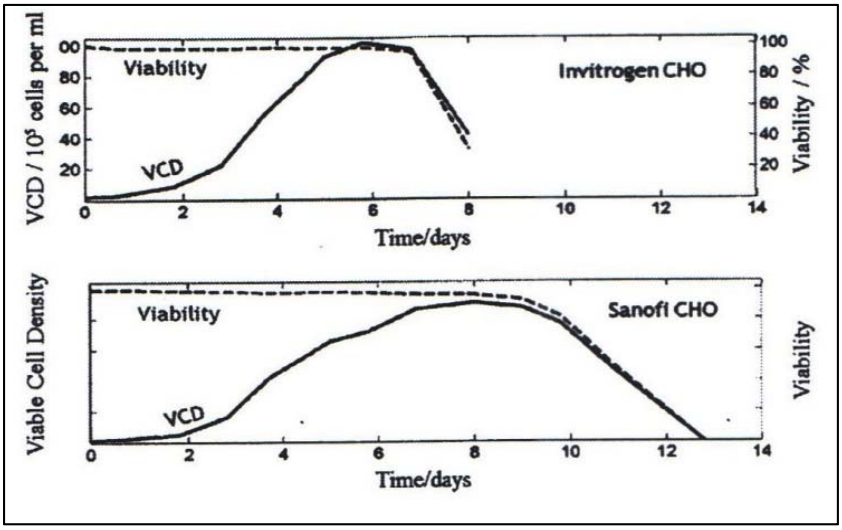

Fig. 4. Growth curves for Invitrogen and Sanofi hamster ovary cell cultures. Sanofi cell line is optimized for antibody production and hence has a longer stationary phase. VCD, viable cell density.

Each measurement consisted of ten spectral acquisitions. For D-glucose, the mean of area under Raman peak at 1121 cm^{-1} was used for signal calculation, while for sodium lactate, the mean of area under Raman peak at 855 cm^{-1} was used. Median standard deviation across the whole Raman spectrum of ten Raman spectra was used for noise estimation.

Figure 4(a) shows the Raman spectra obtained from Invitrogen HO cell culture supernatants on each of the 10 days, while Fig. 4(b) shows the same for Sanofi HO cell culture supernatants for 13 days. PBS Raman spectrum has been subtracted from each day's supernatant Raman spectrum for better visualization. The difference in Raman spectra on each day can clearly be observed. Main Raman peaks at 853 cm^{-1}, 900 cm^{-1} (broad), 1021 cm^{-1}, 1064 cm^{-1}, 1128 cm^{-1}, 1276 cm^{-1}, 1352 cm^{-1}, 1412 cm^{-1}, and 1458 cm^{-1} were observed.

The growth curves for Invitrogen and Sanofi HO cell cultures respectively are shown in Fig. 5. The Sanofi HO cell culture has a longer stationary phase because it has been optimized for antibody production.

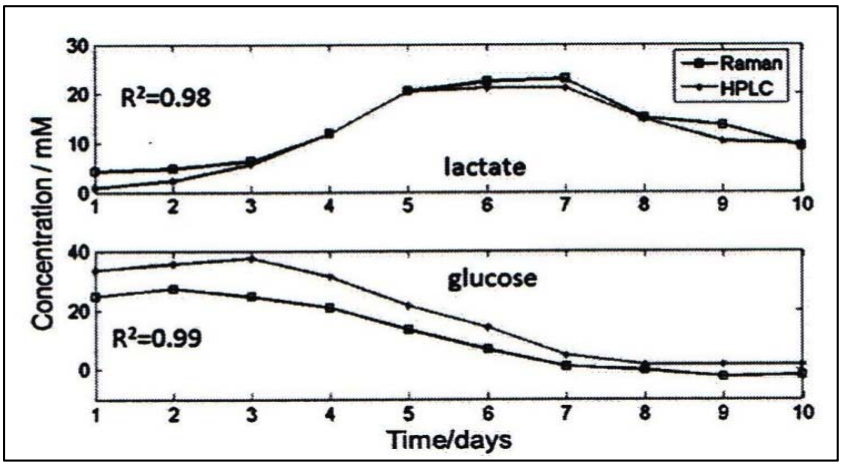

Fig. 5. Prediction of glucose and lactate concentrations in invitrogen hamster ovary cell culture supernatants using Raman spectroscopy and explicit model-based classical least squares (CLS) analysis. The error bars were equivalent to 2 mM concentration for Raman measurements and 0.5 mM concentration for high performance liquid chromatography (HPLC) measurements (Abu-Absi et al., 2011).

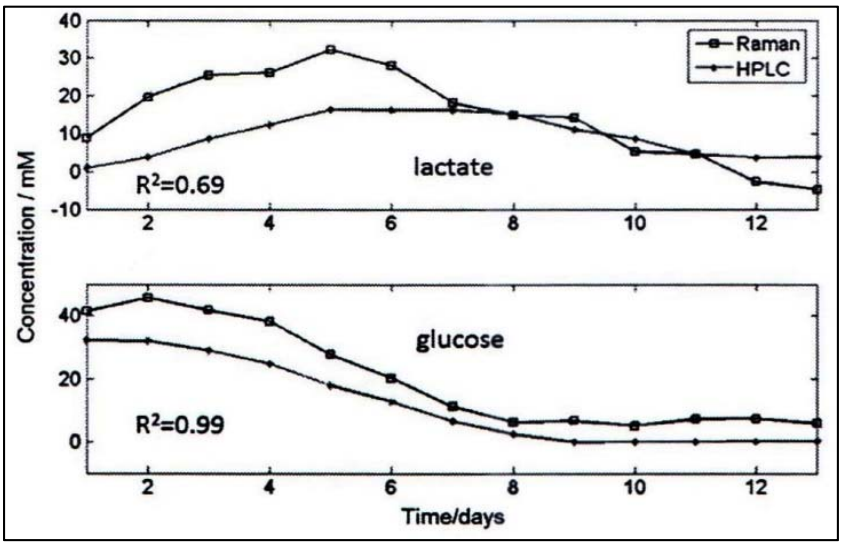

Fig. 6. Prediction of glucose and lactate concentrations in Sanofi hamster ovary cell culture supernatants using Raman spectroscopy and explicit model-based classical least squares (CLS) analysis. HPLC, high performance liquid chromatography (Abu-Absi et al., 2011).

Figure 6 shows the glucose and lactate concentration prediction in Invitrogen HO cell culture supernatants using Raman spectroscopy and explicit model-based CLS algorithm. Comparison with reference concentration values obtained using HPLC is also shown. The experiment was performed three times, and average values of measurement are plotted. The error bars were equivalent to 2 mM concentration for Raman measurements and 0.5 mM concentration for HPLC measurements. The time required for glucose and lactate concentration measurements using HPLC was about 45 min per sample, after the HPLC was calibrated

with the correct measurement column. Calibration of HPLC with a new column required almost 2h. Because the same column could not be used for glucose/lactate and amino acids, for example, HPLC was found to be labor intensive. On the other hand, time required for Raman measurements was about 8 min per sample after the charged coupled device camera of NIR Raman system had been cooled to -90 °C. Cooling of the camera required about an hour. But once ready, the Raman system could be used to predict concentrations of multiple analytes from a single spectral measurement.

We also used an implicit calibration model and performed partial least squares (PLS) analysis using the Invitrogen HO cell culture supernatant Raman spectra from 10 days and the reference concentrations provided by HPLC. The results of leave-one-out cross validation are shown in Table 1. In comparison with Whelan et al. it is observed that the correlation in glucose and lactate concentration predictions obtained using implicit calibration model is comparable, but the standard error for lactate concentration prediction is significantly less in our case. The correlation in concentration prediction using CLS is also comparable with slightly higher standard errors of prediction compared with PLS.

In Fig. 4, we show the glucose and lactate concentration prediction in Sanofi HO cell culture supernatants using Raman spectroscopy and explicit model-based CLS algorithm. Comparison with reference concentration values obtained using HPLC is also shown. The slightly different prediction results for lactate in Figs 5 and 6 are because of the fact that we had used the same smoothing and basis spectra-based CLS model for both Invitrogen and Sanofi cell lines. These cell lines have different metabolism, as shown in Fig. 5, and the CLS algorithm can be sensitive to high frequency system noise or experimental noise because of the pseudo-inversion of the measurement matrix. One way to improve this in the future would be to introduce techniques such as regularization.

CONCLUSIONS

We have shown for the first time that the detection of glucose and lactate concentrations with high accuracy is possible in HO cell culture supernatants using Raman spectroscopy and explicit model-based CLS algorithm. Ehis corresponds to the data obtained by Yamamoto Y.S. et al. (Yamamoto Y.S. et al. (2011). When exact composition of the nutrient media is unknown, a deterministic Raman spectral library of pure components can be created by acquiring Raman spectra from credible nutrient media constituents and HO cell culture metabolites. Residuals obtained after CLS analyses can be used to identify missing components or those present in excess. An algorithmic sieve thus generated provides a revised Raman spectral library that can be used for metabolite concentration predictions (Goh, 2013; Vogel, 2002). Other HO cell culture metabolites can also be monitored after improving the detection limit of the system, and it will require the use of respective HPLC columns to provide reference data values. Once validated, Raman spectroscopy-based concentration measurements require about an order of magnitude less time comparatively are not labor intensive and have the advantage of ease of automation (Lee et al., 2004). Singh et al. also describe this circumstance in their works (Schroder et al., 2004). We believe that our algorithm and approach can be used for rise of quality analytical technology. It also assists in the development of processes and promotes their intensification.

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Mürəkkəb mühitlər üçün Raman spektroqrafiyası: biofarmakoloji tətbiq

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Tədqiqat daxilində dağsiçanının yumurtalıq hüceyrələri kolbada yetişdirilərək 10 gün ərzində inkubatorda 5%-li CO_2 məhlulunda yetişdirilmişdir. Toplanmış 2ml supernatant 20°C dondurucu kamerada saxlanmışdır. Supernatantda qlükoza və laktatın konsentrasiyası yüksək effektivlikli maye xromatoqrafiyada təyin edilmişdir, eyni nümunə mühitin parçalanmanın kombinasiyasının ölçülməsi üçün də istifadə edilmişdir. Biz raman septroqrafiyasından və klassik ən kiçik kvadrat modelindən istifadə etməklə dövrü fermentasiya rejimində kolbada yetişdirilən qlükoza və lakatatın kosentasiyasını təyin etdik. Dağsiçanının yumurtalıqlarının hüceyrələri kultur metabolitləri və qidalı mühitin təmiz komponentlərinin kombinasiyalı dağılma spektrlərinin əldə edilməsilə təmiz komponentlərinin kombinasiyalı dağılma spektlərinin bazası yaradılmışdır. Bu bazaya yalnız cihazın təyin etdiyi həddən yuxarı olan analizatorlar daxil edilmişdir. Klassik ən kiçik kvadrat təhlilindən sonra alınan qalıqlar çatışmayan komponenetlərin aşkar edilməsi və bazanın düzəldilməsi üçün istifadə edilmişdir. Belə ki, mürəkkəb kimyəvi qarışıqlardan kombinasiyalı dağılmanın spektral bazasının alınması üçün alqoritm yaradılmışdır. Etalon qlükoza və laktat konsentrasiyalrının alınması üçün yüksək effektivlikli maye xromatoqrafiyadan istifadə edilmişdir. Biz raman spektroqrafiyasının köməkliyilə dağsiçanın yumurtakıq hüceyrəsinin kulturlarının supenatantlarında qlükoza və laktatı təyin etdik.

Açar sözlər: *Raman spektroskopiyası, bioreaktorlar, ən kiçik kvadratlar klassik metodu, qlükozanın aşkarlanması, laktatın aşkarlanması*

Рамановская спектроскопия для сложных сред: биофармацевтическое применение

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Клетки яичников хомяка в течение 10 дней выращивали в колбе, помещенной в инкубатор, где культивирование осуществлялось при 5%-ом CO_2 и температуре 37°C. Собранные 2 мл супернатанта хранились в морозильной камере при температуре 20°C. Концентрации глюкозы и лактата в супернатанте оценивали на аппарате высокоэффективной жидкостной хроматографии (ВЭЖХ). Тот же образец использовали для немедленного получения результатов измерений комбинационного рассеяния. С использованием рамановской спектроскопии и алгоритма классических наименьших квадратов на основе модели в супернатантах культуры клеток яичников хомяка, выращенных в колбах в режиме периодической ферментации, с высокой точностью определены концентрации глюкозы и лактата. Библиотека детерминированных спектров комбинационного рассеяния чистых компонентов была создана путем получения спектров комбинационного рассеяния достоверных компонентов питательных сред и метаболитов культур клеток яичников хомяка. В эту библиотеку были включены только анализаторы с концентрациями выше предела обнаружения прибора. Остатки, полученные после анализа классических наименьших квадратов, были использованы для выявления недостающих компонентов и создания исправленной библиотеки. Таким образом, был создан алгоритм для получения подходящей спектральной базы комбинационного рассеяния из сложной химической смеси. Концентрации глюкозы и лактата в супернатантах культуры клеток яичников хомяка определили с помощью рамановской спектроскопии.

Ключевые слова: *Рамановская спектроскопия, биореакторы, классический метод наименьших квадратов, обнаружение глюкозы, обнаружение лактата*

Journal of Life Sciences & Biomedicine, vol. 3(76), No 1, p. 77-83 (2021)

Investigation of the interaction of polyene antibiotics with cholesterol

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Received: April 11, 2021; Received in revised form: May 12, 2021; Accepted: June 09, 2021

One of the compounds affected by membranes is polyene antibiotics. Amphotericin B, nystatin, mycoheptin, and levorin are mainly classified in the polyene class and distinguished by high biological activity. The high sensitivity of polyene antibiotics to membranes is due to the cholesterol they contain. The main feature of polyene antibiotics is the formation of structural ion channels by combining with cholesterol in the membranes. The interaction of polyene antibiotics with cholesterol has been demonstrated by obtaining ultraviolet (UV) spectra. Polyenes have three primary absorption spectra and range from 370 nm to 430 nm. Amphotericin B and levorin complex with cholesterol reduce the maximum amplitude of UV absorption spectra. The results show that cholesterol molecules combine with the double-bond chain systems of amphotericin B and levorin to reduce the maximum amplitude of UV absorption spectra. UV spectrum of dimethyl-sulfoxide molecules has been obtained. Its absorption spectrum ranges from 240 nm to 250 nm. The absorption spectrum of dimethyl sulfoxide molecules at these waves is related to the presence of the disulfide S=O group.

Keywords: *Levorin, amphotericin B, cholesterol, ultraviolet spectrum, cholesterol-polyene complex*

INTRODUCTION

One of the most significant problems of modern biophysics is to study at the molecular level of selective conductivity for ions and organic compounds in cell membranes. It knowns that the transport of ions and organic compounds through membranes is carrying out through channels of molecular size. Polyene class compounds are representing by amphotericin B, nystatin, mycoheptin, candidiasis, and levorin molecules. Among the antibiotics studied, amphotericin B and levorin are distinguishing by great membrane activity (Cavassin et al., 2021). The use of these antibiotics in research is not accidental. The main feature of polyene antibiotics is to make structural ion channels of molecular size in the membranes (Kamiński, 2014). Cellular channels have a high selective conductivity for ions, substrates, nucleic acids in molecular size (Samedova et al., 2018). The most significant characteristic of PAs is that they are very responsive to cholesterol molecules present in membranes (Kamiński, 2014; Srinivasarao et al., 2018). The primary solvent for PA is dimethyl sulfoxide (DMSO). It is using in many fields of molecular biology and biochemistry. Studies have shown for the first time that antibiotics are very soluble in water in a complex with DMSO and have high biological activity. The essential purpose of the article is to show the formation of the complex between PA and cholesterol in experimental studies using the method of UV spectroscopy.

MATERIALS AND METHODS

Bilayer lipid membranes are using to study the effect of PA on membranes at the molecular level. Bimolecular lipid membranes (BLM) are the essential representatives of living membranes. Membranes are making of phospholipids and present in the brain tissues of large and small horned animals. The formation of lipid membranes in the hollow

https://doi.org/10.29228/jlsb.10
Available online 30 June 2021

part of a glass made of Teflon material reflects on paper (Samedova et al., 2018). The ultraviolet spectrophotometer was used in the study to determine the absorption spectrum of PA. Amphotericin B and levorin UV spectra were determined using a T92 + UV / VIS spectrometer.

RESULTS AND DISCUSSION

It shows that the interaction of polyene antibiotics with cholesterol and other sterols (ergosterol, 7-dehydrocholesterol) in lipids and cell membranes results in the formation of structural ion channels with different permeability (Fei et al., 2012). Sterins are one of the main structural components of living cell membranes. UV spectroscopy is using to determine the biological activity of antibiotics (Vyazmin et al., 2011). The B spectra of levorin and amphotericin differ in the three main absorption spectra. The absorption spectra of antibiotics range from 370 nm to 430 nm. UV absorption spectrum reflects the characteristic spectra of polyenes belonging to this class. UV spectroscopy based on the irradiation of a substance with monochromatic UV radiation, and the absorption spectra vary with wavelength. It is basing on the identification of individual substances, as well as their quantitative measurement. UV radiation has a biological effect on living organisms. UV radiation penetrates the tissues to a depth of 0.5-1.0 mm, activating biochemical processes. Many morpho-physiological and biochemical parameters of plant cells change under the influence of UV radiation. These changes depend on the stage of tissue development, its genotype, and radiation conditions (radiation duration and spectral composition) (Vyazmin et al., 2011). The main feature of PAs is that they create an absorption spectrum that differs by three maximums in UV waves. Levorin macrolactone ring has seven double bonds and incorporates a polyene chromophore in the UV spectrum of levorin - 358 nm-360 nm, 378 nm-380 nm, and 400 nm-403 nm. The UV spectrum at these wavelengths is typical of heptaen antifungal antibiotics (Cavassin et al., 2021). Levorin is composed of the components levorin A and levorin B (Szczeblewski et al. 2017). Levorin A and B as aromatic heptaen differ from each other in elemental composition and UV spectra (Szczeblewski et al., 2017). Some physicochemical properties of levorin components A and B are given in Table 1. In addition, Table 1 presents the UV spectra of levorin A and levorin B. In comparison, the difference between levorin A and levorin B is 5-6 nm.

Table 1. Physicochemical properties of levorin A and B

General formula	**Levorin A** $C_{59}H_{93}O_{22}N_2$	**Levorin B** $C_{52}H_{98}O_{23}N_2$
UV absorption spectrum		
λ, nm	340 358 378 400	450 790 900 800
E	342 363 382 406	375 620 980 950
The composition of the elements		
C	60,43	59,72
H	7,89	7,87
N	2,38	2,24
Neutralization coefficient	1180	1238
Distribution factor	0,8	7,6
Nitrogenous part of the molecule	p-aminoacetophenone, mycosamine	p- aminoacetophenone, mycosamine

Figure 1 shows the UV absorption spectra of levorin A and levorin B in DMSO. Figure 2 shows the UV absorption spectra of levorin and candidiasis in methanol.

As shown in Fig. 1 and Fig. 2, the UV absorption spectrum of levorin depends on the solvents of the PA molecules. In the DMSO, the UV absorption spectrum of levorin varies in the wavelength range of 325–425 nm (Fig/ 1), while in the methanol, the UV absorption spectrum of levorin varies in the wavelength range of 360–400 nm (Fig. 2). The UV absorption spectra of levorin and candidiasis in methanol are the same. The results show that this is due to the differences between the chromophore in polyenes and their macrolactone ring. Thus, unlike candidiasis, the p-aminoacetophenone group in levorin is located in the hydrophobic part of the molecule.

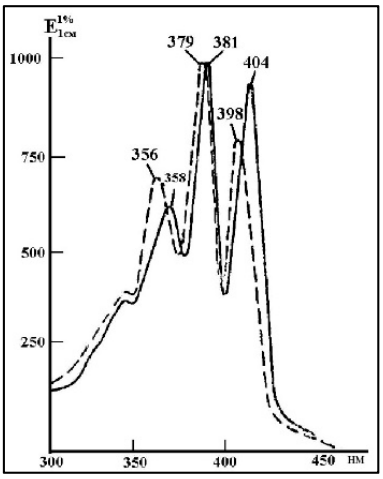

Fig. 1. UV absorption spectra of levorin A and levorin B in dimethyl sulfoxide.

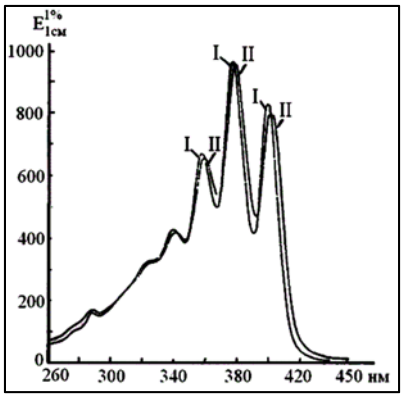

Fig. 2. UV absorption spectra of levorin and candidiasis in methanol: I - levorin; II – candidiasis.

Figure 3 shows the UV absorption spectrum of levorin and amphotericin B at different concentrations.

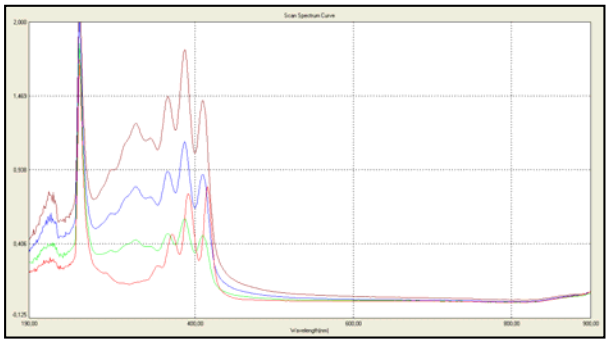

Fig. 3. UV absorption spectra of levorin were obtained in DMSO at different concentrations.

In Figure 3 (red curve), the UV absorption spectrum obtained at the final concentration of levorin 10^{-5} M by adding 0.3 ml and 3 ml of DMSO from the essential solution of levorin at a concentration of 1 mg/ml.

The blue curve was obtained by adding 0.2 ml and 3 ml of DMSO from the essential solution of levorin at a concentration of 1 mg/ml to obtain a UV absorption spectrum at a concentration of levorin $7 \cdot 10^{-6}$ M. The UV absorption spectra was obtained by adding 0.1 ml and 3 ml of DMSO to the green solution of levorin at a concentration of 1 mg/ml and levorin at a concentration of $3 \cdot 10^{-6}$ M. A bright red line was obtained by adding 0.03 ml and 3 ml of DMSO from the essential solution of levorin at a concentration of 1 mg/ml to obtain a UV absorption spectrum at a concentration of levorin $1 \cdot 10^{-6}$ M. As shown in Figure 3, the concentration of levorin increases with the amplitude of the UV-maximum absorption spectrum. Levorin absorption spectra range from 370 nm to 430 nm. UV absorption spectra reflect the characteristic spectra of polyenes belonging to this class. UV spectra of PAs obtained at different concentrations are one of the methods of reflecting their biological activity. Although polyenes are well soluble in DMSO, they are in the form of thin colloidal dispersions in water, whereas molecules in ethyl, methyl alcohol solutions, and water find their presence in disperse form (Pinisetty et al., 2012).

Figure 4 shows the UV absorption spectra of levorin derivatives, N-diacetyl-levorin sodium salt, levorin succinyl sodium salt, and levorin soluble in water and methanol.

Figure 4 shows that levorin derivatives are distinguishing by their biological activity. The highest biological activity shows by the sodium salt of levorin in methanol, spectrum 3, but the biological activity in water is low. The amphoteric nature of levorin is due to the presence of one carboxyl and two amine groups in its molecule. It allows its derivatives to remain in both alkaline and acidic environments. Figure 5 shows the UV absorption spectrum of DMSO.

One tub contains 3 ml of ethanol and the other tub contains 3 ml of DMSO. Temperature 24ºC. As can be seen in Figure 5, the absorption spectrum of the DMSO is reflected in the near-UV absorption waves. The UV spectrum of the DMSO is selected with a maximum absorption between 240 nm-250

nm. The gain spectrum of dimethyl sulfoxide molecules in these waves is due to the presence of the disulfide S=O group. Study of the complex formed by cholesterol with polyenes by UV spectroscopy.

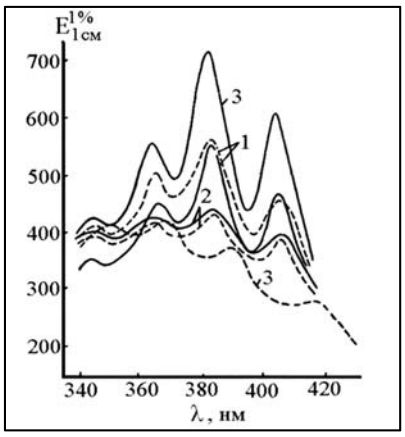

Fig. 4. UV absorption spectra of levorin derivatives soluble in water and methanol. - - in water; - methanol; 1 – N-diacetyl sodium salt of levorin; 2 – levorin succinyl sodium salt; 3 – sodium salt of levorin.

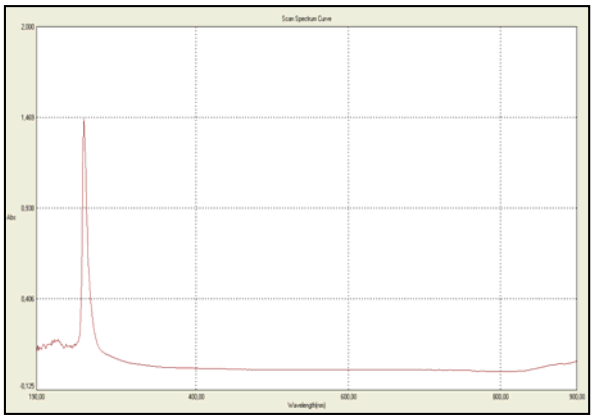

Fig. 5. UV differential absorption spectrum of DMSO.

PA is characterized by a maximum of three absorptions due to the presence of a double-bonded molecule in the chromophore part in water and organic solutions. Changes in the UV absorption spectrum observed during the interaction of PA with cholesterol. An increase in the number of double bonds in a polyene molecule causes a change in the UV absorption spectrum. The addition of cholesterol or other sterols to an antibiotic solution leads to a decrease in the UV absorption spectrum, resulting in the formation of a complex between cholesterol and PA. The presence of sterols does not change the UV absorption wavelength of polyenes and only changes the maximum UV absorption.

It has been shown that the addition of cholesterol to the aqueous solution of the Philippines changes the UV spectrum, but this member of the spectrum does not change in the solvents. No change in the UV spectrum occurs when cholesterol is added to an organic solvent. According to the effectiveness of interaction with cholesterol, PA is in the following order: Philippine > amphotericin B > etruscomycin > pimarisin > nystatin. The structure of sterols often determines their interaction with polyenes. Thus, sterols containing the 3β-OH group are more sensitive to polyenes than sterols containing 3α-OH or 3-keto groups. For polyenes to interact with sterols, the antibiotic C17 must form a hydrogen bond with the 3β-OH group of the sterins molecule. Table 2 shows the maximum UV absorption depending on the number of double bonds of the polyene. Figure 6 shows the UV absorption spectra of amphotericin interacting with B cholesterol.

Table 2. Maximum UV absorption of polyenes depending on the number of double bonds.

	Number of double bond	The three maximum absorption in the UV, nm	Color
Trien	3	-	-
Tetrans	4	291, 304, 318	Light yellow
Pentans	5	317, 331, 350	Yellow
Hexans	6	340, 358, 380	Yellowish-orange
Heptans	7	361, 382, 405	Orange

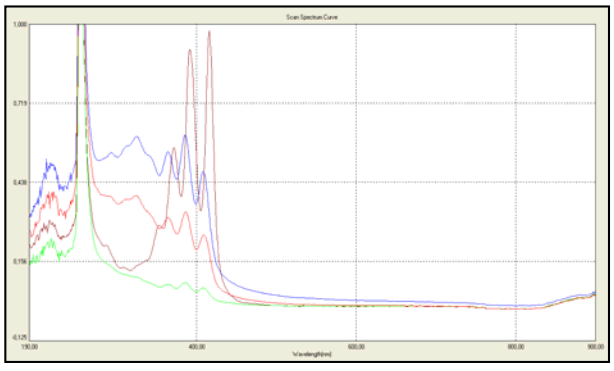

Fig. 6. UV absorption spectra of the interaction of amphotericin with B cholesterol.

In Fig. 6, a dark red line - UV absorption spectrum was obtained by adding 0.03 ml of amphotericin B dissolved in 1 mg/ml DMSO to 3 ml of DMSO in the first tub. 3 ml of ethanol solution was added to the second tub. The blue line in Fig. 6 shows that the UV absorption spectrum was obtained by adding 0.03 ml of amphotericin B dissolved in 1 mg/ml DMSO to the first tub and 0.5 mg of cholesterol in 3 ml of DMSO. 3 ml of ethanol solution was added to the second tub. In Figure 6, a bright red line - UV absorption spectrum was obtained by adding 0.03 ml of amphotericin B dissolved in 1 mg/ml DMSO to the first tub and 1 mg of cholesterol in 3 ml of DMSO. 3 ml of ethanol solution was added to the second tub. In Figure 6, the green line - UV absorption spectrum was obtained by adding 0.03 ml of amphotericin B soluble in 1 mg/ml DMSO to the first tub and 2 mg of cholesterol in 3 ml of DMSO. 3 ml of ethanol solution was added to the second tub.

The results in Figure 6 show that amphotericin interacts with B cholesterol to reduce the concentration of amphotericin B, which is reflected in the UV absorption spectra.It is known that DMSO molecules facilitate the delivery of drugs from biological membranes to the cell. However, the effects of DMSO on membranes have not yet been fully studied (Lee et al., 2016).

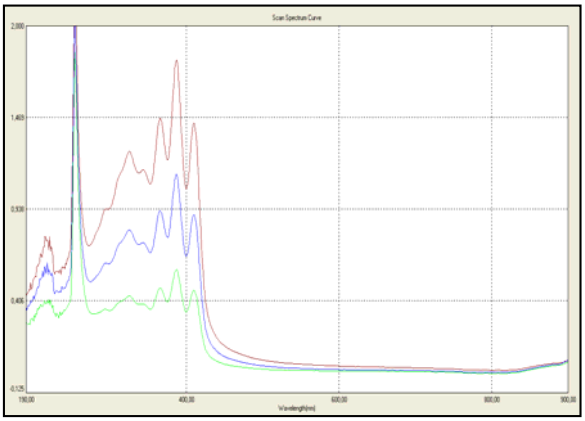

Fig. 7. UV absorption spectra obtained from the interaction of levorin with cholesterol.

It recently has been shown by molecular-dynamic modeling that DMSO creates water pores in biological membranes (Pinisetty et al., 2012). The effect of DMSO on the diffusion of Ca2 + ions from cell membranes has been studied(Jacl et al., 2014). The increase in DMSO-induced Ca2 + permeability did not alter the increase in Ca2 + permeability due to the effects of K-channel blockers and K-Na-ATF-aza. This means that water pores form in the cell membranes induced by DMSO, and Ca2 + ions are transferred to the cells through these pores. In addition, the permeability of Ca2 + ions increases significantly due to the high concentration of DMSO, which indicates the selectivity of water pores induced by DMSO. Thus, these studies suggest that DMSO can induce water pores in cell membranes and, in turn, facilitate the transport of biologically active substances to cells.

Figure 7 shows the UV absorption spectra of levorin were obtained from the interaction with cholesterol.

In Fig. 7 a dark red line - 0.03 ml of levorin solution dissolved in 1 mg/ml DMSO added to 3 ml of DMSO in the first tub. 3 ml of ethanol was added to the second tub.

The blue line in Fig. 7 - 0.03 ml of levorin solution dissolved in 1 mg/ml DMSO and 1 mg of cholesterol in 3 ml of DMSO added to the first tub. 3 ml of ethanol added to the second tub.

The green line in Fig. 7 - 0.03 ml of levorin solution dissolved in 1 mg/ml DMSO and 3 mg of cholesterol in 2 ml of DMSO added to the first tub. 3 ml of ethanol added to the second tub.

As shown in Fig. 6 and Fig. 7, amphotericin B and levorin form a complex with cholesterol, reducing the maximum amplitude of UV absorption spectra. Increased cholesterol further lowers the maximum of the UV absorption spectra. The results show that cholesterol molecules combine with the double communication systems of amphotericin B and levorin to gradually lower the maximum absorption spectra of UV. These studies confirm the complex formation of amphotericin B and levorin with cholesterol and the molecular model of the channel (Kaminski, 2014; Cavassin et al., 2021).

RESULTS

The ultraviolet spectra of amphotericin B and levorin show that amphotericin B differs from 370 nm to 420 nm and levorin from 368 nm to 410 nm with three essential absorption spectra, which are due to the presence of a chromophore chain in the molecule. Amphotericin B and levorin complex

with cholesterol reduce the maximum amplitude of UV gain spectra. The results show that cholesterol molecules combine with the double communication systems of amphotericin B and levorin to reduce the maximum amplitude of UV gain spectra. The ultraviolet spectrum of dimethyl sulfoxide molecules was obtained. Its gain spectrum ranges from 240 nm to 250 nm. The gain spectrum of dimethyl sulfoxide molecules in these waves is due to the presence of the disulfide S=O group.

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Polien antibiotiklərinin xolesterinlə qarşılıqlı təsirinin tədqiqi

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Membranlara təsir göstərən birləşmələrdən biri polien antibiotikləridir. Polien sinfinə daxil olan və yüksək bioloji aktivliyi ilə seçilən əsasən amfoterisin B, nistatin, mikoheptin və levorindir. Poliyen antibiotiklərinin membranlara olan yüksək həssaslıgı onların tərkibindəki xolesterinlə baglıdır. Polien antibiotikləri əsas xüsusiyyəti membranlarda xolesterinlə birləşərək struktur-ion kanallarının yaradılmasıdır. Polien antibiotiklərin xolesterinlə qarşılıqlı təsiri ultrabənövşəyi (UB) spektrlərinin alınması ilə göstərilmişdir. Polienlər

üç əsas udlu spektri ilə fərqlənir və 370 nm – 430 nm çərçivəsində dəyişir. Amfoterisin B və levorin xolesterinlə kompleks yaradaraq UB udlu spektrlərinin maksimum amplitudasını aşağı salır. Alınan nəticələr onu göstərir ki, xolesterin molekulları amfoterisin B və levorinin qoşa rabitə sistemləri ilə birləşərək UB udma spektrlərinin maksimum amplitudasını azaldır. Dimetilsulfoksid molekullarının UB spektri alınmışdır. Onun udma spektri 240 nm – 250 nm dalğalarının uzunluqu arasındadır. Dimetilsulfoksid molekullarının göstərilən dalğalarda udma spektri disulfid S=O qrupunun mövcud olması ilə bağlıdır.

Açar sözlər: *Levorin, amfoterisin B, xolesterin, ultrabənövşəyi spektri, xolesterin-polien kompleksi.*

Исследование взаимодействия полиеновых антибиотиков с холестерином

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Одним из соединений, влияющих на мембраны, являются полиеновые антибиотики. В основном это амфотерицин В, нистатин, микогептин и леворин, которые относятся к классу полиенов и отличаются высокой биологической активностью. Высокая чувствительность полиеновых антибиотиков к мембранам обусловлена содержащимся в них холестерином. Главной особенностью полиеновых антибиотиков является создание структурно-ионных каналов в мембранах путем их соединения с холестерином. Взаимодействие полиеновых антибиотиков с холестерином показано путем изучения ультрафиолетовых (УФ) спектров. Полиены различаются по трем основным спектрам поглощения, которые варьируют в диапазоне 370 Нм – 430 Нм. Амфотерицин В и леворин снижает максимальную амплитуду УФ спектра, благодаря взаимодействию с холестерином. Уменьшение максимальной амплитуды спектров УФ поглощения есть результат взаимодействия холестерина с системой двойных связей амфотерицина В и леворина. Показано, что УФ-спектр молекул диметилсульфоксида находится в диапазоне длин волн 240 Нм – 250 Нм. Предполагается, что полученный спектр поглощения молекул диметилсульфоксида в указанных длинах волн обусловлен наличием в молекуле дисульфидной S=O группы.

Ключевые слова: *Леворин, амфотерицин В, холестерин, ультрафиолетовый спектр, холестерин-полиеновый комплекс*

Journal of Life Sciences & Biomedicine, vol. 3(76), No 1, p. 84-90 (2021)

Classification and productivity of winter pastures in Lankaran-Mugan botanical-geographical region

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Received: August 31, 2020; Received in revised form: March 31, 2021; Accepted: June 09, 2021

In order to ensure food security of the population in Azerbaijan, it is important to protect natural forage plants, effectively use, improve and prevent the process of biodiversity degradation. From this point of view, we aimed to study the parameters of semi-desert and desert formations, fodder quality, grazing norms, ecological assessment of semi-desert and desert formations, which are a valuable source of fodder for livestock in the Caspian coastal areas and vegetation of Lankaran administrative districts (southern part of the Caspian coast). The vegetation of the botanical-geographical region is spread below sea level - below 10 meters (in Masalli region), as well as at an altitude of 27 m to 150 m. During the ecological-geobotanical researches in the Lankaran-Mugan botanical-geographical area of Azerbaijan, phytocenoses with predominance of *Petrosimonieta brachiata* and *Artemisietum lerchiana-Ephemerosum* were registered in the desert and semi-desert vegetation types, which are mainly a source of natural fodder in winter pastures. A classification scheme for 6 formation groups and 8 associations was prepared, and an "Ecological-geobotanical map of the southern part of the Caspian coast. Scale 1: 10,000" was compiled. In the course of the study, endemic species of the Caucasian area - *Iris musulmanica* Fomin, *Salsola nitraria* Pall, *Symphytum caucasicum* M. Bieb., and endemic species of the Azerbaijan area - *Bellevalia zygomorpha* Woronow and Tragopogon macropogon C.A.Mey were identified.

***Keywords**: Ecosystems, phytocenosis, formation, association, dominant, subdominants, endemic*

INTRODUCTION

As indicated in the Law on "State Land Cadastre, Land Monitoring and Land Management", State Program on Rational Use of Summer and Winter Pastures, Hayfields and Prevention of Desertification in the Republic of Azerbaijan, as well as, in the action plan of the "Strategic Roadmap for the production and processing of agricultural products in the Republic of Azerbaijan" approved by Presidential Decree No. 1138 dated December 6, 2016, geobotanical research in pastures has become actual.

The vegetation of winter pastures is spread at an absolute height of -22 to 50 meters above sea level.

Climatic conditions are mild-warm and steppe type with dry summers; The average annual temperature is 14.4°C and the annual rainfall reaches 300 mm.

Vegetation groups are found in saline, saline gray-meadow, carbonate alluvial-meadow, meadow-swamp and sandy soils.

The main aim of the presented research was to determine the structure, productivity, nutrition and capacity of wormwood-ephemeral formation *Artemisietum-Ephemerosum* of semi-desert phytocenosis and *Petrosimonieta brachiata* formation of the most widespread desert vegetation in the state-owned winter pastures which is a source of natural fodder for the development of nomadic small horned animals, mainly in the Lankaran-Mugan region.

In connection with the purpose of the scientific topic were conducted geobotanical researches in the spring, autumn and winter seasons of 2019 in the desert and semi-desert vegetation spread on the southern Caspian coast, as well as in the winter pastures of Lankaran-Mugan region. Moreover,

https://doi.org/10.29228/jlsb.11
Available online 30 June 2021

was planned to determine the following tasks. In the next must be resolved:

- recording the species composition and structure of phytocenoses;
- compilation of modern classification;
- determination of productivity on plant groups;
- determination of nutrition (feed quality) based on biochemical analysis;
- calculation of the load and capacity of pasture area.

MATERIALS AND METHODS

Within the territory of the Republic of Azerbaijan, plant groups on natural forage areas within the South Caspian coast, Lankaran-Mugan botanical-geographical region (Gurbanov, 2018) are spread in saline, saline gray-meadow, carbonate alluvial-meadow, meadow-swamp and sandy soils. The Lankaran-Mugan botanical geographical area is considered to be a source of natural fodder for the development of nomadic sheep-breeding, as well as a widespread desert plant in state-owned winter pastures. Species composition, structure, productivity, nutrition and capacity of *Petrosimonieta* and semi-desert *Artemisietum-Ephemerosum* formation were studied (Shukurov et al., 2008). Based on the results of phytocenoses productivity and feed quality, it was determined that the relevant geobotanical indicators of the area change depending on soil and climatic conditions.

Therefore, before analyzing the dynamic variability of the productivity of the selected "Research Objects", we studied the soil and climatic conditions characteristic of the region, based on the annual results of meteorological stations. (Abdullayev, 2007; Shikhlinsky, 2009).

During the research, geobotanical materials collected during the field research were analyzed by the route method, numerous herbariums were assigned to "Flora of Azerbaijan" on the basis of systematic taxa, and the names of the species were specified according to Cherepanov (1995), Askerov (2011), WFO (2021).

"Field geobotany" (Lavrenko, 1959-1976), "Methods of geobotanical research of natural forage areas" (Hajiyev et al., 1995), "Res. Instructions on geobotanical research of natural forage areas of the Republic of Azerbaijan" and "Methodical instructions on geobotanical research of natural forage areas of Azerbaijan" (Agaguliyev, 2001) were used. The productivity of winter pastures and rural pastures used as a source of fodder for the development of livestock in the administrative districts, the quality of fodder were studied and the capacity of pastures was determined.

The area is characterized by drought, as well as the widespread use of desert and semi-desert vegetation in the ecological conditions of desertification. One of the factors contributing to the desertification process is the degradation of vegetation due to overgrazing of natural forage areas, as well as fuel, construction, etc. cutting of trees and shrubs for purposes (Ibadullayeva et al., 2012; Hatamov, 2000). The refore, due to anthropogenic and man-made influences in the vegetation of the studied region, shrubs have become very sparse, pasture productivity has decreased and nutrients have been depleted.

Information on the improvement of natural winter pastures in the country, including florostatic and geobotanical studies of semi-desert and desert plants (Hajiyev, 1995; Aliyev, 1965; Mailov, 1984; Agadjanov, 1967; Gurbanov et al., 2012; Gurbanov, 2004; Ibadullayeva, 2011; Shukurov et al., 2008).

RESULTS AND DISCUSSION

According to the "General scheme of use of natural fodder lands of the Republic of Azerbaijan till 2005" and "Map of lands of large-scale administrative territory of Lankaran-Mugan region", the total area of winter pastures of the region is 22476,0 ha; of which 12552.0 ha (55.8%) are explored areas and 9924.0 ha (44.2%) are unexplored areas (Table 1).

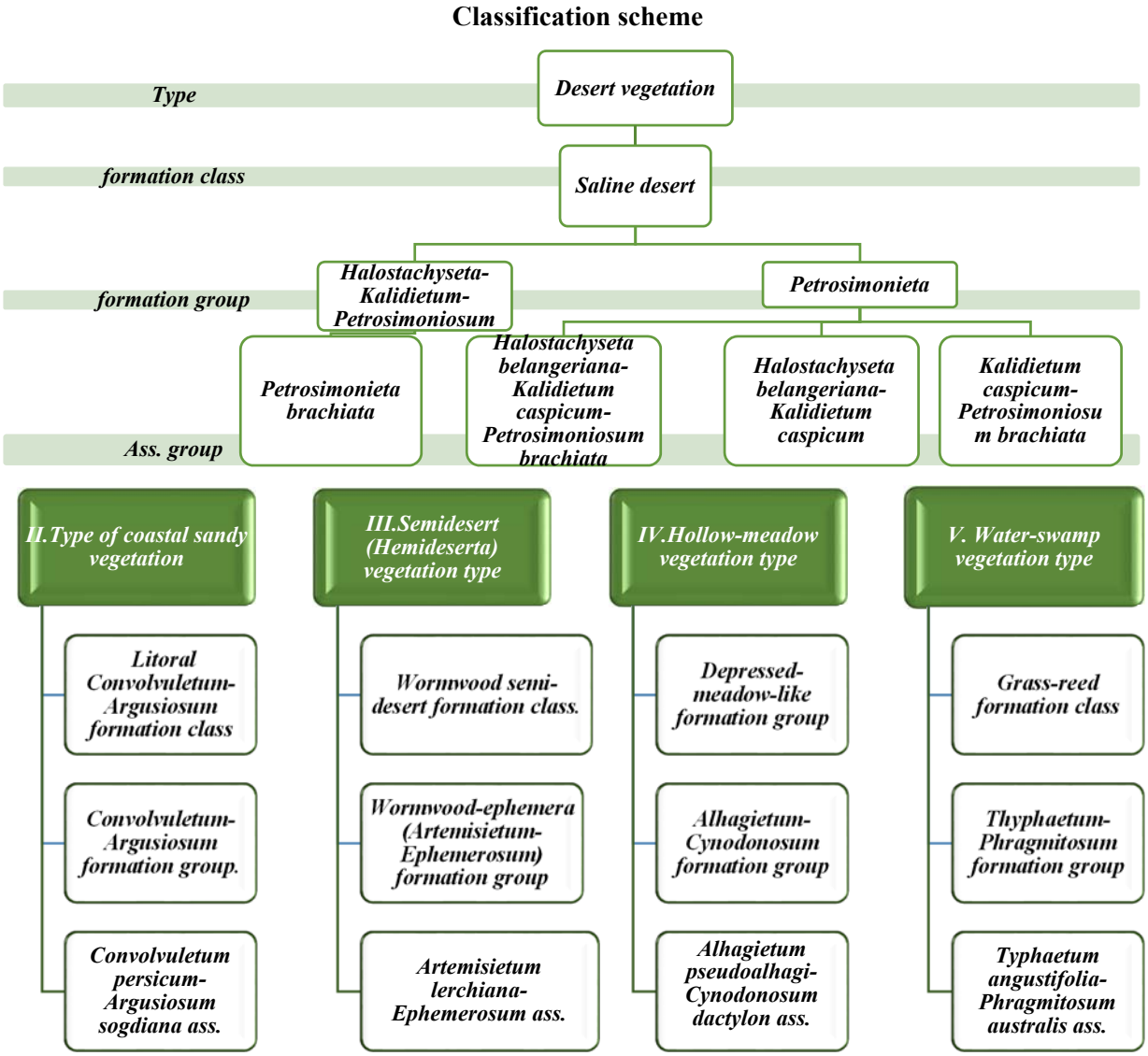
As shown in the table, the area of *Petrosimonieta brachiata* formation is 4550.0 ha (20.2%) and the area of wormwood-ephemeral *Artemisietum lerchiana-Ephemerosum* is 8002.0 ha (35.6%). And first of all, was a task to complete of "Ecological-geobotanical map of the southern part of the Caspian coast (scale 1:10.000)". According

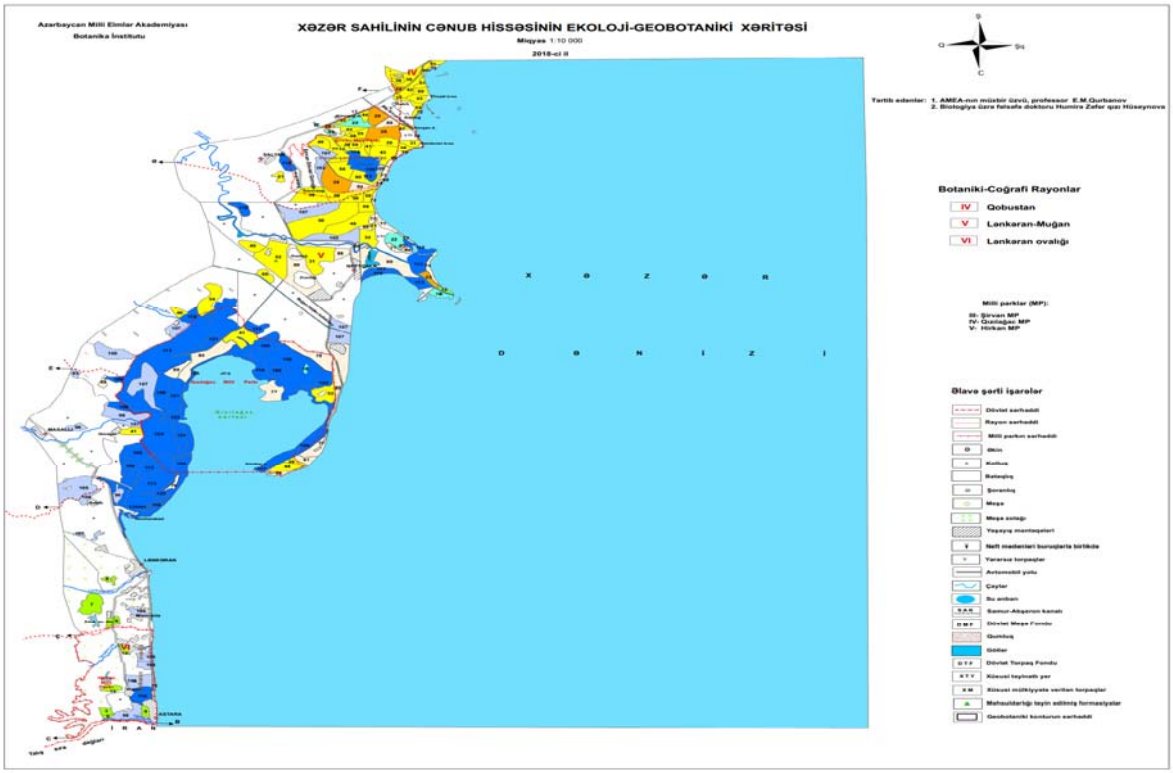
to geobotanical instructions and modern methodology, our research on the southern Caspian coast, as well as in the Lankaran-Mugan region, allowed us to classify the vegetation found in winter pastures.

According to the results of phytocenological research, 5 plant types, 5 formation classes, 6 formation groups and 8 associations are widespread in the winter pastures of the region.

Table 1. Classification and areas of winter pasture vegetation

Counter№	Classification index	Types and formations (dominant and subdominant species)	Area within the border	
			with ha	with %
1	S-III-31	Desert Vegetation (*Petrosimonieta brachiata*)	4550,0	20,2
2	S-III-34	Semidesert vegetation (*Artemisietum lerchiana-Ephemerosum*)	8002,0	35,6
Explored areas			12552,0	55,8
Unexplored areas			9924,0	44,2
Total area			22476,0	100,0





Map 1. Ecological-geobotanical map of the southern part of the Caspian coast. **Scale1: 10,000; 2018.** *Institute of Botany of the National Academy of Sciences of Azerbaijan; Compiled by: Corresponding Member of ANAS E.M. Gurbanov, Doctor of Philosophy in Biology H.Z. Huseynova*

A brief description of the species composition and structure of the studied *Petrosimonieta* and *Artemisietum-Ephemerosum* formations is given below.

Petrosimonieta phytocenosis is found as an indicator formation in saline soils. 16 species were recorded in the formation composition. Of these, 2 species (12.5%) belong to shrubs, 1 species (6.3%) to small semi-shrub, 6 species (37.5%) to cereal grass and 7 species (43.7%) to various grass species.

The monodominant of this formation is *Petrosimonia brachiata (*Pall.) Bunge., The abundance of which is estimated at 3-4 points.

According to the geobotanical description, the phytocenosis is single-storey and the average height of grass cover reaches 10-30 cm. The overall design or projective coverage corresponds to 40-60%. The productivity of the formation is 8.6 cent/ha in dry mass ingested.

The average annual productivity of the *Petrosimonieta* formation was (20.IV) -2.05s / ha (23.3%) cereal grasses, 1.2s / ha (13.9%) legumes in the spring of the year (for 2019) and 2.8 s / ha (32.6%) and 2.6 s /ha (30.2%) various grass species in the fall.

As a result of biochemical analysis, hygroscopic moisture, crude ash, crude protein, crude fat, crude cellulose were detected, as well as nitrogen-free extractives, feed unit and assimilated protein were calculated based on the results of these analyzes. Therefore, the productivity and feed unit for the identified formations was considered the main criterion in the "plant block". In this regard, the formations are divided into quality groups (good, medium and low categories) according to their nutritional value and productivity. Based on the biochemical parameters of phytocenoses, the conversion of crude ash (A1), protein (P1), fat (F1) and nitrogen-free extractives (N1) in each formation is calculated according to the following formula:

Table 2. Area of winter pastures by formations, productivity, nutrition and pasture capacity

№	Name of the formation	Type of pasture	Area within the border		Productivity (in dry mass cent/ha)	In 100 kg of dry feed, in kg		Pasture capacity (at the expense of the main herd)	
			ha	%		Fodder unit	Assimilated protein	one ha	Total area
1	Petrosimonieta	Saline	4550,0	20,2	8.6	32,8	3,3	1,0	4550
2	Wormwood-ephemeral	Clean	8002,0	35,6	10,4	44,2	4,5	1,7	13603
Explored area			12552,0	55,8					
Unexplored area			9924,0	44,2					
Total area			22476,0	100,0					

$$P_1 = \frac{P \text{ x } 100}{d \cdot m};$$

P_1 - absolute dry matter protein (in%), P - crude protein per 100 g of dry mass in air (in%), d·m - dry matter (in%) in the mass of edible dry grass.

It should be noted that A1, F1, N1 are calculated in the same way as above.

According to the laboratory analysis of the main fodder plants, the biochemical composition of the phytocenosis has a hygroscopic moisture of 12.0%, ash in absolute dry matter 9.4%, protein 6.8%, fat 2.4%, cellulose 33.9%, NFE-35.5%, the feed unit per 100 kg of feed is 32.8 kg and the assimilated protein is 3.3 g (Table 2).

Taking into account the nutrition of the formation in the field of *Petrosimonieta* of the South Caspian coast fodder unit (per 100 kg of fodder) productivity (8.6 s/ha), grazing period (210 days), daily fodder norm of cattle (1.3 fodder units), the load of *Petrosimonieta* (1 head per hectare), the capacity was determined to be 4550 heads.

2. *Artemisietum Ephemerosum* phytocenosis is found in saline gray-meadow soils. There are 21 species of plants in the formation, of which 1 species (4.8%) is semi-shrub, 1 species (4.8%) is small semi-shrub, 7 species (33.3%) are cereal grasses, and 12 species (57.1%) are various species grass.

Abundance of edificator of the formation, as well as subdominant type *Artemisia lerchiana* Web. 2-3 points; dominant species-(*Eremopyrum orientale* (L.) Jaub.et Spach) *Lolium rigidum* Gaudin., *Rabbit barley* (*Hordeum leporinum* Link.), etc. are ephemerals, the abundance of which is 3-4 points.

According to the structure of the phytocenosis, the tier is two-storied; On the first floor there are *Artemisia lerchiana* and on the second floor there are ephemerals mentioned earlier. The average height of grass cover reaches 10-40 cm. The total project coverage is 70-90%.

The productivity of the formation is 10.4 s / ha per dry ingested mass.

The productivity of this formation by botanical groups in the spring of 2019 was 2.2 s/ha (21.1%) of cereal grass, 1.4 s/ha (13.5%) of legumes; 3.8 s/ha (36.6%) in autumn and 3.0 s/ha (28.8 s/ha) various species grass in winter.

The biochemical composition of the phytocenosis in which forage crops are found is 13.0% of hygroscopic moisture, 6.6% of ash in absolute dry matter, 8.9% of protein, 2.7% of fat, 26.8% of cellulose, 42.0% of NFE; the feed unit of 100 kg of fodder plant is 44.2 kg and the assimilated protein is 4.5 g.

Taking into account the nutrient content of the formation (feed unit per 100 kg of feed), productivity (10.4 s/ha), grazing period (210 days), daily feed rate (for cattle), the load of winter pasture is 1.7 heads per hectare, and the total area is 13603 heads was determined.

Thus, it is recommended to graze 18,153 heads of small cattle in the winter pastures surveyed in the Neftchala region on the South Caspian coast.

Based on the geobotanical characteristics of desert and semi-desert vegetation spread on the studied South Caspian coast, we recommend the following measures for scientifically and practically efficient use and improvement of winter pastures:

- carrying out autumn (partial) grazing by individuals and legal entities with the application of pasture rotation;

- implementation of root and surface improvement measures in pastures subject to salinization and salinization;
- sowing of valuable fodder (wild and cultivated) plants adapted to soil and climatic conditions, as well as provision of organic and mineral fertilizers in accordance with agro-technical rules;
- -proper (efficient) use of pastures during the vegetation period of fodder crops after vegetation restoration.

The study analyzed the classification of desert, coastal sandy, semi-desert, meadow and wetland vegetation types found in winter pastures in Lankaran-Mugan region up to formation class, formation group and association groups and determined their phytocenological composition.

The productivity of some formations found in the region was studied, the amount of dry fodder mass was determined.

The application of these mentioned measures in the pastures of the South Caspian coast will create a basis for the protection of natural phytocenoses and wild flora, as well as the protection of the environment.

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Lənkəran-Muğan botaniki-coğrafi rayonunun qış otlaqların təsnifatı və məhsuldarlığı

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Azərbaycanda əhalinin ərzaq təhlükəsizliyini təmin etmək üçün təbii yem sahələri bitkiliyinin qorunması, biomüxtəlifliyin səmərəli istifadəsi, yaxşılaşdırılması və degradasiya prosesinin qarşısının alınması üçün elmi-praktiki əsasların işlənilməsi vacibdir. Bu baxımdan Xəzər sahili Lənkəran-Muğan botaniki-coğrafi ərazisində heyvandarlığın dəyərli yem mənbəyi sayılan yarımsəhra və səhra fitosenozlarının məhsuldarlığının dinamikası, yem keyfiyyəti, otarma norması və torpaqlarının ekoloji qiymətləndirilməsi üzrə parametrlərinin araşdırılması qarşıya məqsəd olaraq qoyulmuşdur. Cənubi Xəzər sahilinin Lənkəran-Muğan botaniki-coğrafi rayonunun qış otlaqlarında ekoloji-geobotaniki tədqiqatlar ilk dəfə tərəfimizdən aparılmışdır. Tədqiqat nəticəsində ərazisində qeydə alınan köçəri qoyunçuluğun inkişafı üçün təbii yem mənbəyi hesab olunan, eləcə də dövlət mülkiyyətində saxlanılan qış otlaqlarında ən geniş yayılmış səhra bitkiliyinin qışotuluq (*Petrosimonieta*) və yarımsəhra fitosenozun yovşanlı-efemerlik (*Artemisietum-Ephemerosum*) formasiyalarının növ tərkibi, quruluşu, məhsuldarlığı, qidalılığı və tutumu öyrənilmişdir. Qeyd edilən formasiyaların qış otlaqlarında təsifastına əsasən 5 bitkilik tipi, 5 formasiya sinfi, 6 formasiya qrupu və 8 assosiasiyalarda təmsil olunduğu aşkar olunub. Eyni zamanda "Xəzər sahilinin cənub hissəsinin ekoloji-geobotanik xəritəsi" (Miqyas 1: 10 000) tərəfimizdən tərtib edilmişdir. Tədqiqat zamanı ərazinin bitkiliyində Qafqaz areallı endemiklərdən–*İris musulmanica, Salsola, nitraria, Seymphytum caucasicum*, Azərbaycan areallı endemiklərdən–*Bellavalia zigomorfa* və *Tragopogon macropogon* növləri aşkar olunmuşdur.

Açar sözlər: *Ekosistemlər, fitosenoz, formasiya, assosiasiya, dominant, subdominantlar, endemik*

Классификация и продуктивность зимних пастбищ Ленкорань-Муганского ботанико-географического района

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Для обеспечения продовольственной безопасности населения в Азербайджане важно защищать естественные кормовые растения, эффективно использовать, улучшать и предотвращать процесс деградации биоразнообразия. С этой точки зрения наша цель заключалась в изучении параметров полупустынных и пустынных фитоценозов, качества кормов, норм выпаса, экологической оценке полупустынных и пустынных фитоценозов, которые являются ценным источником кормов для скота в прибрежных районах Каспия, а также оценке растительности административных районов Ленкорань-Муганской ботанико-географической зоны (южная часть побережья Каспия). В ходе эколого-геоботанических исследований в Ленкорань-Муганской ботанико-географической зоне Азербайджана были зарегистрированы фитоценозы с преобладанием *Petrosimonieta brachiata и Artemisietum lerchiana-Ephemerosum* в пустынном и полупустынном типах растительности, которые в основном являются источником естественных кормов на зимних пастбищах. Разработана классификационная схема для 6 групп формаций и 8 ассоциаций, а также «Эколого-геоботаническая карта южной части побережья Каспийского моря. Масштаб 1: 10000». В ходе исследования были выявлены виды эндемиков Кавказского ареала - *Iris musulmanica, Salsola, nitraria, Seymphytum caucasicum* и эндемики Азербайджанского ареала - *Bellavalia zygomorph и Tragopogon macropogon*.

Ключевые слова: *Экосистема, фитоценоз, формация, ассоциация, доминанты, субдоминанты, эндемики*

Journal of Life Sciences & Biomedicine, vol. 3(76), No 1, p. 91-98 (2021)

The species composition and quantitative indicators of the sedentary birds, forming winter ornithocomplexes in the mountain-forest belt of Talish

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Received: May 31, 2020; Received in revised form: June 07, 2021; Accepted: June 16, 2021

The article presents materials about the species composition, the number, the density, the distribution of the sedentary birds and the factors affecting them in the mountain-forest belt of Talish in winter in 2013-2016. 54 species belonging in 6 orders are noted in the part of the winter ornithocomplexes of the mountain-forest belt of Talish. The total number of the 54 species of the sedentary birds amounted to 149781 individuals. Of the 54 species that we noted, 8 species are rare, 17 are ordinary, 29 are numerous. 54 registered bird species have a global and national conservation status and are included in the lists of the CITES, Bonn and Bern Conventions. Only 6 (11.1%) of the 54 bird species have no conservation status. According to the species composition and the total number of the winter ornithocomplexes, the main dominant birds are the species belonging to the order of the Sparrows *(Passeriformes)*. These birds make up 66.7% (36 species) of all registered species and 84.3% (126209 individuals) of the total number of the birds. The dominant species in terms of the number of the individuals is the Common Greenfinch *(Chloris chloris)*. The main limiting factors are the felling the trees due to the construction of gas pipelines and roads to settlements, the creation of the recreation and the tourism zones in the forests, grazing and illegal hunting of the birds. Such an anthropogenic impact leads to a gradual reduction in the number of the habitats and places for feeding of vulnerable species.

Keywords: *Talish Mountains, sedentary birds, species, number, density, factor*

INTRODUCTION

The continuous, multi-year research work has not been conducted about the sedentary birds in the mountain-forest belt of Talish. E.Menetries (1830) made observations around Lankaran and Talish and gave a vertical distribution table of the birds (Menetries, 1832). N.Dinnik described the birds which he recorded in Lankaran and Talish Mountains in his articles written in 1889 and 1912 (Dinnik, 1899). In 1980, Ch.Aghayeva was satisfied with the general registration of the birds in the forest strip of Talish (Aghayeva, 1980). T.Karimov (2004) recorded a Cinereous Vulture (Karimov et al., 2019). The data of these authors are outdated in terms of modernity and do not allow to assess the current state of ornithofauna in the region. That is why, there is a need for research work to assess the current state of ornithofauna in the region.

Taking into account the above-mentioned, we studied the distribution, the species composition and the number of the sedentary birds forming winter ornithocomplexes in the mountain-forest belt of Talish.

MATERIALS AND METHODS

The researches were conducted in the 12 stationaries with a total area of 60 km^2 in winter of 2013-2016 (Figure). 36 expeditions were organized to the research area and 110 working days were spent to the observations and registrations. The species of the birds were identified according to the identification books

https://doi.org/10.29228/jlsb.12
Available online 30 June 2021

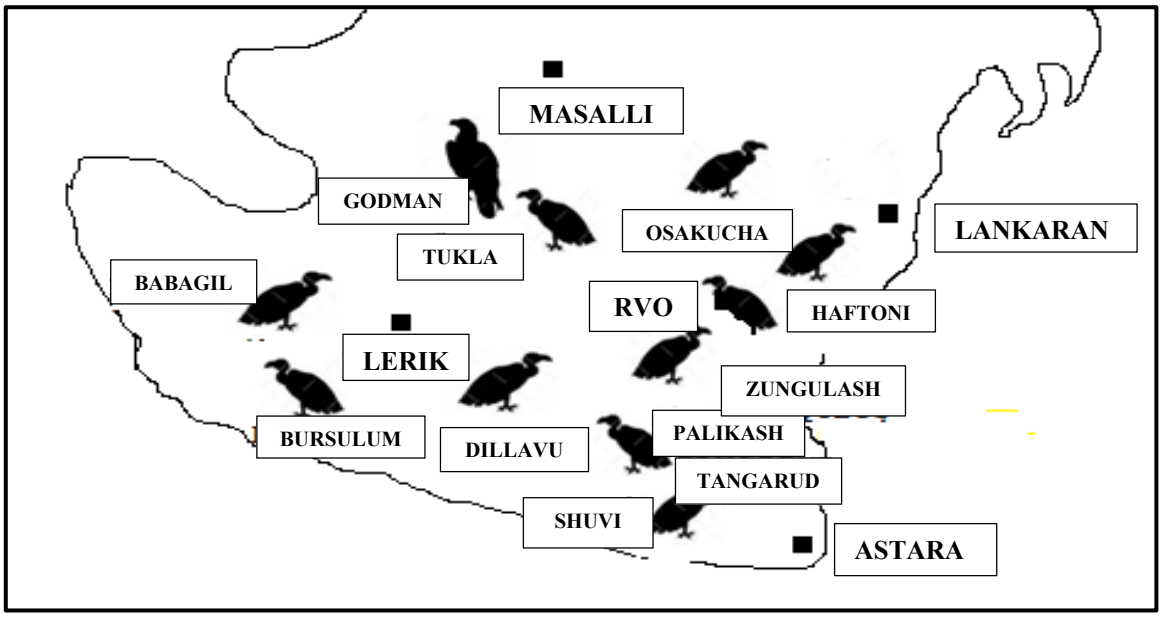

Fig. The schematic map of the research area.

(Mustafayev et al., 2005; Hermann et al., 1995) and the sounds of the birds. Route and the stationary observation methods were used (Sultanov et al., 2008). The category of the species by population density is based to A.P.Kuzyakin (Kuzyakin, 1962) and to G.T.Mustafayev (Mustafayev, 1985): a population of 0.1-0.9 individuals per 1 km^2 was accepted rare; a population of 1-9 individuals was accepted as ordinary, and a population of more than 10 individuals per area was accepted as numerous.

RESULTS AND DISCUSSION

During research, 54 species of the birds with a total number of 149781 individuals were recorded in winter in the mountain-forest belt of Talish (Table). In addition to 6 species, other species are the birds with protected status.

Of the 54 sedentary species, 8 species are rare, 17 are ordinary and 29 are numerous. Due to the differences the natural conditions of the stationaries where the research was conducted, we present a separate analysis of the ornithofauna there.

Zungulash. It is located in the North of the Astarachay bed. The habitat biotopes of the birds consists of the high tier forests, from the shrubberies, from the gardens next to the yard and from the plantations.

We have recorded 50 of the 54 species of the birds in this area which we recorded in winter in the mountain-forest belt of Talish. In 2013-2016, in the mountain-forest belt of Talish, 17.5% (26160 individuals) of the total number of the birds (149781 individuals) settled in this area in winter (Table).

Shuvi. This stationary is located in the Astara region. The habitat biotopes of the birds consists of mainly from the shrubberies.

We have recorded 48 species of the 54 species of the birds in this area which we recorded in winter in the mountain-forest belt of Talish. In winter, 16.3% (24389 individuals) of the total number of the individuals (149781 individuals) settled in this area (Table).

Tangarud. This stationary is located 18 km North from the city of Astara, on the Baku-Astara highway, at the foot of the Talish Mountains.

We have recorded 50 species of the 54 species of the birds in this area which we recorded in winter in the mountain-forest belt of Talish. In 2013-2016, in the mountain-forest belt of Talish, 7.4% (11073 individuals) of the total number of the birds (149781 individuals) settled in this area in winter (Table).

Palikash. This stationary is located 40 km from the center of the Astara region.

We have recorded 46 of the 54 species of the birds in this area which we recorded in winter in the mountain-forest belt of Talish. In 2013-2016, in the mountain-forest belt of Talish, 5.5% (8299 individuals) of the total number of the birds (149781 individuals) settled in this area in winter (Table).

Haftoni. This stationary is an urban settlement located 12 km West of the Lankaran district.

We have recorded 47 species of the 54 species of the birds in this area which we recorded in winter in the mountain-forest belt of Talish. In 2013-2016, in the mountain-forest belt of Talish, 15.3% (22866 individuals) of the total number of the birds (149781 individuals) settled in this area in winter (Table).

Rvo. This stationary is located on the Lankaran-Lerik highway, South-West of the center of the Lankaran region, near the mountain of Ballabur. We have recorded 49 of the 54 species of the birds in this area which we recorded in winter in the mountain-forest belt of Talish. In 2013-2016, in the mountain-forest belt of Talish, 14.3% (21446 individuals) of the total number of the birds (149781 individuals) settled in this area in winter (Table).

Osakucha. This stationary is located 17 km West of the city of Lankaran, at the foot of the Talish Mountains, on the bank of the Veravulchay.

We have registered 42 species of the 54 species of the birds in this area which we recorded in winter in the mountain-forest belt of Talish. In 2013-2016, in the mountain-forest belt of Talish, 4.6% (6910 individuals) of the total number of the birds (149781 individuals) settled in this area in winter (Table).

Table. The quantitative indicators of the sedentary birds in winter in the mountain-forest belt (individual) (2013-2016)

№	Species	Protection status	Astara				Lankaran			Lerik			Masalli		The total average number of the birds	The total average density of the birds
			Zungulash (7 km²)	Shuvi (6 km²)	Tangarud (5 km²)	Palikash (5 km²)	Haftoni (4 km²)	Rvo (7 km²)	Osakucha (3 km²)	Bursulum (4 km²)	Babagil (6 km²)	Dillavu (4 km²)	Tukla (4 km²)	Godman (5 km²)		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1.	Eurasian Goshwak-*Accipiter gentilis*	RBA, CITES, Bern, Bonn	3	4	3	0	3	3	0	4	3	5	4	2	8,5	0,56
2.	Common Buzzard-*Buteo buteo*	CITES, Bern, Bonn	8	0	11	6	4	7	0	12	0	14	7	5	18,5	1,23
3.	Golden Eagle-*Aquila chrysaetos*	RBA, CITES, Bern, Bonn	0	0	2	0	0	3	0	4	0	3	2	0	3,5	0,23
4.	Black Vulture-*Aegypius monachus*	IUCN Red List, RBA, CITES, Bern, Bonn	0	0	4	0	0	5	0	6	0	5	0	0	5	0,33
5.	Griffon Vulture-*Gyps fulvus*	RBA, CITES, Bern, Bonn	5	0	2	0	0	0	4	11	0	5	0	0	6,75	0,45
6.	Common Pheasant-*Phasianus colchicus*	Bern	6	0	0	2	0	0	0	4	2	5	0	0	4,75	0,31

Continued table

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
7.	Common Wood Pigeon-*Columba palumbus*	-	78	6	18	21	0	0	0	48	61	9	28	15	71	4,73
8.	Rock Dove-*Columba livia*	Bern	2500	1400	700	91	1180	1100	200	850	580	970	50	129	2437,5	162,5
9.	Collared Dove-*Streptopelia decaocto*	Bern	10	29	0	0	4	8	14	0	0	0	9	3	19,25	1,28
10.	Eurasian Eagle Owl-*Bubo bubo*	CITES, Bern	5	4	5	0	4	2	0	5	1	3	0	2	7,75	0,51
11.	Long-eared Owl-*Asio otus*	CITES, Bern	0	5	0	6	2	4	6	4	5	6	4	0	10,5	0,7
12.	Little Owl-*Athene noctua*	CITES, Bern	11	7	13	0	17	21	11	7	10	16	0	6	29,75	1,98
13.	Tawny Owl-*Strix aluco*	CITES, Bern	23	12	2	8	21	19	0	0	4	14	4	0	26,75	1,78
14.	Eurasian Green Woodpecker-*Picus viridis*	Bern	1900	800	1211	1190	1200	250	950	670	581	618	280	100	2437,5	162,5
15.	Black Woodpecker-*Dryocopus martius*	Bern	17	6	28	4	2	0	11	19	10	12	8	0	29,25	1,95
16.	Great Spotted Woodpecker-*Dendrocopos major*	Bern	112	98	90	13	110	28	4	110	21	98	31	0	178,75	11,91
17.	Syrian Woodpecker-*Dendrocopos syriacus*	Bern	18	21	9	0	18	0	7	11	0	14	7	5	27,5	1,83
18.	Lesser Spotted Woodpecker-*Dendrocopos minor*	Bern	180	300	400	50	150	120	210	200	300	172	140	60	570,5	38,03
19.	Crested Lark-*Galerida cristata*	Bern	250	150	60	48	34	290	0	90	85	8	15	60	272,5	18,16
20.	Calandra Lark-*Melanocorypha calandra*	Bern	280	140	58	98	11	83	109	218	100	100	58	80	333,75	22,25
21.	WoodLark-*Lullula arborea*	Bern	304	280	630	110	215	298	300	285	218	315	298	7	815	54,33
22.	Jay-*Garrulus glandarius*	Bern	109	98	101	93	128	84	100	180	100	18	49	40	275	18,33
23.	Magpie-*Pica pica*	-	28	34	6	41	90	13	20	18	7	4	14	10	71,25	4,75
24.	Eurasian Jackdaw-*Coloeus monedula*	-	90	63	0	110	98	60	0	0	0	0	38	14	118,25	7,88
25.	Hooded Crow-*Corvus cornix*	-	58	60	30	26	48	54	32	43	24	22	25	28	112,5	7,5
26.	Raven-*Corvus corax*	Bern	13	8	14	11	5	6	0	19	19	8	6	4	28,25	1,88

Continued table

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
27.	White-throated Dipper-*Cinclus cinclus*	Bern	7	12	23	7	27	39	18	24	38	32	0	0	56,75	3,78
28.	Wren-*Troglodytes troglodytes*	Bern	91	72	4	19	71	62	41	23	18	24	0	0	106,25	7,08
29.	Dunnock-*Prunella modularis*	Bern	300	370	50	19	200	218	58	83	28	82	12	20	360	24
30.	Goldcrest-*Regulus regulus*	Bern, Bonn	218	281	147	58	300	218	48	50	81	32	11	6	362,5	24,16
31.	European Robin-*Erithacus rubecula*	Bern, Bonn	310	250	144	58	280	300	57	180	100	30	21	10	435	29
32.	Ring Ouzel-*Turdus torquatus*	Bern, Bonn	300	310	108	92	250	270	9	52	41	22	50	36	385	25,66
33.	Song Thrush-*Turdus philomelos*	Bern, Bonn	350	390	150	110	215	300	64	52	60	28	211	250	545	36,33
34.	Mistle Thrush-*Turdus viscivorus*	Bern, Bonn	110	128	190	78	210	180	30	31	38	22	79	39	283,75	18,91
35.	Long-tailed Tit-*Aegithalos caudatus*	Bern	231	281	28	139	220	180	70	42	53	48	70	68	357,5	23,83
36.	Caspian Tit-*Poecile hyrcanus*	Bern	6	7	8	1	3	3	0	0	0	0	0	0	7	0,46
37.	Coal Tit-*Periparus ater*	Bern	660	590	260	320	450	500	48	90	31	28	113	120	802,5	53,5
38.	Great Tit-*Parus major*	Bern	1670	1700	870	290	1600	1570	409	210	115	156	280	230	2275	151,66
39.	Eurasian Nuthatch-*Sitta europaea*	Bern	1686	1900	910	310	2600	2000	100	38	44	61	70	81	2450	163,33
40.	Western Rock Nuthatch-*Sitta neumayer*	Bern	103	90	250	180	82	110	90	98	84	110	38	40	318,75	21,25
41.	Wallcreeper-*Tichodroma muraria*	Bern	38	41	18	29	39	21	14	18	21	22	10	9	70	4,66
42.	Eurasian Treecreeper-*Certhia familiaris*	-	1610	1715	500	500	1700	1915	250	210	370	120	400	310	2400	160
43.	House Sparrow-*Passer domesticus*	-	2500	2400	500	800	3000	2941	1000	300	350	109	1000	600	3875	258,33
44.	Eurasian Tree Sparrow-*Passer montanus*	Bern	1400	970	250	700	250	180	650	600	470	100	370	460	1600	106,66
45.	Rock Sparrow-*Petronia petronia*	Bern	13	21	73	47	0	15	62	74	18	110	0	0	108,25	7,21
46.	Common Chaffinch-*Fringilla coelebs*	Bern	2300	2800	360	280	2050	2000	161	209	400	280	700	810	3087,5	205,83
47.	Common Greenfinch-*Chloris chloris*	Bern	3000	2900	1000	800	3000	2730	500	160	210	180	900	720	4025	268,33
48.	Eurasian Siskin-*Spinus spinus*	Bern	1000	1200	500	300	950	1000	160	175	181	148	100	86	1450	96,66

Continued table

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
49.	European Goldfinch-*Carduelis carduelis*	Bern	388	450	200	380	410	300	200	90	75	89	100	78	690	46
50.	Eurasian Linnet-*Linaria cannabina*	Bern	1100	965	215	310	600	780	510	200	215	150	350	285	1420	94,66
51.	Eurasian Bullfinch-*Pyrrhula pyrrhula*	Bern	0	0	50	14	0	31	80	50	58	29	0	0	78	5,2
52.	Hawfinch-*Coccothraustes coccothraustes*	Bern	700	950	770	410	915	900	270	600	715	410	150	120	1727,5	115,16
53.	Corn Bunting-*Emberiza calandra*	Bern	55	60	50	70	60	135	28	39	51	23	70	64	176,25	11,75
54.	Rock Bunting-*Emberiza cia*	Bern	6	11	48	50	40	90	5	18	20	5	0	0	73,25	4,88
Total			**50 species 17,5 % (26160 individuals)**	**48 species 16,3 % (24389 individuals)**	**50 species 7,4 % (11073 individuals)**	**46 species 5,5 % (8299 individuals)**	**47 species 15,3 % (22866 individuals)**	**49 species 14,3 % (21446 individuals)**	**42 species 4,6 % (6910 individuals)**	**50 species 4,4 % (6534 individuals)**	**46 species 4 % (6016 individuals)**	**51 species 3,3 % (4894 individuals)**	**43 species 4,1 % (6182 individuals)**	**40 species 3,3 % (5012 individuals)**	**37445,25**	
			149781 indviduums													

Note: RBA-The Red Book of Azerbaijan; **IUCN Red List-**The International Union for Conservation of Nature (IUCN) Red List; **CITES, Bern, Bonn-**conventions.

Bursulum. This stationary is located 70 km from the center of the Lerik district.

We have recorded 50 species of the 54 species of the birds in this area which we recorded in winter in the mountain-forest belt of Talish. In 2013-2016, in the mountain-forest belt of Talish, 4.4% (6534 individuals) of the total number of the birds (149781 individuals) settled in this area in winter (Table).

Babagil. This stationary is located 40 km to the Lankaran-Lerik highway, by the Lankaranchay in the Lerik district.

We have recorded 46 species of the 54 species of the birds in this area which we recorded in winter in the mountain-forest belt of Talish. In 2013-2016, in the mountain-forest belt of Talish, 4% (6016 individuals) of the total number of the birds (149781 individuals) settled in this area in winter (Table).

Dillavu. This stationary is located in the Lerik district. We have registered 51 species of the 54 species of the birds in this area which we recorded in winter in the mountain-forest belt of Talish. In 2013-2016, in the mountain-forest belt of Talish, 3.3% (4894 individuals) of the total number of the birds (149781 individuals) settled in this area in winter (Table).

Tukla. This stationary is located in the Masalli district.

We have recorded 43 species of the 54 species of the birds in this area which we recorded in winter in the mountain-forest belt of Talish. In 2013-2016, in the mountain-forest belt of Talish, 4.1% (6182 individuals) of the total number of the birds (149781 individuals) settled in this area in winter (Table).

Godman. This stationary is located in the Masalli district.

We have recorded 40 species of the 54 species of the birds in this area which we recorded in winter in the mountain-forest belt of Talish. In 2013-2016, in the mountain-forest belt of Talish, 3.3% (5012 individuals) of the total number of the birds (149781 individuals) settled in this area in winter (Table).

In general, the trophic conditions of the biotopes, the anthropogenic factors, the ecological and the ethological characteristics (the adaptation of the species to the specific conditions, the number of the enemies and the feed rivals, the number of shelters, the feed objects and access to them, the anthropogenic impacts, etc.) were influenced to the number and the density of the species in the researching stationary. The dominance of the Sparrows is due namely to their better adaptation to the natural conditions of the mountain-forest belt of Talish in the researching stationary.

RESULTS

1. 54 species belonging to the 6 groups were registered as part of the winter ornithocomplexes of the mountain-forest belt of Talish. The total number of the 54 species of the sedentary birds was 149781 individuals.
2. Of the 54 species that we recorded, 8 species are rare, 17 are ordinary, and 29 are numerous.
3. 54 registered species of the birds have global, national protection status and were included to the lists of CITES, Bonn and Bern conventions. Only 6 (11.1%) of the 54 species of the birds do not have protection status.
4. The main dominant birds according to the species composition and the total number of winter ornithocomplexes are the species belonging to the order Sparrows *(Passeriformes)*. These birds constitutes 66.7% of all registered species (36 species) and 84.3% of the total number of the birds (126209 individuals). The dominant species according to the number of the individuals is the Common Greenfinch (*Chloris chloris)*.
5. It was determined that the feeding (trophic conditions), protection from the enemies, overnight stay, the recreation conditions, including the anthropogenic factors as recreation in forest areas, tourist centers, cattle grazing, illegal bird hunting, road construction affects to the species composition and the density in the ornithocomplexes. All this factors, first of all, have a negative impact to the trophic relationships with the biotope and the numbers of the species that are more sensitive to the anthropogenic impacts and the threats.

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Talışın dağ-meşə qurşağında qış ornitokomplekslərini formalaşdıran oturaq quşların növ tərkibi və kəmiyyət göstəriciləri

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Məqalədə Talışın dağ-meşə qurşağında qışda 2013-2016-cı illərdə oturaq quşların növ tərkibi, sayı, sıxlığı, yayılması və onlara təsir edən amillər haqqında materiallar təqdim edilmişdir. Talışın dağ-meşə qurşağının qış ornitokomplekslərinin tərkibində 6 dəstəyə mənsub 54 növ qeydə alınmışdır. 54 növ oturaq quşun ümumi sayı 149781 fərd təşkil etmişdir. Qeydə aldığımız 54 növün 8-i nadir, 17-si adi saylı, 29-u isə çoxsaylıdır. Qeydə alınan 54 növ quş qlobal, milli mühafizə statuslarına malikdir, CITES, Bonn və Bern konvensiyalarının siyahılarına daxil edilmişdir. 54 növ quşun yalnız 6-sı (11,1%-i) mühafizə statusuna malik deyildir. Qış ornitokompleklərinin növ tərkibinə və ümumi saylarına görə əsas dominant quşlar Sərçəkimilər *(Passeriformes)* dəstəsinə mənsub olan növlərdir. Bu quşlar qeydə alınan bütün növlərin 66,7%-ini (36 növ), quşların ümumi sayının 84,3 %-ini (126209 fərd) təşkil edir. Fərdlərinin sayına görə dominant növ Adi yaşılcadır (*Chloris chloris)*. Müəyyən edildi ki, ornitokomplekslərdə quşların növ tərkibinə və sıxlığına yemlənmə (trofik şərait), düşmənlərindən qorunma, gecələmə, istirahət şəraitləri, o cümlədən, meşə zonalarında istirahət, turizm mərkəzlərinin, mal-qara otarılması, qanunsuz quş ovu, yol çəkilişi kimi antropogen amillər təsir gostərir. Bütün bu amillər, ilk növbədə, antropogen təsirlərə, təhlükələrə daha həssas olan növlərin biotopla trofik əlaqələrinə, saylarına mənfi təsir göstərir.

Açar sözlər: *Talış dağları, oturaq quşlar, növ, say, sıxlıq, faktor*

Видовой состав и показатели количества оседлых птиц, формирующих зимние орнитокомплексы в горно-лесном поясе Талыша

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В статье представлены материалы о видовом составе, численности, плотности, распространении оседлых птиц и влияющих на них факторах в горно-лесном поясе Талыша в зимние периоды 2013-2016 годов. В составе зимних орнитокомплексов Талышского горно-лесного пояса отмечены включенные в 6 отрядов 54 вида. Общее число особей из 54 видов оседлых птиц составило 149781. Из 54 видов, отмеченных нами, 8 видов, являются редкими, 17 – обычными, 29 – многочисленными. 54 зарегистрированных вида птиц имеют глобальный национальный охранный статус и включены в списки CITES, Боннской и Бернской конвенций. Лишь 6 (11,1%) из 54 видов птиц не имеют охранного статуса. По видовому составу и общему количеству зимних орнитокомплексов основными доминирующими птицами являются виды, относящиеся к отряду Воробьиных *(Passeriformes)*. Эти птицы составляют 66,7% (36 виды) от всех зарегистрированных видов и 84,3% (126209 особей) от общего числа птиц. Доминирующим видом по количеству особей является Обыкновенная зеленушка *(Chloris chloris)*. Основными ограничивающими факторами являются вырубка деревьев в связи со строительством газопроводов и дорог к населенным пунктам, создание зон отдыха и туризма в лесных массивах, выпас скота и незаконная охота на птиц. Такое антропогенное воздействие приводит к постепенному сокращению количества местообитаний и мест кормежки уязвимых видов.

Ключевые слова: *Талышские горы, оседлые птицы, вид, численность, плотность, фактор*

Journal of Life Sciences & Biomedicine, vol. 3(76), No 1, p. 99-107 (2021)

Impact of illumination on plant Photosystem II at different temperatures

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Received: October 22, 2020; Received in revised form: June 12, 2021; Accepted: June 18, 2021

The photochemical activity of photosystem II (PSII) illuminated at different temperatures (20-55°C) has been studied. The photochemical activity of PSII preparations incubated at different temperatures in the dark decreased sharply in the range of 40-55°C, and was relatively stable at temperatures 20-40°C. The photochemical activity of PSII decreased with relatively monotonous kinetics in preparations processed at different temperatures and exposed to light, but in the end, it was higher than the activity observed in samples incubated in the dark. The effects of glycerin and sucrose on the PSII inhibition due to the exposure to different temperatures in darkness, and after temperature-light treatment were studied. The photochemical activity of PSII was measured after the incubation of the samples in a solution containing 50% glycerol (volume) or 1 M sucrose for 5 minutes in the dark at a certain temperature or under high light intensity at the same temperature. The photochemical activity of the PSII complex was found to be partially preserved in samples incubated in a glycerin-containing solution, at high temperatures, in both dark and light. The addition of sucrose to the solution resulted in a higher degree of protection of the photochemical activity of PSII.

Keywords: *Photosystem II, temperature, light, inhibition*

Abbreviations: ***BBY*** *((Berthold, Babcock, Yocum) Thylakoid membrane fragments enriched with PSII),* ***Cyt b$_{559}$*** *(Cytochrome b$_{559}$),* ***PSII*** *(Photosystem II),* ***F$_V$ (F$_0$)*** *(Variable (initial) fluorescence of chlorophyll),* ***Q$_A$, Q$_B$*** *(Plastoquinones, electron acceptors),* ***MES*** *(4-Morpholinye-ethane-sulfonic acid), organic buffer),* ***P$_{680}$*** *(Primary donor of the electron in PSII),* ***Pheo*** *(Pheophytin),* ***RC*** *(Reaction center),* ***Y$_Z$, Y$_D$*** *(Tyrozines, electron donors of P680)*

INTRODUCTION

Environmental factors such as drought, salinity, and high temperature as well as toxic organic and inorganic substances intruding the environment due to technogenic disasters affect metabolic processes in plants and retard their development, thereby creating a serious danger to the ecosystem, agriculture and the development of the whole society (Krupa and Baszynski, 1995; Raven et al., 1999; Bertels and Sunkar R., 2005; Hasanuzzaman et al., 2013). Different mechanisms are involved in the response of plants to each of the extreme factors. However, under natural conditions, several factors affect the plant simultaneously and depending on the physiological state of the plant, the factor type, the duration and strength of the factor, more complex patterns of the response may occur (Havaux, 1992; Murata et al., 2007; Takahashi and Murata, 2008). One of the main targets affected by extreme factors in the plants is the photosynthetic process providing energy and oxygen for living things. Therefore, the study of the mechanisms of the effects of environmental factors on the functional activity of photosynthetic membranes, which is important for clarifying the balance of life processes on Earth, has become one of the substantial problems of modern biology.

Of the photosynthetic complexes, photosystem II (PSII) is more sensitive to unfavorable physicochemical factors, and the effect of the above factors

https://doi.org/10.29228/jlsb.13
Available online 30 June 2021

on the photosynthetic apparatus of plants is mostly determined by PSII (Murata et al., 2007). PSII of plants, algae, and prokaryotic cyanobacteria is composed of ~30 proteins including light-harvesting antenna proteins, and numerous cofactors. Some of these cofactors are involved in light absorption and electron transfer, thereby providing the realization of the PSII function. The photochemical core of PSII includes PsbA (D_1), PsbD (D_2), PsbB (CP47), PsbC (CP43) and Cyt b_{559} proteins, and redox cofactors such as chlorophyll dimer P_{680}, pheophytin (Pheo), plastoquinones Q_A and Q_B, and tyrosine residues Y_Z (D_1-Tyr^{161}) and Y_D (D_2-Tyr^{161}). The main function of this complex is to catalyze the oxidation of water. This function is due to the cooperative action of the components of the PSII photochemical core (P_{680}, Pheo, Q_A, Q_B), Y_Z, and the Mn_4CaO_5 cluster. PsbO,P,Q (PsbO,U,V in cyanobacteria) proteins form the lumen domain of the PSII complex and participate in the stabilization of the Mn_4CaO_5 cluster (Shen, 2015).

In the initial stage of the energy conversion, the electron is transferred from the excited chlorophyll molecule (P_{680}*) to pheophytin for ~3 ps and the unstable $P_{680}^{\bullet+}Pheo^{\bullet-}$ pair is formed. In the subsequent stage, the electron is transferred from $Pheo^{\bullet-}$ to plastoquinone (Q_A) for ~200 ps and from tyrosine Y_Z to $P_{680}^{\bullet+}$ for 20-260 ns (Feyziyev, 2019). The oxidized tyrosine ($Y_Z^{\bullet+}$) receives an electron from the Mn_4CaO_5 cluster, which is the inorganic core of the water oxidation center. In general, PSII acts as "water- plastoquinone oxidoreductase" and catalyses the oxidation of two water molecules, ultimately resulting in the release of molecular oxygen to the atmosphere and 4 protons to the lumen of thylakoids. (Muh and Zouni, 2011).

The paper presents an *in vitro* study of the simultaneous effects of two different factors - high temperature and light on the photochemical activity of plant PSII preparations.

MATERIALS AND METHODS

PSII membrane preparations (BBY particles) were used in the study (Berthold et al., 1981; Völker et al., 1985). The experiment was carried out in the medium containing 50 mM MES-NaOH (pH6.1), 20 mM NaCl, 3 mM $MgCl_2$, and other components (glycerine, sucrose) were added when needed. The PSII preparations were incubated at different temperatures (in the range of 20–55°C) for 5 min in the dark or at a light intensity of ~80 μmol photon $m^{-2}s^{-1}$. After the inhibitory incubation period, the sample was diluted 3 times with the cold buffer solution, and fluorescence was measured.

The concentration of chlorophyll was determined spectrophotometrically measuring the optical density of its 80% acetone extract at 645 and 663 nm (MacKinney, 1941). The intensity of initial, variable, and maximum fluorescence of PSII (F_0, F_V, and F_M, respectively) was measured in an optical spectrometer supported by phosphororscope. The intensity and wavelength of measuring (which excite the fluorescence) and actinic (which excite the charge separation) light were ~5 μmol photon $m^{-2}s^{-1}$, λ=490 nm, and ~10^3 μmol photon $m^{-2}s^{-1}$, λ>650 nm, respectively. The concentration of chlorophyll in samples was 15 μg/ml.

The measurements were made in 3-6 repetitions, average values and standard deviations were determined.

RESULTS

We evaluated the photochemical activity of the isolated PSII membranes by measuring variable chlorophyll fluorescence. According to the measurements, the F_V/F_M value for the isolated FSII membranes was ~ 0.75 (75%). Since the value and kinetics of variable fluorescence characterize the electrons transfer from P_{680} to plastoquinone (Q_A) in PSII, it is widely used to evaluate the photochemical reaction proceeding at the reaction center (Kalaji et al., 2017; Feyziyev, 2019).

The isolated PSII membrane fragments were exposed to different temperatures in the dark and light for 5 min, and then variable fluorescence was measured in these samples. Figure 1 shows the kinetic changes of fluorescence at different temperatures.

As seen in the figure, variable fluorescence of chlorophyll was higher at room temperature (20°C) in both dark- and light-incubated preparations with values a little lower for the light-incubated ones (Curves 1 and 1a). This suggests that short-term incubation at room temperature did not affect the photochemical activity of the PSII preparations and that

the insignificant decrease in variable fluorescence of chlorophyll observed in the illuminated samples was most likely due to slight photoinhibition of PSII.

An increase in temperature caused a decrease in the intensity of variable fluorescence of chlorophyll in the dark and under illumination. These effects of temperature and light are demonstrated in figure 1 (curves 2 and 2a) for 45℃. As shown in the figure the intensity of variable fluorescence in illuminated preparations was lower than in the dark-incubated samples.

A further increase in temperature was accompanied by a slightly different effect. Thus, measurements at 52℃ showed that the dark-incubated samples almost completely lost their photochemical activity, while the intensity of variable fluorescence in the illuminated samples still remained high (curves 3 and 3a). This indicates that the illuminated PSII membrane fragments partially retain their photochemical activity. It is clear that both thermoinhibition and photoinhibition occur at this temperature. However, it is likely that there exists a mechanism that positively influences the intensity of variable fluorescence - the photochemical activity of PSII. It can be assumed that this mechanism is the photoactivation of the Mn cluster inactivated thermally (Bao and Burnap, 2016).

Figure 2 shows the temperature dependence of the photochemical activity of PSII membranes incubated in the dark and under illumination in the temperature range of 20-52.5℃. According to the figure, the photochemical activity of PSII defined as the F_V/F_M ratio decreases with increasing temperature, both in the dark- and light-treated samples at different temperatures. However, this inhibition occurred in different ways: in dark-incubated samples, a slight decrease in photochemical activity occurred when rising the temperature up to ~45℃ and in the subsequent short temperature range, a sharp decrease in the PSII photochemical activity was observed.

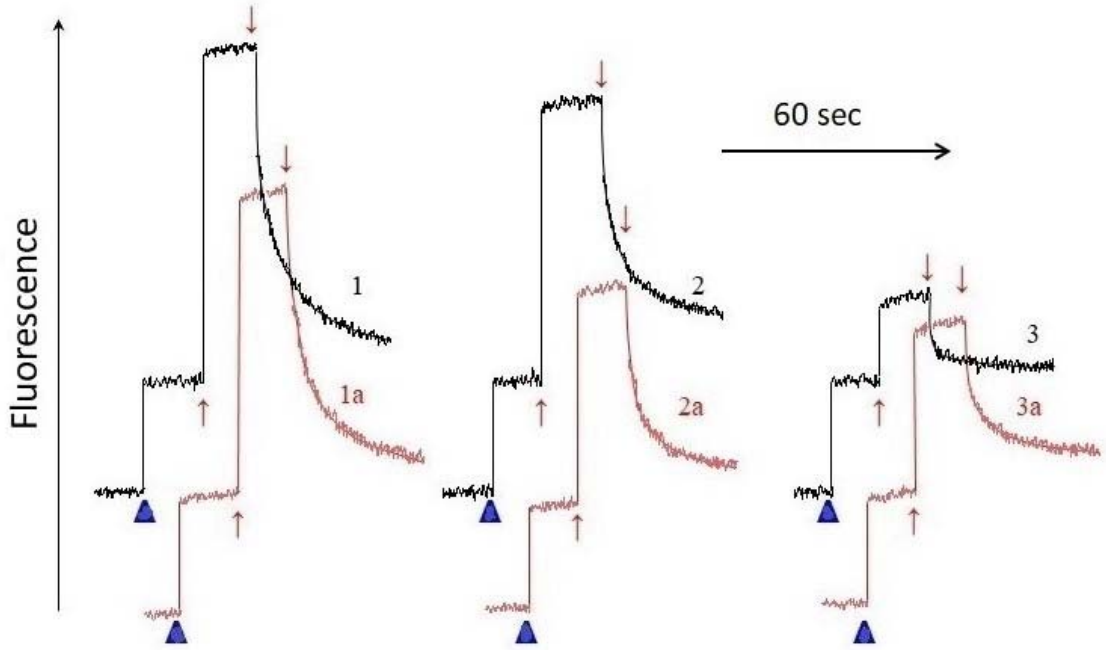

Fig. 1. Variable fluorescence of chlorophyll in PSII membrane fragments (BBY-particles) incubated at different temperatures in the dark and under illumination. PSII membrane fragments were incubated for 5 min at 20℃; 45℃ and 52℃ - in the dark (1, 2, and 3, respectively) or under illumination (1a, 2a, and 3a, respectively).
▲ – measuring light (490 nm; ~5μ mol photon $m^{-2}s^{-1}$) on; ↑ (↓) – actinic light (λ>650 nm; ~1000 μ mol photon $m^{-2}s^{-1}$) on (off).

Whereas, in the light-incubated samples, a monotonous decrease in PSII photochemical activity was detected with increasing temperature in the whole considered temperature range. Although in the temperature range of 20-35°C, its value was in the same order as that of the dark-incubated preparations, in the temperature range of 35-50°C, it was lower than the activity typical of dark-incubated samples. Finally, the photochemical activity of PSII in the illuminated preparations was significantly higher than in the dark-treated samples.

All three processes – thermal inhibition and photoinhibition, and photoactivation – can be assumed to occur simultaneously in PSII membranes illuminated in the temperature range of 20-55°C, and that contrary to the dark-treated samples, the photochemical activity inherent in illuminated samples is determined by the balance of these three processes. Heat-induced inhibition and photoinhibition of preparations play a leading role in this balance at temperatures > 40°C. However, at temperatures >50°C, illumination demonstrated a clear effect on the photochemical activity of PSII samples (Fig. 2, red symbols) and the activity increased due to contribution to the photoactivation process. At temperatures <40°C, all three processes can be assumed to be equally probable.

Thus, the inhibition that occurs at high temperatures as a result of lighting is partially eliminated. We have studied the effect of glycerin and sucrose on the heat-induced inhibition occurring in the dark and light. These compounds are known to protect biological molecules from damage and are used to ensure their stability. For example, in our research, high concentrations (0.3-0.4 M) of sucrose are used in the preparatory isolation process (conducted at a temperature of 4°C). The results of our study are shown in Figure 3A (glycerin) and 3B (sucrose). The amount of glycerin and sucrose in the incubation medium was 50% and 1.0 M, respectively.

As seen in Figure 3, a photochemical activity of PSII remained almost stable in the glycerin-containing medium in the temperature range of 20-45°C, both in the dark and light. A gradual decrease (20-25%) occurs only at >45°C (45-55°C) temperatures.

A similar pattern was observed in the sucrose-containing medium: in this case, the photochemical activity of PSII remained stable in the temperature range of 20–45°C both in the dark and light and decreased by ~ 15% at subsequent >45°C temperatures (Fig. 3B).

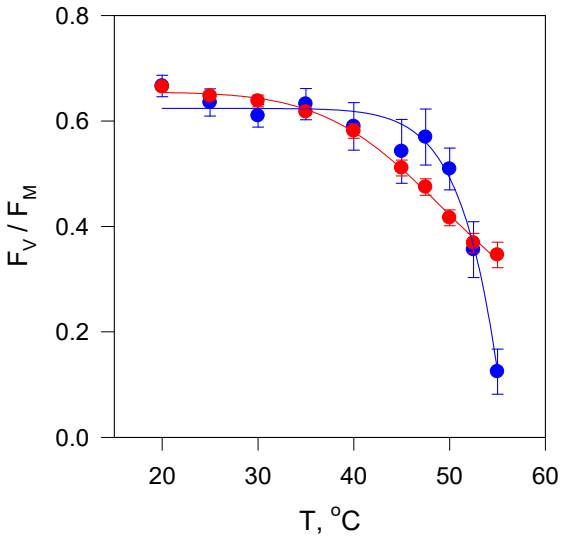

Figure 2. Temperature-dependent changes in the photochemical activity of the PSII preparations (BBY-particles) incubated at different temperatures in the dark and under illumination. The treatment of the samples at various temperatures in the dark (blue symbols) and under illumination (red symbols) was performed for 5 min. The light intensity was ~80 µmol photon $m^{-2}s^{-1}$. The experiments were conducted in the 50 mM MES-NaOH (pH 6.1) buffer solution containing 20 mM NaCl, 3 mM $MgCl_2$. The PSII membranes were incubated in the dark and light at various temperatures for 5 min and then diluted 3 times with the cold buffer solution and the measurements were made. The chlorophyll concentration was 15 µg/ml. The measurements were made in 3 repetitions, average values and standard deviations were calculated.

As seen in Figure 3, in both cases, the temperature factor plays a key role in inhibition, as the change in photochemical activity during lighting is almost indistinguishable from that observed in the dark-treated preparations. Thus, in these two cases, the roles of photoinhibition and photoactivation are not clear. It can be assumed that in both cases, the mechanism of heat-induced inhibition does not involve the electron transport of PSII on the donor side. For this reason, photoactivation of the catalytic center (Mn cluster and its immediate surroundings) where water is oxidized does not occur.

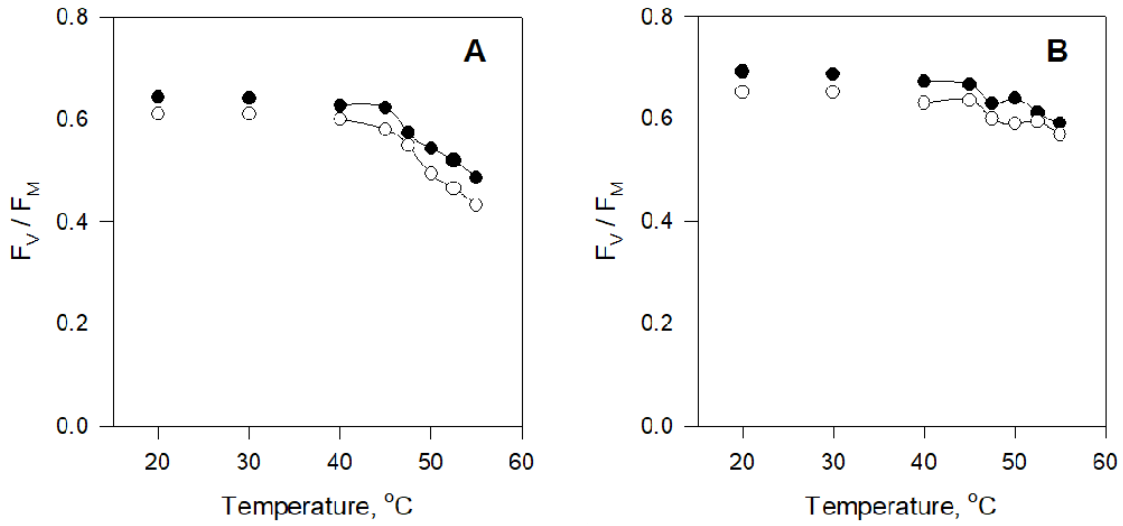

Figure 3. The effects of 50% (volume) glycerine (A) and 1M sucrose (B) on changes of photochemical activity of PSII at different temperatures in dark-incubated (black symbols) and light-incubated samples (light symbols). The measurements were made in 3 repetitions. Other parameters are shown in Fıgure 2.

Thus, the results of our research can be summarized as follows: (i) PSII complexes are inhibited both in the dark and light in the temperature range of 20°C–55°C. They gradually lose their photochemical activity with increasing temperature *in vitro*; (ii) The inhibitory effects of heat and lighting at high temperatures are not synergic: at temperatures >45°C, the thermal inhibition is partially eliminated by lighting; (iii) The compounds such as glycerin and sucrose prevent the inhibition of PSII preparations by both heat and the combined effects of heat and lighting.

DISCUSSION

The interesting aspects of PSII for researchers are related to the individual properties of this complex. Thus, first of all, PSII performs a very important biological function, such as the catalytic oxidation of water, and enriches the atmosphere with oxygen. The formation and existence of aerobic life on Earth are directly related to the activity of PSII. Second, this complex is the main target of many stress factors (heat, salinity, intense illumination, etc.), pollutants (heavy metals, organic compounds), and herbicides. This is attributed to the very fine structure of the PSII complex, the presence of high-affinity sites for organic and inorganic compounds. Third, in recent years, the creation of artificial devices based on the structure and function of biological complexes is in the focus of the alternative energy source projects. Along with other photosynthetic complexes, PSII may have a significant share in the realization of such concepts. Thus, the quantum yield of the initial photochemical reaction that takes place at its reaction center demonstrates a very high value (≥98%).-The primary electron donor of PSII in its oxidized form ($P_{680}^{\bullet+}$) has a very high (~ 1.2 V) potential that increases the interest for its practical usage. On the other hand, protons released to lumen as a result of the PSII activity, form an electrochemical gradient, besides ATP synthesis, they can be directed to the other metabolic processes (Feyziev, 2010; Barber and Tran, 2013; Maitra et al., 2014; Shen, 2015).

In all considered cases, the use of the properties of the PS II complex for practical purposes is limited due to its resistance to harmful effects of the environment, especially high temperatures, intense lighting, and pollutants. Given that these factors often coexist and act simultaneously, the combined effects of heat and light on PSII have been studied *in vitro*.

PSII is known to be inhibited under high temperatures as well as high light intensity (Yamashita et al., 2008; Yamomoto, 2001; 2016; Yin et al.,

2010). The inhibition depends on the duration of plant exposure to these factors. In our experiments, the exposure time was 5 min. This period is sufficient for the preparation to undergo heat shock *in vitro*. Changes in the PSII complex under the influence of heat are observed mainly at the biochemical level – proteins, lipids, enzymes, pigments, etc. As the incubation temperature of the preparation rises, a sharp decrease in the photochemical activity of the preparation, especially at high temperatures, occurs (Peters et al., 1999; Zhang et al., 2012).

In our experiments, the intensity of variable fluorescence decreased sharply starting from temperatures >45°C. This change under high temperatures may be primarily due to the loss of stability of the Mn cluster, as a result of perturbations on the donor side of PSII leading to dissociation of PsbO,P,Q proteins, and the elimination of this cluster from the binding site at very high temperatures (Bao and Burnap, 2015). Since such inactivation of the Mn cluster at very high temperatures is not reversible, the electron transport activity of the complex is completely inhibited.

Another mechanism of heat-induced inhibition occurs at the reaction center. This process is initiated by heat-induced damage to the lipid phase of the membrane or direct damage to RC proteins (Tsonev and Hirosaka, 2003). However, the role of these two mechanisms in the inhibition of photochemical activity (variable fluorescence) of PSII is not known, and these two processes can be assumed to occur with equal probability at high temperatures.

Several mechanisms can be involved in photoinhibition of PSII under high light intensity. These include extreme reduction of electron acceptor Q_A, the formation of a triplet ($^TP_{680}$ or $^3P_{680}$) or oxidized form of chlorophyll in RC ($P_{680}^{\bullet+}$), and inhibition of the PSII donor side. Photoinhibition can also occur in low-intensity lighting, and in this case, even the quantum yield of the process is expected to be higher.

However, in contrast to photoinhibition, which occurs in the PSII complex, there is also a positive photoactivation mechanism. This mechanism is always active in the process of *in vivo* assembly of the PSII complex, ensuring the reassembly of the water oxidation catalytic center during the *de nova* synthesis of proteins. In PSII complexes, which donor side is inhibited by the elimination of the Mn cluster *in vitro*, in addition to other factors, a photoactivation phase is required for the reassembly of the Mn cluster and restoration of electron transport (oxygen-evolving activity) (Bao and Burnap, 2015). Thus, in PSII, both inhibition and recovery processes can occur during illumination.

Our results can be explained by the abovementioned mechanisms of heat-induced inhibition, photoinhibition, and photoactivation. Thus, in our experiments, the share of heat thermoinhibition and photoinhibition mechanisms in the general inhibition of electron transport is small on the donor side of PSII at temperatures ≤45°C. Therefore, the photoactivation effects are not visually observed in this temperature range. However, at temperatures > 45°C, the perturbation in the Mn cluster of PSII caused by temperature is likely to be eliminated by photoactivation during lighting. Thus, due to photoactivation, the complete thermal inhibition of the photochemical activity of PSII is prevented. However, in the end, photochemical activity (electron transport) does not fully recover under illumination. A simple explanation for this is the possibility of irreversible changes in the membrane structure and RC under high temperatures, as well as the activation of an inhibitory mechanism at the expense of $P_{680}^{\bullet+}$ during photoinhibition.

This scheme can explain the maintenance of physiological activity for a long time and the survival of plants under high temperature and light intensity. Thus, in this case, along with the antioxidant system, the photoactivation process is probably one of the main protection mechanisms.

The observed protective role of the compounds such as glycerin and sucrose against high temperature and combined effect of high temperature and lighting is a feature of each compound demonstrated against heat-induced inhibition as well as photoinhibition of the PSII complex. Thus, a slight inhibition of PSII occurs in the medium containing these compounds, therefore, there is almost no need for photoactivation, and this phenomenon is not observed.

ACKNOWLEDGMENTS

This work was supported by the Science Development Foundation under the President of the Republic of Azerbaijan – **Grant № EIF-BGM-4-RFTF-1/2017-21/20/3**, and by the Presidium of Azerbaijan National Academy of Sciences – **Grant dated on 15.03.2017**.

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Müxtəlif temperaturlarda bitkilərin ikinci fotosisteminə işıqlanmanın təsiri

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Fotosistem II (FSII) kompleksinin fotokimyəvi aktivliyi müxtəlif temperaturlarda (20-50°C) işıqlandırılaraq öyrənilmişdir. Qaranlıqda, müxtəlif temperaturlarda inkubasiya olunmuş FSII membran fraqmentlərinin fotokimyəvi fəallığının 40-55°C intervalında kəskin azalması, aşağı temperaturlarda (20-40°C) isə nisbətən sabit olması müşahidə olunmuşdur. Müxtəlif temperaturlarda işlənilmiş və işığın təsirinə məruz qalmış nümunələrdə FSII-nin fotokimyəvi fəallığı nisbətən monoton kinetika ilə azalmış, lakin sonda qaranlıqda inkubasiya edilmiş nümunələrdə müşahidə olunan fəallıqdan yüksək olmuşdur. Qliserin və saxarozanın FSII-nin temperatur (qaranlıq) və birgə temperatur/işıqlanma inhibirləşməsinə təsiri də öyrənilmişdir. Bunun üçün FSII membran fraqmentləri 50% qliserin (həcm) və ya 1 M saxaroza əlavə edilmiş məhlulda 5 dəq müddətində müəyyən temperaturda qaranlıqda və ya həmin temperaturda işıqda inkubasiya edilmişdir. Qliserin əlavə edilmiş mühitdə yüksək temperaturlarda həm qaranlıqda, həm də işıqda inkubasiya edilmiş nümunələrdə FSII kompleksinin fotokimyəvi fəallığı qismən mühafizə olunmuşdur. Məhlula saxarozanın (1M) əlavə olunması isə FSII kompleksinin fotokimyəvi fəallığının qliserinə nisbətən daha yüksək dərəcədə mühafizə olunması ilə nəticələnmişdir.

Açar sözlər: *Fotosistem II, temperatur, işıq, inhibirləşmə*

Влияние освещения на фотосистему II растений при разных температурах

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Исследована фотохимическая активность фотосистемы II (ФСII) растений при разных температурах (20-50°C). В препаратах ФСII, инкубированных в темноте при разных температурах, наблюдалось резкое снижение фотохимической активности в диапазоне 40-55°C и относительная стабильность при температурах 20-40°C. Фотохимическая активность ФСII снижалась относительно монотонной кинетикой в препаратах, обработанных при разных температурах на свету, но в итоге была выше,

чем активность, наблюдаемая в образцах, инкубированных в темноте. Было изучено влияние глицерина и сахарозы на ингибирование ФСII, вызванного воздействием температуры (темнота) и совместного влияния температуры и света. Фотохимическую активность ФSII измеряли после инкубации мембранных фрагментов в растворе, содержащем 50% глицерина (объем) или 1 М сахарозы, в течение 5 минут в темноте при определенной температуре или на свету при той же температуре. Установлено, что фотохимическая активность ФСII частично сохраняется в образцах, инкубированных в глицеринсодержащем растворе при высоких температурах как в темноте, так и на свету. Добавление сахарозы (1М) к раствору приводило к более высокой степени защиты фотохимической активности ФСII.

Ключевые слова: *Фотосистема II, температура, свет, ингибирование*

Journal of Life Sciences & Biomedicine, vol. 3(76), No 1, p. 108-115 (2021)

Comparative analysis of *KRAS* and *NRAS* gene mutations in colorectal cancer

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Received: April 06, 2021; Received in revised form: May12, 2021; Accepted: June 21, 2021

Identifying the genetic profile of cancer by tumor biopsy has made progress in precision medicine. The study of the genetic profile of a tumor can help to choose the optimal treatment at the right time and identification of the cause of drug resistance. It is known that the biopsy is an invasive procedure, and it has some risks. That is why the development of non-invasive methods such as liquid biopsy is required. It is possible to determine circulating cancer cells (CTC) and circulating free tumor DNA (cfDNA) fragments with this technique. In the present study, *KRAS* and *NRAS* codon 12 and 13 mutations were compared in biopsy-derived DNA and liquid biopsy-derived cfDNA. The study included 26 patients with colorectal cancer. DNA extraction has been performed in Human Genetics Laboratory of the Genetic Resources Institute of ANAS, from biopsy material and plasma. Five (19.2%) mutations were detected in tumor DNA samples and 2 (7.7%) mutations in plasma cfDNA in the *KRAS* gene. Totally 3 missense mutations were detected in the *NRAS* gene. Two of these mutations (7.7%) were identified in tissue DNA samples and one of these (3.8%) in cfDNA. It was determined that the incidence of *KRAS* gene mutations in both tissue DNA and cfDNA samples was higher than *NRAS* gene mutations. Obtaining cfDNAs by liquid biopsy and particularly analyzing RAS gene family play a significant role in the early diagnosis, anti-EGFR therapy, selection of the right drugs, resistance, and the prognosis of the disease.

Keywords: *Cancer, liquid biopsy, gene, cfDNT, exon*

INTRODUCTION

Colon cancer is the second most common malignant tumor in the world and the second most common cause of death (Sung et al., 2021). Studies estimate that by 2030, the number of newly diagnosed patients worldwide will be 2.2 million and the number of deaths will be 1.1 million (Arnold et al., 2020). The disease is asymptomatic in the early stages, so it is important to use screening tests for the early detection of malignant tumors as well as precancerous lesions (Zhang et al., 2019; Shaukat et al., 2021). The American Cancer Society recommends a yearly colonoscopy and stool blood test for control after age 50 (Shaukat et al., 2021). Long-term inflammatory diseases of the intestines, smoking, alcohol, malnutrition and some other factors play an important role in the etiology of the disease (Bayramov and Safiyeva, 2019). Studies have indicated that increasing the intake of red meat and fats in the daily diet, reducing the consumption of low-fiber foods, as well as fruits and vegetables increase the risk of disease (Larsson and Wolk, 2006; Haggar and Boushey, 2009; Aune et al., 2011). Factors such as obesity, lack of physical activity, diabetes, family history of disease also increase the risk of developing colon cancer (Haggar and Boushey, 2009; Cho et al., 2012; Deng et al., 2012). There are various oncogenes, tumor suppressor genes, cell cycle control genes, as well as epigenetic and genetic changes in DNA repair genes that play an important role in the molecular pathogenesis of colon cancer (Mahasneh et al., 2017).

Liquid biopsy has been used to analyze the genetic profile of circulating tumor cells (CTC) and

https://doi.org/10.29228/jlsb.14
Available online 30 June 2021

cell-free DNA fragments (cfDNA) and to monitor cancer in a non-invasive procedure (Normanno et al., 2018). Determination of nucleic acids in blood plasma and serum by minimally invasive methods has led to the emergence of modern approaches in the diagnosis and prognosis of colon cancer (Chen və Zhao, 2019). The heterogeneity of the malignant tumor has led to some difficulties in the choice of treatment or the use of tissue biopsy to monitor the disease (Wills et al., 2018). However, the liquid biopsy technique has the potential to eliminate the problem of tumor heterogeneity and to obtain information about the entire cancer genome (Cheung et al., 2018).

cfDNAs are small derivative DNA fragments that may contain mutations specific to cancer tissue and can be easily obtained from venous blood by liquid biopsy (Vymetalkova et al., 2018). The *KRAS*, *HRAS*, and *NRAS* genes are members of the RAS oncogenic family. The proteins encoded by these genes play important roles in cell division, differentiation, apoptosis and other cellular processes. Several organizations, such as the European guidelines and the National Comprehensive Cancer Network Guidelines for Oncology (NCCN) and the Food and Drug Administration (FDA), advise that patients with colorectal cancer who have any *KRAS* or *NRAS* gene mutations should not be treated with anti-EGFR monoclonal antibodies (Hamzehzadeh et al., 2018). The FDA has approved EGFR antibody therapy for patients without mutations in the 12th and 13th codons of the *KRAS* gene. The European Agency for Evaluation Medicinal Products (EMEA) has reported the use of cetuximab and panitumumab in colon cancer based on the analysis of exons 2, 3 and 4 of the *KRAS* and *NRAS* genes (Cutsem et al., 2009; Van Cutsem et al., 2011).

Analysis of genetic changes in the RAS (*KRAS*, *NRAS* and etc.) gene family and other oncogenes in plasma DNA can optimize the choice of target therapeutic drugs and anti-epidermal growth factor receptor (EGFR) therapy, especially for metastatic colorectal cancer. The aim of the current study was to compare *KRAS* and *NRAS* gene mutations in cfDNA fragments taken from blood plasma by liquid biopsy and tumor DNA samples obtained from tissue biopsy material in patients diagnosed with colorectal cancer.

MATERIALS AND METHODS

The study included 26 patients diagnosed with colon cancer at the Educational-Surgical Clinic of Azerbaijan Medical University. Preoperative blood samples were taken from the EDTA tube to obtain cfDNA from the patients included in the study. Patient information such as age, sex, diagnosis, results of pathohistological analysis, etc. registered at the clinic. DNA extraction from blood plasma and tumor biopsy was performed in the Laboratory of Human Genetics of the Genetic Resources Institute of ANAS according to QIAamp DNA Micro Kit protocol. Quantitative and qualitative indicators of DNA were also measured in Nanodrop (Thermo Scientific, 2000). For PCR amplification of *KRAS* and *NRAS* genes in a volume of 25 µl; 2.5 µl 10xPCR buffer, 2.5 µl $MgCl_2$ (50 mM), 0.25 µl dNTP mix (20 mM), 0.5 µl primers (10 pmol/µl), 0.25 µl Taq polymerase (5 U/µl), 2 µl genomic DNA (50 ng/µl) and 16.5 µl dH_2O were used, respectively.

The PCR (Applied Biosystems, USA) cycle conditions consisted of an initial denaturation step at 95˚C for 5 min, followed by 35 cycles at 95˚C for 45 sec, at 54˚C for 45 sec (*KRAS*) or at 58˚C for 30 sec (*NRAS*) and at 72˚C for 2 sec, with a final elongation step at 72˚C for 5 min. After 1.5% agarose gel electrophoresis, PCR amplicons were purified, followed by PCR for sequencing using the BigDye™ Terminator v3.1 Cycle Sequencing Kit. After this procedure, exon 2 of the *KRAS* and *NRAS* genes (codons 12 and 13) was analyzed with the Sanger 3730xL genome sequencer (Applied Biosystems) and the results were compared with the reference genome to identify mutations.

RESULTS AND DISCUSSION

Blood and tissue biopsies of 26 patients were analyzed in the current study. Fifteen (57.7%) of the study group were men and 11 (42.3%) were women. The age range was 39-84, and the average age was 61.9. The pathohistological results of the patients were T2 in 23.1%, T3 in 53.8% and T4 in 23.1%. Tumor grades were determined by pathohistological analysis of 7.7% G1, 73.1% G2 and 19.2% G3. Demographic and clinical parameters of patients are presented in Table 1.

A total of 7 mutations in the *KRAS* gene have been identified. Of these mutations, 5 (19.2%)

were found in tumor DNA samples and 2 (7.7%) in cfDNA. Four of the mutations in the tumor DNA (GGT>GAT, GGT>GTT, GGT>TGT and GGT>TTG) were found in the 12th codon of the 2nd exon of the *KRAS* gene, respectively.

Table 1. Demographic and clinical information of the study group

Characteristic	Study group N=26 (%)
Gender	
Male	15 (57.7%)
Female	11 (42.3%)
Age	
Range	39-84
Average	61.9±10.3
Tumor Stage	
T2	6 (23.1%)
T3	14 (53.8%)
T4	6 (23.1%)
Tumor Grade	
G1	2 (7.7%)
G2	19 (73.1%)
G3	5 (19.2%)

The 12th (GGT) and 13th (GGC) codons of the KRAS gene encode the amino acid glycine. As a result of mutations, the amino acid glycine was replaced by aspartic (Asp), valine (Val) and cysteine (Cys). The GGC> GGA heterozygous mutation in the 13th codon of the *KRAS* gene caused the conversion of valine to asparagine.

Table 2. Mutation spectrum of *KRAS* gene and their localization

Tumor	Codon	Mutation	Amino acid substitution
T1	12	GGT→GAT	Gly→Asp
T4	13	GGC→GAC	Gly→Asp
T6	12	GGT→GTT	Gly→Val
T12	12	GGT→TGT	Gly→Cys
T19	12	GGT→GTT	Gly→Val
Plasma			
P6	12	GGT→GAT	Gly→Asp
P21	12	GGT→GAT	Gly→Asp

Only in the cfDNA of the two samples a heterozygous mutation was detected in codon 12, and no mutation was found in the tissue DNA (Table 2). Figure 1 shows the electropherograms of sequencing analysis.

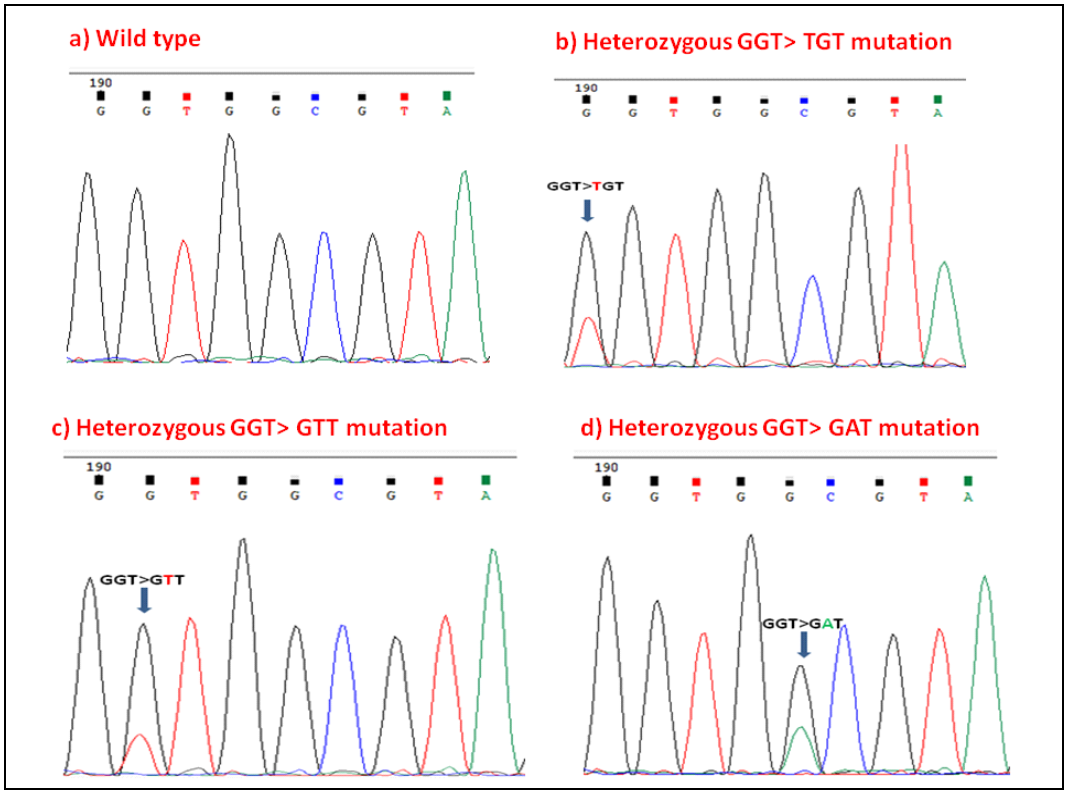

Fig. 1. Sanger sequence electropherogram of the 2nd exon of the *KRAS* gene.

Table 3. The spectrum of mutations in the 2nd exon of the *NRAS* gene and their localization

Tissue	Codon	Mutation	Amino acid substitution
T11	12	GGT→AGT	Gly→Ser
T26	12	GGT→GGG	Gly→Asp
Plasma			
Pl23	13	GGT→TGT	Gly→Cys

A total of 3 missense mutations were detected in 26 patients analyzed for the *NRAS* gene. Sequence analysis identified 2 mutations (7.7%) in tumor DNA and 1 (3.8%) mutation in cfDNA (Table 3). The missense mutation in tumor DNA samples was found in the 12th codon of the *NRAS* gene. In cfDNA (Pl-23), a missense mutation (GGT>TGT) was found in codon 13 of the *NRAS* gene, which caused the conversion of glycine amino acid to the cysteine. Figure 2 shows electrographic images of the *NRAS* gene.

One patient with a GGT>GAT missense mutation in exon 2 of the *KRAS* gene reported liver metastasis, while other patients with the mutation reported no organ metastasis. In cfDNA, in a patient with a GGT>TGT mutation of the *NRAS* gene, the tumor metastasized to both the liver and gallbladder. No metastases were reported in other patients with mutations in this gene.

Liquid biopsy is a minimally invasive technique used in recent years that allows for easy obtaining and analysis of circulating tumor cells (CTCs) and cfDNAs (Marrugo-Ramírez et al., 2018). Isolation analysis of cfDNA in blood plasma has advantages such as early diagnosis, monitoring of the response to therapy, in particular, clarification of the molecular mechanisms of drug resistance (Siravegna et al, 2014). Several biomarkers with widespread clinical use; *KRAS*, *NRAS*, *BRAF* mutations, Human Epidermal Growth Factor Receptor 2 (HER2) microsatellite instability (MSI), DNA repair (MMR) genes, etc. play an important role in choosing the optimal treatment (Afrăsânie et al., 2019). Mutations in codons 12 and 13 of KRAS gene exon 2 are found in 35-45% of cases of colorectal cancer and are considered to be the main predictor of resistance to anti-EGFR treatment (Therkildsen et al., 2014).

In our study, we analyzed 26 patients with colorectal cancer, 19.2% of *KRAS* gene mutations were identified in the biopsy material of patients, and 7.7% of missense mutations were detected on cfDNA. The frequency of mutations in the *NRAS* gene was 7.7% in cancer tissues and 3.3% in cfDNA samples, respectively. Erve et al. found that 54% of 100 patients had a *KRAS* gene mutation, 3% had a cfDNA mutation in a liquid biopsy, and no mutation was found in the *NRAS* gene (Erve et al., 2020).

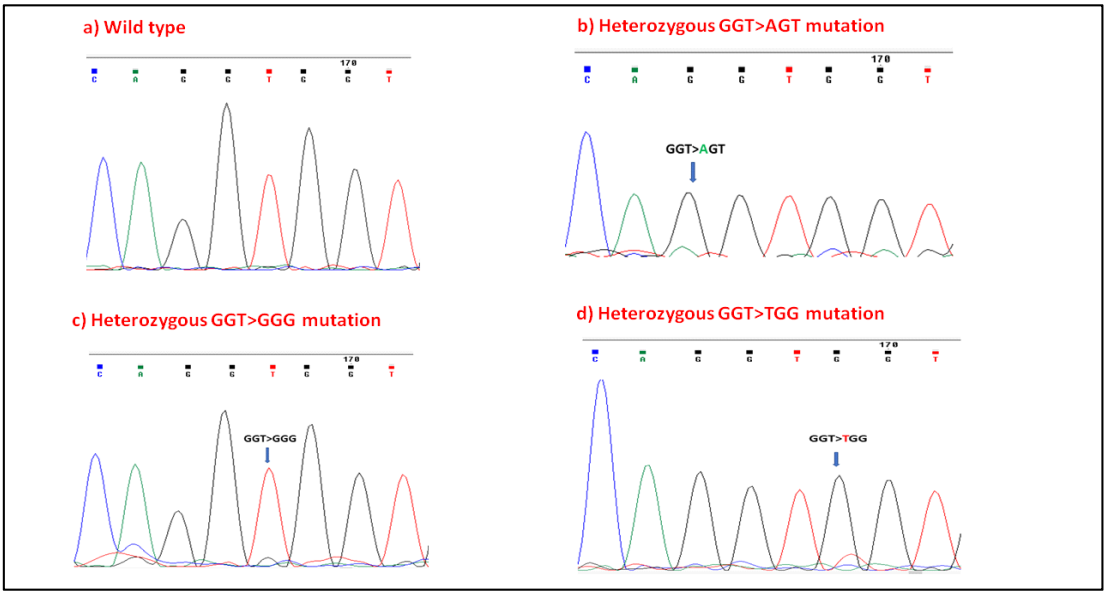

Fig. 2. Sanger sequence electropherogram of the 2nd exon of the *NRAS* gene.

In a study carried out in China in 2018, 50 genes were analyzed in both tumor tissue and cfDNA, a KRAS gene mutation was found in 27.7%, and liquid biopsy was recommended as a non-invasive diagnostic method (Yang et al., 2018). Takeda and colleagues found 47 mutations in cfDNA samples, of which 20 mutations were found only in cfDNA and not in tissue DNA (Takeda et al., 2019). Also, in our study, 2 mutations in the *KRAS* gene and 1 mutation in the *NRAS* gene were found only in cfDNA. This is explained by tissue heterogeneity and the specificity and sensitivity of the liquid biopsy. A study by the University of Montpellier in France found that the frequency of *KRAS*, *NRAS* and *BRAF* gene mutations was higher in cfDNA than in tumor DNA, suggesting that these tests could replace tissue biopsy (Thierry et al., 2017).

In conclusion, in our current study, we compared *KRAS* and *NRAS* gene mutations in both tissue DNA and cfDNA using liquid biopsy in patients diagnosed with colon cancer for the first time, using Sanger sequencing technology. *KRAS* gene mutations were found more frequently in patients compared to the *NRAS* gene. Analysis of *KRAS* gene mutations will enable the selection of the right treatment and obtaining optimal results, especially in patients with metastases. The use of liquid biopsy in medicine may be important in terms of early diagnosis, monitoring, treatment options, time-saving, and detection of new diagnostic biomarkers.

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Yoğun bağırsağın bəd xassəli törəmələrində *KRAS* və *NRAS* gen mutasiyalarının müqayisəli tədqiqi

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Toxuma biopsiyası nəticəsində xərçəng toxumasının genetik profilinin öyrənilməsi fərdiləşdirilmiş tibb sahəsində yeni irəliləyişlərin əldə olunmasına imkan yaratmışdır. Törəmənin genetik profilinin öyrənilməsi vaxtında düzgün müalicə seçimini və dərman rezistentliyi səbəbinin aşkarlanmasını təmin edə bilir. Buna baxmayaraq, biopsiya prosedurunun invaziv olması və müəyyən riskləri əhatə etməsi maye biopsiyası kimi invaziv olmayan metodların inkişaf etdirilməsinə zəmin yaratmışdır. Bu üsulla qanda sirkulyasiya edən xərçəng hüceyrələrini (CTC) və sərbəst şiş DNT (cfDNT) fraqmentlərini analiz etmək mümkündür. Cari tədqiqat işində biopsiya ilə alınan törəmə DNT-si və maye biopsiyası ilə əldə olunan cfDNT-də *KRAS* və *NRAS* genlərinin 12-ci və 13-cü kodonunda baş verən mutasiyalar müqayisəli tədqiq edilmişdir. Tədqiqat işinə yoğun bağırsaq xərçəngi diaqnozu qoyulan 26 xəstə daxil edilmişdir. Genetik analizlərin aparılması üçün biopsiya materialından və qan plazmasından AMEA Genetik Ehtiyatlar İnstitutu, İnsan Genetikası Laboratoriyasında DNT ekstraksiyası həyata keçirilmişdir. *KRAS* geni üzrə 26 şiş toxuması DNT nümunələrində 5 (19,2%) mutasiya, plazma cfDNT nümunələrində isə 2 (7,7%) mutasiya aşkar edilmişdir. *NRAS* geni üzrə isə ümumilikdə 3 missens tipli mutasiya aşkar edilmişdir. Analiz nəticəsində məlum olan mutasiyaların 2-si (7,7%) toxuma DNT nümunələrində, biri (3,8%) isə cfDNT-də müəyyən edilmişdir. *KRAS* gen mutasiyalarının həm toxuma DNT nümunələrində, həm də cfDNT-lərdə *NRAS* geni ilə müqayisədə rastgəlmə tezliyi yüksək olmuşdur. cfDNT-lərin maye biopsiyası vasitəsilə əldə olunması və xüsusilə də *RAS* gen ailəsinin analiz edilməsi erkən diaqnoz, anti-EGFR terapiyası, doğru dərman preparatlarının seçimi, rezistentlik və eləcə də xəstəliyin proqnozlaşdırılması baxımından mühüm əhəmiyyət kəsb edir.

Açar sözlər: *Xərçəng, maye biopsiyası, gen, cfDNT, ekzon*

Сравнительный анализ мутаций генов KRAS и NRAS при колоректальном раке

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Определение генетического профиля рака с помощью биопсии опухоли позволило достичь прогресса в сфере персонализированной медицины. Изучение генетического профиля рака может обеспечить правильный выбор лечения в нужное время и выявить причину лекарственной устойчивости. Известно, что биопсия - это инвазивная процедура и она сопряжена с определенными рисками. Вот почему необходима разработка неинвазивных методов, таких как жидкостная биопсия. С помощью этого метода можно проанализировать циркулирующие раковые клетки (CTC) и циркулирующие фрагменты свободной опухолевой ДНК (cfDNA). В этом исследовании сравнивали мутации в 12-м и 13-м кодонах генов KRAS и NRAS в ДНК, полученной из биопсии, и в cfDNA, полученной из жидкостной биопсии. В данное исследование были включены 26 пациентов с колоректальным раком. Выделение ДНК производилось в Лаборатории Генетики Человека Института Генетических

Ресурсов НАНА из биопсийного материала и плазмы. В гене KRAS 5 мутаций (19,2%) были обнаружены в образцах ДНК опухолевой ткани и 2 мутации (7,7%) в образцах cfDNA плазмы. Всего в гене NRAS обнаружено 3 миссенс-мутации. Две из этих мутаций (7,7%) были идентифицированы в образцах ДНК тканей и одна из них (3,8%) в cfDNA. Было установлено, что частота мутации гена KRAS, как в тканевой ДНК, так и в образцах cfДНК была выше, чем частота мутации гена NRAS. Получение cfDNA с помощью жидкостной биопсии и, особенно, анализ семейства генов RAS играет основную роль в ранней диагностике, терапии против EGFR, выборе правильных лекарств, устойчивости, прогнозировании заболевания.

Ключевые слова: *Рак, жидкая биопсия, ген, cfDNT, экзон*

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NƏŞRİYYATI

Nəşriyyatın direktoru: *Səbuhi Qəhrəmanov*
Kompüter tərtibçisi: *Rəvanə İlmanqızı*
Bədii tərtibat: *Şəlalə Məmməd*

Formatı 60x84 $^{1}/_{8}$
Həcmi 14,5 ç.v.
Tirajı 300

Ünvan: Bakı şəh., İstiqlaliyyət küç. 28