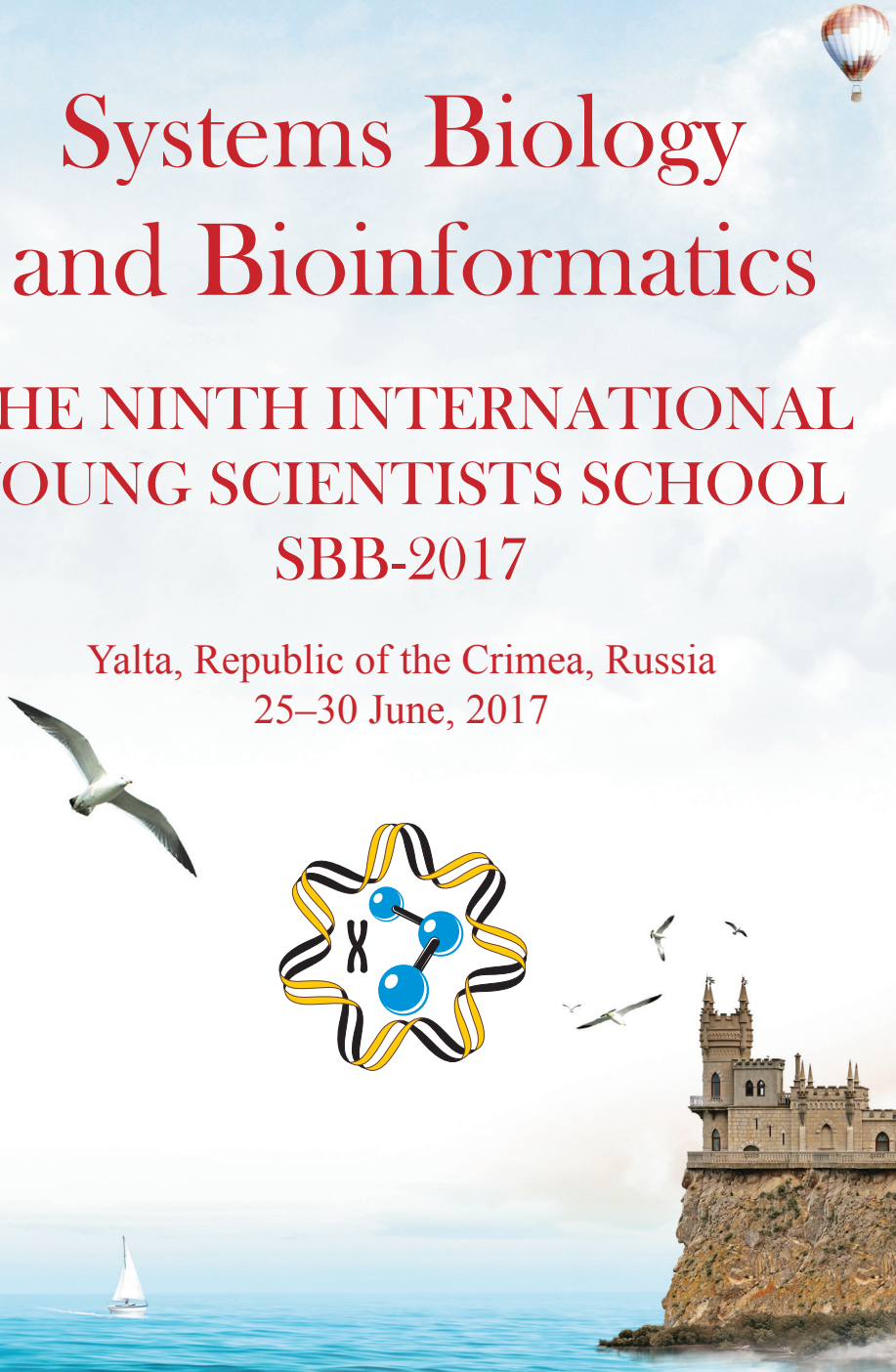
Systems Biology and Bioinformatics

THE NINTH INTERNATIONAL YOUNG SCIENTISTS SCHOOL SBB-2017

Yalta, Republic of the Crimea, Russia
25–30 June, 2017



ABSTRACTS

ХИММЕД

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ПРОЕКТИРОВАНИЕ И СТРОИТЕЛЬСТВО ЛАБОРАТОРИЙ РАЗЛИЧНОГО ПРОФИЛЯ, ПОЛНОЕ ОСНАЩЕНИЕ И ОБЕСПЕЧЕНИЕ РАСХОДНЫМИ МАТЕРИАЛАМИ

ПРОДУКЦИЯ:

- химические реактивы со склада и на заказ по каталогам;
- лабораторное оборудование;
- аналитические приборы, хроматография;
- биохимия;
- собственное производство органических растворителей различной степени очистки для использования в химическом производстве, в лабораторной практике, растворителей высокой степени очистки для хроматографии;
- химикаты и оборудование для микроэлектроники.

НАШИ ПОСТАВЩИКИ:

Компания «Химмед» успешно работает более 25 лет. Собственная сеть логистики компании позволяет без временных затрат осуществлять регулярные поставки грузов между складом в Германии и складами в Москве и Подмосковье.

Инновации и новейшие технологии в области биохимии и биотехнологии – предмет особого внимания для наших специалистов. Именно поэтому мы сможем обеспечить научные исследования любого уровня материалами, использование которых обеспечит максимально эффективный результат.

Сотрудники компании с готовностью ответят на все интересующие вас вопросы и помогут сориентироваться во всем многообразии биохимической продукции от российских и зарубежных производителей.

Давние и взаимовыгодные отношения, сложившиеся с нашими партнерами, делают возможным формирование конкурентоспособных цен для клиентов компании «Химмед».

Среди наших поставщиков: R&D, Merck, Neogen, Cayman, DUCHEFA BIOCHEMIE, BioWest и многие другие.

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Nikita Botanical Gardens – National Scientific Center of the Russian Academy of Sciences"

Federal State Budget Scientific Institution "All-Russian National Research Institute
of Viticulture and Winemaking "Magarach" of the Russian Academy of Sciences"

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Novosibirsk
ICG SB RAS, 2017

УДК 575
S95

Systems Biology and Bioinformatics : the Ninth International Young Scientists School SBB-2017 (Yalta, Republic of the Crimea, Russia, 25–30 June, 2017); Abstracts = Системная биология и биоинформатика : девятая международная школа молодых ученых SBB-2017 (Ялта, Республика Крым, Россия, 25–30 июня 2017 г.); тезисы докладов / Compilers: O. Petrovskaya, Y. Orlov, S. Zubova. – Novosibirsk : ICG SB RAS, 2017. – 85 p. – ISBN 978-5-91291-031-9.

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ISBN 978-5-91291-031-9

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Sponsors

Russian Foundation for Basic Research
Grant # 17-34-10135

Российский фонд фундаментальных исследований
Грант № 17-34-10135

Federal Agency for Scientific Organizations
(FASO Russia)

Федеральное агентство научных организаций
(ФАНО России)

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ООО «Химмед Сибирь»

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Институт создан в 1957 г. в числе первых институтов Сибирского отделения АН СССР. В настоящее время ИЦиГ СО РАН – мультидисциплинарный, многопрофильный биологический институт, который по праву считается одним из ведущих научных учреждений биологического профиля в России. В мае 2017 г. закончился второй этап реорганизации Федерального исследовательского центра Институт цитологии и генетики Сибирского отделения РАН. На сегодняшний день ФИЦ включает три филиала:

Сибирский научно-исследовательский институт растениеводства и селекции (СИБНИИРС),
Научно-исследовательский институт клинической и экспериментальной лимфологии (НИИКЭЛ),
Научно-исследовательский институт терапии и профилактической медицины, НИИТПМ.

Миссия ИЦиГ СО РАН. Решение приоритетных задач развития научно-технологического комплекса РФ в области генетики и селекции растений, генетики и селекции животных, генетики человека и биотехнологии на основе методов молекулярной генетики, клеточной биологии и биоинформатики.

Стратегическая задача. Проведение полных циклов исследований от генерации фундаментальных знаний до прикладных разработок в области генетики и селекции растений, генетики и селекции животных, генетики человека и биотехнологии на основе методов молекулярной генетики, клеточной биологии и биоинформатики, имеющих приоритетное значение для решения задач агропромышленного, биотехнологического, медико-биологического и фармацевтического комплексов России.

Кадровый состав. На 1 июня 2017 г. в ФИЦ ИЦиГ СО РАН 88 научных подразделений, в которых работает 1394 человека, в том числе 513 научных сотрудников, из которых 187 сотрудников в возрасте до 39 лет, 2 советника РАН, 8 академиков РАН, 4 члена-корреспондента РАН, 90 докторов наук, 296 кандидатов наук.

Аспирантура и ординатура. На 1 июня 2017 г. в аспирантуре ФИЦ ИЦиГ СО РАН обучается 77 аспирантов и 18 ординаторов.

Публикации. Институт активно печатается в российских и зарубежных журналах и является в российской биологии одним из признанных лидеров. За 2016 г. опубликовано 188 статей, зарегистрированных в международной базе Web of Science (WoS), общее количество статей в рецензируемых журналах – более 440. В 2016 г. статьи сотрудников института цитировались в WoS 4143 раза.

Инфраструктура ИЦиГ СО РАН. УНУ «Центр генетических ресурсов лабораторных животных (ЦГР)» и семь центров коллективного пользования (http://www.bionet.nsc.ru/uslugi/).

Имущественный комплекс. Земельный участок площадью 35 тыс. га, закрепленный на праве постоянного пользования; 85 тыс. м^2 рабочих площадей, расположенных на территории Советского района г. Новосибирска, Барышевского сельского совета Новосибирской области, в Искитимском и Черепановском районах НСО, в пос. Краснообск НСО.
Федеральный исследовательский центр ИЦиГ СО РАН приглашает к сотрудничеству научные и коммерческие организации!

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Никитский ботанический сад основан в 1812 г. и с первых дней существования способствовал ускоренному развитию сельскохозяйственного производства юга России на основе интродукции, акклиматизации, селекции и широкого распространения плодовых, цветочных, декоративных, эфиромасличных, лекарственных и других полезных растений, изучения и активного использования местных растительных ресурсов. В «копилке» Никитского ботанического сада – свыше 1150 наград, 70 из них получены на международных выставках, включая дореволюционные (376 медалей разного достоинства, из которых 63 – зарубежные). В 1962 г. Государственный Никитский ботанический сад удостоен ордена Трудового Красного Знамени. В 1995 г. в честь Сада была названа малая планета в поясе астероидов за номером 4480 – «Никитиботания».

Родоначальник отраслей и институтов. Никитский ботанический сад – родоначальник таких отраслей народного хозяйства России, как виноградарство, эфиромасличное растениеводство, табаководство, южное декоративное садоводство, южное и субтропическое плодоводство. На базе Сада получили жизнь такие известные отраслевые институты, как Институт винограда и вина «Магарач», Институт эфиромасличных и лекарственных растений, опытные станции овощебахчевых и лекарственных растений, табаководства. Никитское училище садоводства стало самостоятельным техникумом (ныне – Крымский агропромышленный колледж).

В Саду работает более 800 человек: доктора и кандидаты наук, научные сотрудники и мастера садово-паркового хозяйства. Здесь сформировались известные научные школы в области интродукции и селекции южных плодовых, декоративных и эфиромасличных культур, биотехнологии и биохимии растений, сельскохозяйственной акарологии, экологии многолетнего растениеводства, фитоценологии, альгологии. Работает аспирантура и докторантура, специализированный ученый совет по защите докторских и кандидатских диссертаций, совет молодых ученых.

Крупнейшее в России хранилище плодовых культур и коллекций. Сад является крупнейшим в нашей стране хранилищем видового и сортового разнообразия южных плодовых культур, включающего более 11 000 сортов персика, абрикоса, алычи, черешни, яблони, груши, айвы, инжира, граната, маслины, зизифуса, хурмы и других культур. Здесь собраны уникальные коллекции декоративных древесных и травянистых растений, насчитывающие более 6 000 видов, и богатейшие сортовые и формовые коллекции более 250 ботанических видов эфиромасличных, лекарственных, пряно-ароматических растений. Впервые введено в культуру более 400 видов новых растений, выведено и передано в народное хозяйство более 800 сортов культурных растений.

Памятник садово-паркового искусства. Всемирную известность принес Саду его уникальный Арборетум (дендрарий). На площади более 40 га сосредоточены коллекции древесных растений мировой флоры. В парках Арборетума представлено свыше 2 000 видов деревьев и кустарников. Никитский ботанический сад как памятник садово-паркового искусства является живым музеем и зеленой сокровищницей под открытым небом. В Верхнем парке расположены розарий, участок хризантем и экспозиции цветочных культур. В апреле здесь проходит «парад тюльпанов». В мае – выставка ирисов, в июне – лилейников, июне-июле – выставка роз и канн, в октябре-ноябре – «бал хризантем». На террасах Нижнего парка расположены маслиновая роща, пальмовая аллея, роща пробкового дуба, роща черного бамбука, растут земляничник мелкоплодный, дуб калифорнийский, дзельква граболистная, сосна Монтезумы, кипарис крупноплодный, платан восточный, 1000-летняя маслина, ива вавилонская, тюльпанное дерево, гинкго двулопастный и другие редкости. По дороге к морю находится кактусовая оранжерея, в которой собрана коллекция из 2 000 видов кактусов и других суккулентов. Рядом с оранжереей расположен экспозиционный участок холодостойких суккулентов, сирингарий и сад клематисов – «Райский сад», оформленный в восточном стиле, вновь открыт Приморский парк (парк Приключений). Один из интереснейших туристических объектов – Государственный природный заповедник «Мыс Мартьян», входящий в состав Никитского ботанического сада.

Приглашаем вас в оазис красоты и гармонии, вечно юный двухсотлетний сад! Уверены, что вы полюбите наш сад так же, как любим его мы.

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История. Институт создан на основании распоряжения Правительства Российской Федерации от 7 сентября 2015 г. № 1743-р и является преемником основанного в 1828 г. по распоряжению новороссийского генерал-губернатора князя М.С. Воронцова при Никитском ботаническом саде опытного виноградно-винодельческого заведения «Магарач». В середине прошлого столетия институт, имея разветвленную сеть опорных пунктов (Степной, Балаклавский, Судакский, Азово-Черноморья, Средневолжский, Сочинский, Приморский опорный пункт по виноградарству во Владивостоке) и филиалов (Грузинский, Московский, Кишиневский, Закавказский, а также в Казахской, Киргизской, Туркменской и Таджикской ССР и т. д.), охватывал своей деятельностью большинство винодельческих и виноградарских районов Советского Союза, а все ведущие институты по виноградарству и виноделию стран бывшего СССР в свое время были созданы как филиалы института «Магарач». С 31.08.89 г. институт был подчинен Президиуму ВАСХНИЛ. В 1992 г. институт «Магарач» вошел в систему Украинской академии аграрных наук как Институт винограда и вина «Магарач». 21.03.14 г. на основании распоряжения Совета министров Республики Крым от 23.12.14 г. № 1480-р институт переименован в Государственное бюджетное учреждение Республики Крым «Национальный научно-исследовательский институт винограда и вина «Магарач».

Кадровый потенциал. Число работающих – 220 человек. Число научных работников – 95 человек, в том числе 1 академик РАН; 1 член-корреспондент Национальной академии аграрных наук Украины; 14 докторов наук; 50 кандидатов наук; 9 профессоров; 34 доцента и старших научных сотрудника. Средний возраст исследователей составляет 49 лет. До 39 лет – 34 человека.

Основная деятельность института. Проведение фундаментальных, поисковых и прикладных научных исследований, опытно-конструкторских работ, внедрение достижений науки и передового опыта, направленных на получение новых знаний в сфере виноградарства и виноделия, способствующих технологическому, экономическому и социальному развитию агропромышленного комплекса.

Основные достижения. Институт ведет разработку научных основ формирования экологически устойчивых агросистем, адаптивных ресурсосберегающих и природоохранных технологий выращивания и хранения винограда, интегрированных систем защиты, средств механизации нового поколения, создания новых генотипов и сохранения генетических ресурсов винограда, разработку нового технологического оборудования в виноделии и ресурсо- и энергосберегающих технологий производства вин, коньяков, а также биологически активных продуктов (из отходов виноделия), осуществляет научное, методологическое и нормативно-технологическое сопровождение вино-градно-винодельческой отрасли, обеспечивающее ее качество, безопасность и конкурентоспособность. Разработаны и внедрены в практику более 60 новых сортов и 4 высокоурожайных клона винограда.

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CONTENTS

CONTROL OVER SOUR ROT DEVELOPMENT ON GRAPES

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Key words: *grapes, harvest losses, sour rot, fungi, bacteria, drosophila, thrips, bactericides, insecticides, fertilizers*

Rationale and Objectives: In recent years the vineyards of Crimea have experienced a progressive development of berry rot pathogens of fungal and bacterial etiology. Sour rot is gaining increasingly greater economic importance as it can ruin up to 80% of the ripening harvest of grapes [1]. The complex of putrefactive fungi and bacteria is represented by either weak or secondary pathogens, whose infections most often penetrate through the wound on the berry. The disease is caused by yeast of the genera *Candida, Hanseniaspora, Pichia, Metschnikowia pulcherrima, Issatchenkia occidentalis, Saccharomycopsis crataegensis, Zygoascus hellenicus, Zygosaccharomyces bisporus, Saccharomycopsis vini, Kloekera api-culata, Torulopsis stelata*, etc. (at least 10 yeast species) and bacteria of the genus *Acetobacter Beijer*. (at least 6 species). In nature sour rot can be caused by both yeast and a mixture of bacteria. Most species are part of the normal microflora living on grape clusters; however under certain conditions they may cause a pathological process [2]. Protective measures against this disease are only being developed; the challenge is the fact that sour rot develops during the period when it is prohibited to use chemical fungicides. Thus, the main purpose of the research was to determine the biotic factors contributing to the development of sour rot on grapes and to find the most effective ways to control it.

Methods: The studies were conducted in 2016 on *Muscat white* variety vineyards ("Livadiya" branch of PJSC "Massandra", Yalta), applying methodological approaches standard for domestic and international practices of viticulture and plant protection [3, 4].

Results: It is important to minimize berry skin trauma in order to prevent the infection of grape berries with sour rot-causing organisms. Hence, we studied the influence of such biotic factors as intensity of the damage to grape berries caused by oidium and thrips on the level of sour rot development. As a result, we established a strong influence of oidium development and berry damage caused by thrips on the level of sour rot development – $r = 0.73$ and 0.74, respectively. We also tested several preparations used to increase the mechanical strength of the skin, control the development of the surface pathogenic microflora, fight fruit fly during berry ripening season, prevent damage to the skin of the berries caused by thrips. The intensity of sour rot development after treatment with potassium, calcium and silicon based preparations, which help reinforce berry skin mechanical strength, at high degree of disease development in the control variant (81.5%) made 31.3, 45.1 and 50, 9%, respectively, which corresponded to the Chorus, WDG chemical standard (34.2%) and exceeded it. Application of domestic growth regulators with bactericidal properties Growth Matrix and Agat-25K, TPS helped restrain the development of sour rot at the level of 40.9 and 53.7%. The development of sour rot on samples after single application of Lannate, LV insecticide for protection against thrips and double application of Confidor Extra, WDG to control fruit fly were 25.6 and 44.7%, respectively.

Conclusion: The present phytosanitary situation in agroecosystems places special demands to the choice of both means and technologies to restrict the severity of the most dangerous types of pathogens and ways to prevent negative environmental consequences. The research conducted in 2016 produced preliminary experimental data on the study of the biotic factors contributing to the development of sour rot in grapes and ways to control it. It was established that treatments with potash fertilizer, a multifunctional preparation Growth Matrix and insecticide Lannate, LV resulted in the minimum sour rot development.

Acknowledgements: The authors thank the Head of research, Dr. Sc. in Agriculture, Aleynikova N.V., the staff of the department of plant protection and physiology at "Magarach" Institute for their support in organizing and conducting the experiments.

References

1. Aleynikova N.V. Modern trends in proliferation of pathogenic organisms in the grapevine communities of Crimea / N.V. Aleynikova, M.N. Borisenko, Ye.S. Galkina, Ya.E. Radionovskaya. Fruit growing and viticulture in the South of Russia. 2016;42(06):119-133.
2. Volkov Ya.A. Myco-complex of rot-causing organisms in grape berries in the south of Ukraine and methods for their control / Ya.A. Volkov, E.P. Stranishevskaya. Yalta, 2011;28.
3. Dospekhov B.A. Field assay methodology. Moscow: Harvest, 1985;336.
4. Guidelines for fungicide registration tests in agriculture / under ed. V.I. Dolzhenko. S.-P., 2009;378.

ESTIMATION OF GENETIC RELATION BETWEEN KUBAN AND CRIMEAN WALNUT CULTIVARS BY MICROSATELLITE MARKERS

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Key words: *persian walnut, polymorphism, microsatellite, molecular identification, genetic similarity*

Introduction: Walnut is one of the most important nut-bearing crops, common in temperate climatic zones around the world. It is valued not only for nutritious fruits, which have high taste qualities, but also for timber with many valuable properties [1].

In walnut breeding, the basic moment is the availability of genetic resources, their evaluation and effective management. The use of molecular markers in the work with geneplasm is an effective tool that allows fast and accurate identification of genotypes, assess genetic diversity and, based on the data obtained, plan the breeding.

Material and Methods: Plant Material. Ten cultivars from Nikita Botanical Gardens (NBG) walnut germplasm collection and eleven cultivars from North Caucasian Regional Research Institute of Horticulture and Viticulture (NCRRIH&V) collection were used in this work.

DNA extraction and SSR amplification. The genomic DNA was extracted from young fresh leaves using large-scale CTAB based method of Murray and Thompson (1980) [2]. Nine primer pairs developed by Woeste et al. (2002) [3] were used to amplify the genomic DNA. To save the time, the markers were arranged into 3 multiplex sets, WGA009, WGA202, WGA276 in the first one, WGA001, WGA069, WGA349, WGA376 in the second one and WGA005, WGA054 in the third. The amplification was performed in the volume of 25 µl with 1 unit of Taq DNA polymerase, 2.5 µl 10X PCR buffer including 2 mM MgCl2, 0,05 mM dNTPs, 0.3 µM each primer pair and 1 µl (30–40 ng) DNA. The PCR reaction was carried out in a 96-well block Mastercycler (Eppendorf), using this program: 4 min at 94 °C, afterward 35 cycles of 30 sec at 94 °C, 45 sec at 58 °C, and 72 °C for 45 sec; followed by a final extension at 72 °C for 5 min.

The PCR products were detected using the ABI3130 Genetic Analyzer and the GeneMapper analysis software (Applied Biosystems). For capillary electrophoresis detection, the forward SSR primers were labeled with the 5'- fluorescence dyes. The size standard used in the sequencer was GS500LIZ (Applied Biosystems). Each reaction was repeated and analyzed twice for confirmation.

Results: Based on the published data 9 SSR loci with the highest polymorphism were selected. Chosen markers were grouped in three multiplex sets, taking into account the size of the products obtained and the primer sequences. The size of the fragments obtained varied from 109 bp in WGA054 to 282 bp in WGA349. The genotypes that showed the presence of one allele were interpreted as homozygous for this locus. The number of observed alleles (Na) per locus ranged from 6 in WGA349 to 13 in WGA276 with an average of 8 alleles. The heterozygosity observed ranged from 0.409 in WGA069 to 0.864 in WGA005, with an average of 0.667. The heterozygosity expected was lower

than observed for WGA005, WGA009 and WGA376. The size differences detected between alleles at a locus ranged from 2 to 34 bp. According to the cluster analysis, two clusters can be identified, the bootstrap values for which are 100.

The first cluster including all cultivars from NCRRIH&V collection except Californian variety Chandler, also this group including two cultivars from NBG collection. The second cluster consists other 8 NBG collection cultivars. The similarity values for the majority of varieties pairs are quite low and range from 0.22 to 0.76, which confirms high heterogeneity of the studied set of cultivars.

The Chandler variety from the USA has been assigned to the first of two clusters. Chandler's similarity with other cultivars is about than 0.47.

Discussion: The data obtained indicate significant differences between the groups of varieties studied. Since most of the varieties from NCRRIH&V and BNS are indigenous and were obtained by selecting prospective seedlings in local populations, it can be assumed that there is a certain genetic distance between these areals.

References

1. Balapanov I. (2014) Biologial traits in walnut breeding. Polythematic Online Scientific Journal of Kuban State Agrarian University. 101:1-15.
2. Murray M., Thompson W. (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research. 10:4321-4325.
3. Woeste K. et al. (2002) Thirty polymorphic nuclear microsatellite loci from black walnut. J. Hered 93:58-60.

SCREENING OF FUNGICIDES AND BIOPREPARATIONS USED FOR PROTECTION OF GRAPEVINE AGAINST MOLD PATHOGENS

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Key words: *grapevine, fungi, rot, active substance, fungicides, biological preparations, efficiency, in vitro*

Rationale and Objectives: The damage to the grapevine plant caused by a complex of pathogens including molds is the factor that constitutes an obstacle to effective viticulture and meeting the popular demand in high-quality table grapes and wine and wine products. The grape mold pathogens – saprophytic fungi living in the soil or epiphytically on the surface of plants – under certain conditions can evolve as facultative parasites with broad specialization profile and are registered on a large number of host plants. Furthermore, depending on composition of the fungi pathogens, decayed berries get moldy and covered with pads of fungi spores of different colors. Black mold is caused by a combination of fungi including *Aspergillus niger* Tiegh, *Rizopus nigricans* Ehr., green mold of vines is caused by fungi of the genus *Cladosporium*, blue mold – fungi of the genus *Penicillium*, pink mold – fungi of the genus *Trichothecium roseum* Fr., *Gliocladium roseum*, *Trichoderma lignorum* [1–3].

The results of surveys conducted in the vineyards of the south coast, south-west and central-steppe Crimea, where winemaking and table varieties are cultivated, have established that when bunches of grapes were affected by a complex of pathogens the share of molds (including *Aspergillus niger* Tiegh, Penicillium sp., *Cladosporium herbarum* Link, etc.) accounted for no more than 7 and 1 % in 2015, while in 2016 this figure reached up to 11.1, 15 and 17.4 %, respectively [4]. The known ways to control mold development on grapes are mainly preventive in nature and aim at protecting berries from damage by insects and other diseases, which is achieved by timely use of insecticides and fungicides. Among the active substances permitted for use on grapes today only captan is known for certain efficacy in controlling mold pathogens [1, 2, 5, 6]. Thus, the research conducted in 2016 aimed at *in vitro* screening of modern fungicides and biologically active preparations of domestic production used to ensure effective control over grape mold pathogens of the genus *Aspergillus niger* v. Tiegh, *Penicillium* sp., *Cladosporium herbarum* Link, *Trichotecium roseum* Link.

Methods: 11 contact, systemic and systemic-contact fungicides currently registered (with the exception of Topsin M, WP) in the Russian Federation for use on vine plants to protect them against mildew, oidium and gray rot (concentrations of the preparations corresponded to those recommended by "The list of pesticides and agrochemicals authorized for use in the territory of the Russian Federation" [5]) and biologically active preparation of domestic production FITOP-FLORA-S in two concentrations were tested in the laboratory. The tests were carried out using the method of measuring fungicide performance under laboratory conditions [7]. Potato-glucose agar (PGA) was used as a nutrient medium. The tested preparation in experimental variations was added to the nutrient medium. Pure PGA was used as control.

Results: The conducted tests established a good fungicidal activity (biological efficiency exceeding 80%) against all the studied fungi both on the 5th and the 10th day of cultivation for the following preparations: Scor EK, Switch WDG and Chorus WDG. The development of *Aspergillius niger, Penicillium* sp., *Trichotecium roseum* was effectively controlled by Malvin WDG and Zummer SC preparations, while against *Cladosporium herbarum* these preparations were highly effective only on the 5th day. Fungicide Topsin M, WP highly effectively (90–100%) controlled the growth of colonies of all fungi on the 5th day; an up to 70 % decrease in its biological efficacy against *Aspergillius niger* and *Cladosporium herbarum* was registered on the 10th day of cultivation. For specialized botryticide Kantus we established biological activity (at 60–70 %) against *Aspergillius niger, Cladosporium herbarum* and *Trichotecium roseum*.

The test of the Russian-made biological preparation FITOP-FLORA-C (in two concentrations) produced on the basis of *Bacillus subtilis* bacterium demonstrated that to the maximum effect (60–70 %) on the 5th and 10th day it inhibited only the growth of *Cladosporium herbarum* and *Trichotecium roseum* fungi colonies when a 9 % concentration of the stock solution of the preparation was used.

Conclusion: Thus, our laboratory tests established the most effective preparations against mold pathogens on grapes the application of which will help control development of pathogenic micromycetes associated with the vine.

References

1. Popushoy I.S. Mycoses of grapevine / I.S. Popushoy, L.A. Marzhina. Chisinau: Shtiintsa, 1989;244.
2. Chichinadze Zh.A. Pests, diseases and weeds on grapevine / Zh.A. Chichinadze, N.A. Yakushina, A.S. Skorikov, E.P. Stranishevskaya. K.: Agrarian Science. 1995;304.
3. Duncan R.A. Population dynamics of epiphytic mycoflora and occurrence of bunch rots of wine grapes as influenced by leaf removal / R.A. Duncan, J.J. Stapleton and G.M. Leavitt. Ptant Pathology. 1995;44:956-965.
4. Aleynikova N.V. Modern trends in proliferation of pathogenic organisms in the grapevine communities of Crimea / N.V. Aleynikova, M.N. Borisenko, Ye.S. Galkina, Ya.E. Radionovskaya. Fruit growing and viticulture in the South of Russia. 2016;42 (06):119-133.
5. List of pesticides and agrochemicals allowed for use in the territory of the Russian Federation. Reference works. M., 2016;880.
6. Annemiek Schilder Management of bunch rot diseases in grapes [Electronic resource] / Plant Pathology, Michigan State University, 2008. Access mode: [http://msue.anr.msu.edu/news/management of bunch rot diseases Grapes]
7. Golyshin N.M. Fungicides in agriculture. M.: Kolos, 1970;61-177.

MORPHOLOGYCAL, ANATOMICAL AND PHYSIOLOGICAL FEATURES OF *IN VITRO* REGENERANTS IN VARIOUS PERSIMMON AND COMMON FIG CULTIVARS

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Key words: *subtropical fruits, plant biotechnology, structural features, water regime, photosynthesis activity*

Fruit crops due to the content of biologically active substances, sugars and antioxidants in their fruits are important for people's health care and the development of food industry. The gene pool of valuable fruit crops in the Nikita Botanical Gardens includes over 6000 cultivars and breeding forms of stone, pome, subtropical and nut-bearing crops [1]. In its turn, importance of *Diospyros kaki* Thunb. and *Ficus carica* L. plants cultivation *in vitro* is caused by particular nutritional value of their fruits [2], sensitivity of plants to the virus diseases damages [3], the problem of obtaining a large scale of health improve planting material and preserving the unique gene pool of these fruits in the Nikita Botanical Gardens. The aim of our studies was to identify some structural and metabolic features in the vegetative organs of some persimmon and common fig cultivars at the regeneration stage under *in vitro* conditions.

Two persimmon cultivars were studied: 'Zolotistaya' (refers to the constant, impetuous and large-fruited cultivars with fruit ripening of early-season), 'Nikitskaya Bordovaya' (constant tart and small-fruited cultivar with fruit ripening of late-season) and seven common fig cultivars: 'Finikovyi', 'Nikitsky Suhofruktovyi', 'Desertnyi' (cultivars used for dry fruit production, self-fertilized, with one generation), 'Sabrutsiya Rozovaya', 'Medovyiy', 'Pomoriyskiy' (cultivars used both – processing and fresh fruits, have one or two generations) and 'Figue Jaune' (caprifig). The isolated sterile vegetative buds were introduced to *in vitro* culture. The persimmon regenerants were cultured for 14 months on MS medium supplemented with 4.0–5.0 mg/l BAP and 0.1–0.3 mg/l IBA, after 4 weeks of the culture rosette of 2–4 leaves were formed and in 6–8 weeks 1–2 microshoot/explant were obtained in the cultivar 'Zolotistaya' and 2–3 microshoot/explant – in 'Nikitskaya Bordovaya' cultivar. For an active regeneration microshoots and microcuttings were transferred to MS medium with 0.8 mg/l zeatin or BAP. Common fig plants were cultured on MS medium with 1.5–2.0 mg/l BAP, 0.2–0.4 mg/l NAA and 0.25 mg/l GA_3. The samples were collected after 6 months culture at 24 ± 1 °C, 16-h photoperiod and light intensity 37.5 μmol $m^{-2}s^{-1}$.

Morphometric measurements were made in 10 replications, the temporary slides were prepared to study the leaf blade structure [4]. The studied material was analyzed using Jenaval (Carl Zeiss, Germany) and AxioScope A.1 (Zeiss, Germany) light microscopes. Such water regime parameters of the leaves in regenerants were determined: the total water content and its fractional composition [5]. A photosynthetic activity was determined by measuring of chlorophyll fluorescence induction parameters [6].

The formed persimmon microplants were 3.8–5.0 cm height and had 6–8 leaves per plant. Formation of the adventitious microshoots occurred at the basal part of the explants. We obtained 6–8 microshoot/explant in the cultivar 'Zolotistaya' and 6–7 –

in the cultivar ‘Nikitskaya Bordovaya’. The leaves were of an oval-lanceolate shape, 1.6–2.8 cm long. The leaf blades in persimmon cultivars were thin (126–145 μm), amphistomatic. A leaf mesophyll was differentiated, a palisade index was low – 0.33. The epidermis was covered with simple 1–2-cell hairs (167–303 μm long), stomata of anomocyte type (80–106 stomata/mm^2). The total water content in leaves was high – 83–91 % and the part of bounded water was 14–28% (higher water holding capacity was noticed in the regenerants of cultivar ‘Nikitskaya Bordovaya’). Photosynthesis was active: $(F_m – F_{st})/F_m$ = 0.54–0.60 a.u.

The common fig regenerants *in vitro* were 1.3–2.3 cm height. A single explant formed 1–5 microshoots with 3–9 leaves (the maximum number of adventitious microshoots was noted in the cultivars ‘Desertnyi’ and ‘Medovyiy’). Leaf blades (2.2 × 1.5 cm) were of a typical fig shape and green, 63–110 μm thick, bifacial, hypostomatic. The chlorenchyma was also differentiated. Better developed palisade tissue was found in the cultivars ‘Nikitsky Suhofruktovyi’, ‘Finikovyi’ and ‘Pomoriyskiy’ (palisade index 0.44–0.53), in some cases isopalisade parenchyma was noted. Cover tissues were represented by a single-layer epidermis covered with a thin cuticle, with multiple simple and glandular trichomes (33–90 μm long). Stomatal apparatus were of an anomocyte type, mainly concentrated on the abaxial leaf surface (216–280 stomata/mm^2).The photosynthetic activity indexes (0.52–0.71 a.u.) indicated the normal functioning of the assimilation apparatus. The total water content in regenerants was 83–88%, where of 22–40% was osmotic and adsorptive water.

The following analysis enables to evaluate the studied persimmon and common fig cultivars as highly adaptive, promising for conservation and cultivation *in vitro*. The leaves of the persimmon regenerants demonstrated clearer mesomorphic structure than the common fig plants, in which a number of xeromorphic features appeared under *in vitro* conditions. Thus, the ability to regulate the water regime and capacity to the active assimilation were also higher in the common fig cultivars.

Acknowledgements: This study was funded by research grant N 14-50-00079 of the Russian Science Foundation.

References

1. Smykov A.V. (2015). Genofond and the creation of southern fruit crops cultivars for Crimea and the South of Russia, Works of the State Nikita Botanical Gardens. 140:19-23.
2. Caliskan O., Polat A. (2012) Morphological diversity among fig (*Ficus carica* L.) accessions sampled from the Eastern Mediterranean Region of Turkey, Turk. J. Agric. For. 36:179-193. DOI 10.3906/tar-1102-33.
3. Mitrofanova I.V., Mitrofanova O.V., Lesnikova-Sedoshenko N.P., Chelombit S.V., Shishkina E.L., and Chirkov S.N. (2016) Phytosanitary status of *Ficus carica* collection orchards in Nikita Botanical Gardens and biotechnology of fig plants regeneration, Acta Horticulturae. 1139:303-310. DOI 10.17660/ActaHortic. 2016.1139.53
4. Pausheva Z.P. (1990). Practicum in plant cytology. Moscow, USSR: Kolos, p. 283.
5. Lishchuk A.I. (1991). Technique for determining of the water holding capacity for dehydration in the leaves of fruit crop trees. Physiological and biophysical methods in the breeding of fruit crops: methodological recommendations. M.: GNBS, 33-36.
6. Stirbet A., Govindjee J. (2011). On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and Photosystem II: Basics and applications of the OJIP fluorescence transient. Journal of Photochemistry and Photobiology B: Biology. 104:236-257. DOI 10.1016/j.jphotobiol.2010.12.010.

COLLOIDAL METHOD OF DISTRIBUTION OF SOI INITIAL MATERIAL WITH HIGH COLD STABILITY

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Key words: *soybean breeding, isolation methods, cold resistance, frost resistance, cytosol, cytocolloids*

Motivation and Aim: Soybean in the southern regions of Russia often is cultivated in climatic zones with a deficit of summer precipitation and frequent summer droughts [1, 2]. The shift in the timing of soybean sowing to earlier periods may be one way of cultivating soybeans in arid zones. There are practically no varieties in the gene pool of cultured soybeans that can germinate at low positive temperatures and withstand probable early spring frosts below minus 3.5 °C. Numerous studies of increased frost and cold resistance of plants found that this feature positively correlates with the mass fraction in the cytoplasm of some water-soluble carbohydrates, amino acids, fatty acids [3]. However, when the concentration of cytoplasm in the tissues of soybean seedlings is 3–4 %, the freezing point of the cellular juice, calculated from the molar mass of sucrose or glucose, will be about minus 0.2–0.3 °C. The increase in the carbohydrate content during the quenching of soybeans to 5–6 % reduces the calculated freezing point, but only to minus 0.3–0.5 °C. But in actual conditions, cytoplasm does not freeze in soybean sprouts for 4–6 hours, even at minus 3–5 °C. This cannot be explained by simply increasing the concentration of known cryoprotective compounds [2]. Therefore, the main goal of our research was to search for additional physiological mechanisms of increased cold resistance and methods for their evaluation, suitable for use in the practical breeding of soy.

Methods and Algorithms: The experiments were conducted in 2012–2016 on the experimental base of VNIIMK, Krasnodar. The soybean variety of Lyra was used as slightly frost-resistant, the Slavia soybean variety as more frost-resistant. Variety of narrow-leaved lupin Smena was highly frost-resistant control. Soybean and lupine were sown in October to determine the frost resistance *in vivo*. All the necessary observations and records were made after the onset of late frost frosts and winter frosts. Sufficient to determine the concentration of the volume of cell sap was obtained from the soybean and lupine plants that had risen in the autumn. Cellular juice of all varieties was frozen at temperatures of minus 5, minus 15 and minus 20 °C. Subsequent defrosting was carried out at room temperature for the next 24 hours to complete the coagulation and sedimentation of cytocolloids. The concentration of cytosols was determined on a refractometer model PR-101a with an error of ± 0.1 abs. %.

Results: Field assessment of soybean shoots in the prewinter (October) sowing showed that individual soybean varieties can survive freezing to minus 4–5 °C. Assuming that the cell juice of the test samples is an ideal aqueous solution, according to Raoul's second law, their freezing points should be directly proportional to the molar concentration of the basic soluble substance, that is, the higher the solution concentration, the lower its freezing point [4]. However, the initial concentration of cellular juice in tissues of soybean plants, which differed markedly in frost resistance in vivo, was practically the

same and varied within 4.2–4.3 %. This parameter for a cold-resistant narrow-leaved lupin was only 3.4 %. Low-molecular weight water-soluble carbohydrates are the main component of cytoplasm. If glucose is taken as the basis of the aqueous solution of the cytoplasm (molar mass 180.16 g/mol), the calculated freezing temperatures in soybean will be only minus 0.45–0.46 °C; In lupine – minus 0.36 °C. The calculated freezing temperatures of the cell juice, where the main component is sucrose (molar mass 342.30 g/mol), at given concentrations will be in soya – minus 0.24 °C; In lupine – minus 0.19 °C. However, in real field conditions, the freezing temperatures of the cytoplasm and the death of soybean and lupine plants turned out to be much lower. The revealed peculiarities of the cytoplasmic reaction to supercooling are most adequately explained by the properties of colloidal solutions. The freezing points of colloidal solutions are much lower than in true solutions, since most of the water molecules in the colloid micelles are in a bound state. Therefore, cytosols do not crystallize when the temperature is lowered to minus 2.5 °C for soy, and up to minus 10 °C for lupine, and they gel without damaging the cell organelles. Thus, the increased cold and frost resistance of individual soybean genotypes can be determined by an increased concentration of colloids in the cytoplasm. Deep freezing of the cytoplasm (up to minus 20 °C) of soy provides complete coagulation and sedimentation of all colloidal fractions of the cytosol. This allows to estimate the content of cytocolloids in the cell juice. This method of evaluation showed that the more cold-resistant genotypes of soy are distinguished by an increased volume of colloidal coagulants. The share of sedimented colloids in lupine was 1.5–2 times higher than in soybean.

Conclusion: The deep freezing of soybean cell sap can evaluate the total volume of sedimented colloidal coagulants. This method is suitable for estimating the initial soybean material for cold and frost resistance.

References

1. Zelentsov S.V., Moshnenko E.V. (2010) Perspektivy ispol'zovaniya sverkhrannikh posevov soi v usloviyakh Krasnodarskogo kraya, Maslichnyye Kul'tury. 1(142-143):87-94.
2. Zelentsov S.V., Moshnenko E.V., Ryabenko L.G., ZelentsovV.S. , Budnikov E.N., Bubnova L.A., Vaylova A.V. (2015) Rol' tsitokolloidov v formirovanii morozo- i zamorozkoustoychivosti yarovykh kul'tur na primere soi i l'na, Problemy sovremennoy nauki i obrazovaniya. 12(42):102-107.
3. Tumanov I.I. (1979) Fiziologiya zakalivaniya i morozostoykosti rasteniy. M.: Nauka, 349.
4. Khmel'nitskiy R.A. (1988) Fizicheskaya i kolloidnaya khimiya. M.: Vysshaya shkola. 174-177.

THE STUDY OF SIBERIAN COLLECTION OF SPRING BARLEY

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Motivation and Aim: Barley (*Hordeum vulgare* L.) is one of the world's earliest domesticated and most important crop plants. The improvement of yield, disease resistance and tolerance to environmental stress are the main tasks of barley breeding programs. DNA-markers for accelerated and more precise selection have been developed in many studies. However, Russian barley germplasm has not been involved in development of agronomically important genetic markers yet, therefore its analysis using association genetics approaches has a potential for developing new markers useful for native barley breeding programs. Prior association studies such as GWAS (Genome-Wide Association Studies) analysis, selection of proper material is needed. The aim of the current study was the comparative assessment of the Siberian collection of spring barley for selection of sub-collection suitable for association studies.

Methods and Algorithms: The plant material was selected from the spring barley collection maintained in ICG (Novosibirsk, Russia). It includes barley varieties widely cultivated in Siberia, as well as potential donors of agronomically important traits, originating from other regions of the Russian Federation or from abroad. The selected varieties were reproduced and preliminary assessed in greenhouse and then were sown in May 2016 at two experimental ICG fields: Field 1 (Latitude: 54.853094 | Longitude: 83.138094) and Field 2 (Latitude: 54.892406 | Longitude: 82.977161). At least 10 plants of each variety were analyzed for the plant height, total tiller number, productive tiller number, grain number per plant, grain weight per plant, awn length, spike density, spike shape, main spike length, 1000 grain weight, emergence date, heading date, date of ripeness. Laboratory tests were performed for drought and salinity. The data were assessed using Microsoft Excel, PASS and R-software.

Results: Overall the results obtained in 2 fields were correlating with the exception of productive tiller number and grain number per plant, which gave lower scores for most varieties in the Field 2. This can be explained by unfavorable conditions (drought) observed just in this field during the tillering phase. After phenotyping qualitative and quantitative traits we reduced number of preliminary chosen 116 varieties to 96 ("plate"–format for future molecular-genetic studies), by exclusion of those demonstrating heterogeneity and by combining varieties with contrast traits phenotype. The final sub-collection included 70 2-row and 26 6-row, 82 hulled and 14 naked varieties, both early and middle-ripening, with plant height from semi-dwarf to normal-sized and with different productivity potential. By laboratory test for drought also contrast forms were observed from sensitive to high tolerant (in case of salinity - from relatively to high tolerant). There was correlation between these traits. In both tests, Altan-Bulag and AC0760258 had outstanding tolerance. However, these laboratory tolerance tests results should be further verified by greenhouse and field experiments.

Conclusion: Field and laboratory tests of varieties from the Siberian spring barley collection allowed compiling a sub-collection suitable for further association studies. Further tests are expedient in both experimental ICG fields to make comparisons and obtain valuable data on genotypes with stable productivity in unfavorable conditions.

Acknowledgements: This study was supported by RSF 16-14-00086.

EVOLUTIONARY RELATEDNESS AND ELECTROSTATIC PROPERTIES OF NAD(P)H:FMN-OXIDOREDUCTASES FROM LUMINOUS BACTERIA IN THE CONTEXT OF BIOLUMINESCENCE REGULATION

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Key words: *coupled enzymes, bacterial luciferase, NAD(P)H:FMN-oxidoreductase, nitroreductase, flavin-dependent oxidoreductase*

Motivation and Aim: Flavin-dependent bi-enzymatic systems are widespread in nature and catalyze redox reactions accompanied by electron transfer process. Bacterial luciferase together with NAD(P)H:FMN-oxidoreductase represent the most extensively studied system of this type in bacteria. Since bioluminescent signal could be easily registered, luciferase and oxidoreductase are widely used in biosensors for ecological monitoring and as a model enzymatic system for investigation of metabolic pathways containing NAD(P)H-dependent enzymes. However, there are evidences for [1] and against [2] complex formation between luciferase and oxidoreductase, and the mechanism of their cooperative work is still under consideration.

The reported study is aimed at investigation of evolutionary relatedness and common properties of the primary and tertiary structures of oxidoreductases, that could make a contribution to interaction with luciferase.

Methods and Algorithms: Complete genomes, scaffolds and contigs of 23 luminous species from NCBI database were analyzed in order to identify primary sequences of oxidoreductases. As a result 14 oxidoreductases of LuxG type, 20 of Fre, 19 of FRG and 20 of FRP type were found. In order to perform a detailed analysis of their evolution a set of oxidoreductases from different bacterial species was collected according to clusters stored in the NCBI Conserved Domains web server. Phylogenetic analysis was performed using MAFFT and PhyML software. FASTML server was used for a common ancestor sequence reconstruction.

To build tertiary structures of oxidoreductases – using SWISS-MODEL server – known crystal structures of oxidoreductases were applied as templates (1QFJ structure for LuxG and Fre oxidoreductases, 1VFR for FRG oxidoreductases, 1BKJ for FRP oxidoreductases). Surface electrostatic potential was described with Apbs software.

Results: The analysis of oxidoreductases' conserved domains revealed that they can be assigned to two families: *flavin-dependent* oxidoreductases (Fre and LuxG types) and *nitroreductases* (FRP and FRG types), what is in a good agreement with previously reported studies of *Vibrio harveyi*, *Vibrio fischeri* and *Photobacterium leiognathi* species. Phylogenetic analysis of nitroreductases indicated low similarity in amino acid composition of the active site. However their structural alignment demonstrated analogous structure of the binding pocket. Phylogenetic tree of flavin-dependent

oxidoreductases shows that LuxG and Fre oxidoreductases of luminous bacteria share a common ancestor. Ancestral sequence reconstruction allowed to determine specific changes leading to the separation of the proteins. Most of the changes unaffected the active site except for the mobile loop responsible for substrate binding.

In spite of lack of structural similarity between flavin-dependent oxidoreductases and nitroreductases that could serve bioluminescent pathway, we found that electrostatic potential distribution on the surface of all oxidoreductases exhibits similar patterns within the area of a hypothetical interface involved into interaction with luciferase.

Conclusion: NAD(P)H-oxidoreductases used in luciferase assays *in vitro* belong to different families and they probably could be involved into diverse metabolic pathways according to the analysis of the gene neighbourhood. However, Fre and LuxG oxidoreductases evolved from the single ancestor, therefore Fre from luminous bacteria could probably serve bioluminescent pathway *in vivo*. Moreover, all studied reductases share a significant similarity in electrostatic potential distribution on the hypothetical interaction interface, what could be indicative of similar interaction mechanisms.

Acknowledgements: The reported study was funded by RFBR according to the research project No. 16-34-00746 mol_a.

References

1. Jeffers C.E. et al. (2003) Complex formation between Vibrio harveyi luciferase and monomeric NADPH:FMN oxidoreductase. Biochemistry. 42(2):529-534. 2.
2. Tinikul R. et al. (2013) The Transfer of Reduced Flavin Mononucleotide from LuxG Oxidoreductase to Luciferase Occurs via Free Diffusion. Biochemistry. 52(39):6834-6843.

EXPANSION OF CICADAE (AUCHENORRHYNCHA) SPECIES IN THE VINEYARDS OF CRIMEA

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Key words: *grapes, invasion, Cicadae, vectors of phytoplasmic diseases*

Rationale and Objectives: Nowadays, invasion of aggressive alien insect species constitute a significant part of global natural changes often leading to significant losses in the biological diversity and economic importance of the ecosystems. At times of changes in the climate, grape growing technology, active import of foreign planting stock and changes in the assortment of pesticides that have occurred in the recent decades the issue of special concern for the viticultural industry of Crimea is monitoring and control over invasive phytophagan species that penetrate the peninsula from places of their mass reproduction. The purpose of our research was to study the composition of species belonging to cicadae (*Auchenorrhyncha*), identify invasive species among them and assess their harmfulness in the vine plantations of Crimea.

Methods and Algorithms: The studies were carried out using methodological approaches applied in domestic and international practice, specifically, they met the requirements stipulated in "Methods for determining diseases and pests of agricultural plants" [1]; "Methodical recommendations for application of phytosanitary control in protection of commercial vineyards against pests and diseases in the South of Ukraine" [2]; "Methodical recommendations for phytosanitary monitoring of the cicadae complex in the vineyards of Crimea" [3].

Results: The following invasive species of cicadae have been identified in the vineyards of Crimea: Japanese grapevine leafhopper *Arboridia kakogawana* Mats [4], North American leafhopper *Scaphoidide titanus* (= *littoralis*) Ball. [5] and buffalo treehopper *Ceresa bubalus Fab.* [6].

A. kakogawana leafhopper has been registered everywhere with persistent reproduction centre in the southern coast of Crimea, where the pest develops from April through October in 3–4 generations. High numbers and harmfulness have been registered in the second half of vegetation (July-September) during development of 2–4 pest generations at the "growth and ripening of berries" stage of the grapevine vegetation period.

In the last few years leafhopper *C. bubalus* has been detected in contained hotbeds in the vineyards of Crimea, but it was characterized by insignificant damage (2–3 specimens/ per bush from the second half of June in the vineyard rows adjacent to trees and shrubs), however, the phytophagus has a tendency to spread.

In the period of 2012–2016 we identified hotbeds of leafhopper *S. titanus* transmission in different vineyards of the south-west and mountain-valley Crimea, with pest numbers amounting from individual specimens up to 40–130 specimens per yellow glue trap per week. This demonstrates successful adaptation of only one vector of the quarantine phytoplasma disease of grapevine "golden yellowing" (Flavescence doree, *Candidatus Phytoplasma vitis* pathogen) in the Crimean vineyards.

Conclusions: Thus, to date, the potentially harmful requiring control pests of the Crimean vineyards are the following progressive invasive species: North American leafhopper – as a possible vector of phytoplasmosis of grapevine "golden yellowing"; Japanese grapevine leafhopper, which causes discoloration of grape leaves in the process of feeding, and buffalo treehopper, which disrupts the normal development of shoots when developing on grape bushes.

Moreover, with the appearance in our region of invasive phytoplasma disease "blackening of wood" of grapevine (Bois noir, causative agent – *Candidatus Phytoplasma solani*), the following identified aboriginal species of cicadae represent potential danger for stable development of the Crimean viticulture: *Hyalestes obsoletus* Sign., *Hyalesthes luteipes* Fieb., *Reptalus melanochaetus* Duf., also capable of spreading the phytoplasma infection. The significance of other cicadae species as grapevine phytophages is not high in Crimea today due to their limited distribution and/or small numbers, which, however, does not reduce the relevance of their further study in the context of the Crimean ampelocenoses.

References

1. Methods to determine diseases and pests on agricultural plants / Trans. from Germ. K.V. Popkova, V.A. Shmyglya. Moscow: Agropromizdat, 1987;224.
2. Methodical recommendations for phytosanitary control in the protection of commercial vineyards against pests and diseases in the South of Ukraine. Simferopol: Polypress, 2006;24.
3. Radionovskaya Ya.E. Methodological recommendations on phytosanitary monitoring of the complex of cicadae in the vineyards of Crimea / Ya.E. Radionovskaya, L.V. Didenko. Yalta: FSBSI "All-Russian National Research Institute of Viticulture and Winemaking "Magarach". 2015;24.
4. Radionovska Ya.E. Invasion and characteristics of the development of Japanese grapevine leafhoppers *Arboridia kakogavana* Mats. in the vineyards of Crimea / Ya.E. Radionovskaya, L.V. Didenko. Quarantine and Plant Protection. 2014;8:5-7.
5. Radionovskaya Ya.E. Evaluating the harmfulness of a new phytophagus Japanese grapevine leafhopper *Arboridia kakogavana* Mats. in the vineyards of Crimea / Ya.E. Radionovskaya, L.V. Didenko. Bulletin of State Nikitsky Botanical Gardens. 2015;114:61-68.
6. Radionovskaya Ya.E. A study of the diversity of the cicadas' species (Auchenorrhyncha) in the vineyards of Crimea / Ya.E. Radionovskaya, L.V. Didenko. Improvement of the effectiveness of innovative processes in horticulture and viticulture: Scientific Papers of the Federal State Budget Scientific Organization "North Caucasian Regional Research Institute of Horticulture and Viticulture". Krasnodar. 2015;8:205-215.

ECOLOGICALLY SOUND PRODUCTION OF GRAPES IN THE CONTEXT OF TODAY'S REPUBLIC OF CRIMEA

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Key words: *grapevine, harmful organisms, biological effectiveness, sprayings*

Rationale and Objectives: Long-term cultivation of grapes in one place leads to violation of the existing level of soil fertility during crop formation, removal of nutrients by the phytomass of the bush, creates conditions for concentration of a large number of pathogens and pests and intensifies their harmfulness. For example, mildew and oidium are the main grapevine diseases on which the whole system of protection of the culture is built. The yield losses during their epiphytoties reach over 60%. Today, at the time of total chemicalization of the agricultural industry, of utmost importance is research in the field of development ecologically sound technologies aimed at reducing the pesticide load on agrobiocenoses and increasing the productivity of plants [1–5].

Methods and Algorithms: During research conducted in the South-West and South coast of Crimea standard for viticulture and plant protection methods were used: route surveys to establish the level of disease spread in commercial vineyards; field studies – to analyze the dynamics of disease development, determine the yield of grapes; laboratory tests – to determine content of sugars and titrated acids in grape juice; mathematical, statistical – to calculate disease spread, determine biological effectiveness of fungicides, detect experimental errors, mathematically process data (the Excel program).

Results: Ecologically sound practices are the ones addressing development and implementation of measures to reduce man-made pressure, neutralize harmful influences on natural environment, preserve the habitats of living organisms, and create conditions for the reproduction of natural resources and restoration of their initial qualitative indicators [6]. Pesticide load reduction in commercial conditions can be achieved by reducing the rate of applied pesticides and working fluid by using modern spraying equipment, surfactants, fertilizers and growth regulators.

During 2013–2015 we tested and confirmed high biological effectiveness and positive impact on the quantitative and qualitative yields of the following preparations: adjuvant of natural origin Kodaside 950, m.e. (2 l/ha), complex fertilizer Nutri-Fait PK and water conditioner Spartan (0,2 l/ha) in tank mixtures of pesticides. The possibility to cut out two treatments when using Kodaside 950, m.e. amid average and high level spread of the two main grapevine diseases (mildew, oidium) without decreasing the biological efficiency has been experimentally proven. This indicator for leaves and bunches averaged 80.7 and 81.1 % respectively, which is considered a good level of protection (according to Golyshin). Sprayings with adjuvant Kodaside 950, m.e. on winemaking varieties Rkatsiteli and Cabernet-Sauvignon resulted in the yield increase that averaged 10 %. With the use of Nutri-Fait PK fertilizer and Spartan conditioner in a tank mixture of chemicals on Cabernet-Sauvignon variety, the yield made 5.7 kg/bush, which is 11.8 % higher than this criterion in control (5.1 kg / bush). The use of these preparations

boosted a significant increase in the total sugars index in the juice of grapes, specifically, up to 21.5 g/100 cm^3, which is 0.9 g/100 cm^3 higher than the same indicator measured in the reference sample.

Conclusion: Thus, international and local practices demonstrate that improvement of the elements of grape cultivation technology through the use of adjuvants in tank mixtures of pesticides is an innovative factor in the intensification of modern agriculture, without which it is impossible to achieve highly productive, stress-resistant and environmentally friendly agricultural production.

References

1. Aleynikova N.V., Avidzba A.M., Didenko P.A. Biological regulation for application of pesticides using modern adjuvant Kodaside. Magarach. Viticulture and winemaking. 2015;1:18-20.
2. Aleynikova N.V., Didenko P.A., Didenko L.V. The elements of an integrated grapevine protection system against major diseases. Viticulture and winemaking. 2016;2:17-19.
3. Aleynikova N.V., Galkina Ye.S., Didenko P.A. Raising the productivity of vineyards through application of liquid fertilizer Nutri-Fait PK and water conditioner Spartan in the conditions of Crimea. Scientific almanac. 2016;11-2(25):465-467.
4. Aleynikova N.V., Didenko P.A. Adjuvant "Kodaside" application for raising the biological effectiveness of fungicides in the protection of grapevines against oidium in the conditions of the Southern coast of Crimea. Magarach. Viticulture and winemaking. 2015;4:35-37.
5. Radionovskaya Ya.E., Didenko L.V. Biological effectiveness of modern-day insecticides in the protection of grapes against leafhopper *Arboridia kakogawana* Mats. Magarach. Viticulture and winemaking. 2015;1:21-24.
6. Yegorov E.A., Petrov V.S., Kuznetsov G.Ya. Ecologically sound management of ampelocenoses using biological methods of agricultural management. Bulletin of Agricultural Sciences. 2013;5:24-25.

THE MANIFESTATION AND PHYTOHORMONE RESPONSE OF LEAF PUBESCENCE GENES IN BREAD WHEAT

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Key words: *high throughput phenotyping, trichomes, bread wheat*

Motivation and Aim: The leaves of many angiosperm species develop trichomes. This trait is known to make a significant contribution to the protection from pests and adaptation to environmental factors in bread wheat. However the genetic basis of wheat trichome formation is poorly understood although a wide variation was found among Triticeae species with different ploidy level. Currently Catalogue of Gene Symbols for wheat contains only two loci associated with this trait: the gene *Hl1* in 4B chromosome and the gene $Hl2^{aesp}$ in 7B chromosome. Molecular function and regulation of these genes are currently not known. The present research sought to establish the individual and joint effect on trichome patterning and growth of each of three wheat leaf pubescence genes (*Hl1,* $Hl2^{aesp}$ and new one – *Hl3*) under normal conditions and phytohormone treatment.

Methods and Algorithms: Various lines carrying *Hl1, Hl3* and $Hl2^{aesp}$ and specially created nearly isogenic lines were used to quantitatively compare leaf pubescence using a modern high throughput phenotyping method (wheatdb.org/lhdetect2; Genaev et al., 2012). This method allows us to obtain rapidly quantitative characteristics of leaf pubescence (length of individual trichomes and their number) among many plants.

Results: Studied genes differed in their effect on trichome formation. *Hl1* and *Hl3* more affected trichome initiation and growth, while $Hl2^{aesp}$ modified mostly trichome length. Their action was independent to a large extent. A model of the action and interaction of *Hl1, Hl3* and $Hl2^{aesp}$ has been proposed to explain the genetic basis of trichome length and number.

Conclusion: The effects of phytohormones on trichome cell growth and initiation while *Hl1, Hl3* and $Hl2^{aesp}$ genes manifestation were explored. The effects of auxin (IAA), gibberellic acid (GA), cytokinins (6-BAP, Kinetin), metyl jasmonate (MeJa), ethylene (ACC) have been investigated and described. Our data revealed a key role of GA and cytokinin signaling pathways in *Hl1* and *Hl2* gene manifestation. At the same time this genes differs in a character of responce to hormone action. This suggests a different position *Hl1, Hl3* and $Hl2^{aesp}$ genes in the network of trichome formation control (Doroshkov et al., 2016).

Availability: LHDetect2 software available at *http://wheatdb.org/lhdetect2*

References

1. Genaev M.A., Doroshkov A.V., Pshenichnikova T.A., Kolchanov N.A., Afonnikov D. (2012). Extraction of quantitative characteristics describing wheat leaf pubescence with a novel image-processing technique. Planta. 236(6):1943-1954.
2. Doroshkov A.V., Afonnikov D.A., Dobrovolskaya O.B., Pshenichnikova T.A. (2016). Interactions between leaf pubescence genes in bread wheat as assessed by high throughput phenotyping. Euphytica. 207(3):491-500.

INTEGRATION AND AUTOMATIC ASSEMBLY OF METABOLIC MATHEMATICAL MODELS

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Key words: *metabolic reconstruction, metabolic model assembly, -omics data integration*

Motivation and Aim: Understanding of structure and dynamics of metabolic networks is an essential step in modern biological and medical studies, such as the design of superproducer strains for biotechnology, and identification of biomarkers for diagnostics of complex metabolic diseases, etc.

In silico approaches have been part of metabolic engineering toolkit since the mid-90s, allowing the costs and the time reduction by hypothesis testing and design of experiments. The performance of such approaches is heavily relying on the quality of the reaction network reconstruction, which by itself is the complicated and time-consuming process. More than a decade of active development of high-quality whole genome scale (WGS) models for prokaryotic species open a way for automatic reconstruction WGS models based on genomic annotation.

The aim of the work is the development of tools and methods for integration and headless assembly of metabolic networks flux models.

Methods and Algorithms: Authors recently developed the BioGraph database, a graph-oriented storage for information about prokaryotic organisms. It contains various -omics data (genomic, proteomic, taxonomic) integrated between each other with graph representation. The properly organized network of structural and semantic similarity relationships between proteins, genes, organisms and reactions makes it possible to construct models for close strains and species using the reference model as a template. We can also extract the existing model from the database for comparison. The model is generated as the file in SBML format, suitable for validation with COBRApy [1] package. The developed tool was tested on the database with several strains of *E. coli* and the iWFL_1372 [2] as a template model.

Results: We developed tools that allow assemble metabolic flux models in headless manner from different *-omics* data such as genomic annotation or proteins and genes similarity. Also the BioGraph database could be used as a source of biological information integrated with graph representation.

Availability: Source code of the software is available on Github: https://github.com/arc7an/scalaBiomeDB

References

1. Ebrahim A. et al. COBRApy: constraints-based reconstruction and analysis for python. BMC systems biology. 7.1 (2013):74.
2. Monk J.M. et al. Genome-scale metabolic reconstructions of multiple *Escherichia coli* strains highlight strain-specific adaptations to nutritional environments. Proceedings of the National Academy of Sciences 110.50(2013):20338-20343.

RIBOSOME AS SELECTOR OF ADAPTIVE RESPONSE IN *MYCOPLASMA GALLISEPTICUM*

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Key words: *Mollicutes, ribosome profiling*

Motivation and Aim: Mycoplasma gallisepticum is a cause agent of avian chronic respiratory disease. This disease has been responsible for major declines in house finch populations, damages commercial poultry industry over the world. Besides that, mycoplasmas are very interesting object for fundamental biology. These bacteria lack cell wall, their genome is reduced and contains few transcription factors. At the same time mycoplasmas demonstrate high adaptive potential. Recently, we demonstrated that transcription about half of coding sequences changed and antisense transcription was activated under heat stress [2]. However proteomic profile underwent few changes and demonstrated little correlation with transcriptional response. The causes of this phenomenon are not clear. The observed massive transcriptional response can potentially have negligible contribution to the stress adaptation [3]. Therefore, we used a combination of RNA-seq and ribosome profiling to dissect adaptive and non-adaptive responses. In addition we aimed to identify the determinants that guide RNAs towards effective translation under the stress.

Methods and Algorithms: Mycoplasma gallisepticum S6 was cultured in liquid medium for exponential phase and then cells were exposed to sublethal heat stress at 46°C for 30 min. Isolation of ribosomes was performed as previously described [1]. Libraries of cDNA were sequenced in SOLiD platform. Read mapping was performed using Bowtie software. Quantitative analysis was performed using R packages as previously described [2].

Results: Under exponential growth, mRNA abundance in the ribosome-bound pool of mRNA showed a correlation of 0.87 with the total RNA pool. But under heat stress correlation drastically decreased. The profile of ribosome-bound mRNA in stress was more similar to the one under control conditions rather than to total mRNA profile. Using the multiple regression method, we built a model to predict mRNA abundance in the ribosome-bound pool of mRNA. Total mRNA abundance, RBS (ribosome binding site) strength, stability of secondary structure near RBS and GC content of the transcript were used as predictor variables. Our model explains 72 and 38% of total variance in exponential growth and heat stress, respectively. Total mRNA abundance was the best predictor for mRNA abundance in the ribosome-bound pool of mRNA under exponential growth, but under heat stress, it was RBS strength.

Ribosome-bound mRNA demonstrated a phenomenon of high upregulation of low-abundant mRNAs in the stress. Previously using RNA-seq we demonstrated that numerous transcriptional terminators decrease efficiency under the stress. This effect results in the terminator read-throung and appending of downstream genes to the transcript. Using these data we found that 40% of low-abundant transcripts are upregulated in ribosome-

bound mRNA due to this mechanism. Our previous study indicate that the heat stress induces large amount of noise to the transcription and the ribosome profiling generally confirms this observation. We propose a method to distinguish adaptive response from the noise-like. In our previous research we showed that the abundance of the majority of transcripts in mycoplasmas is lower than one copy per cell [2]. Using our previous data we calibrated absolute abundance of transcripts identified by RNA-seq to the copy number. We identified that under the stress a small amount of genes increase abundance to 1 copy per cell, while the rest of genes, highly induced in terms of fold-change are still expressed in the amount significantly less than one copy per cell. The latter should be considered as transcriptional noise. The genes involved in adaptive response code for chaperons, cell division proteins, immunoglobulin-binding proteins, and uncharacterized protein.

Conclusion: Under heat stress, transcriptional response in *M.gallisepticum* is characterized as bimodal, not equal changes. The first mode is noise but the second is adaptive response. Ribosome binding to mRNA is every important and critical step for generation specific cell response. Numerous perturbations on the level of transcriptionare not transmitted by ribosomes to the level of translation.

Availability: Deep-sequencing (translatomics) data were uploaded to the NCBI SRA database, accession number PRJNA301561.

Acknowledgements: The work was funded by the Russian Science Foundation grant 14-24-00159, "Systems research of minimal cell on a Mycoplasma gallisepticum model".

References

1. Fisunov G.Y., Evsyutina D.V., Arzamasov A.A., Butenko I.O., Govorun V.M., Profiling of Mycoplasma gallisepticum ribosomes. Acta Naturae. 2015.
2. Mazin P.V., Fisunov G.Y., Gorbachev A.Y., Kapitskaya K.Y., Altukhov I.A., Semashko T.A., Alexeev D.G., Govorun V.M., Transcriptome analysis reveals novel regulatory mechanisms in a genome-reduced bacterium. Nucleic Acids Res. 2014.
3. Fisunov G.Y., Evsyutina D.V., Garanina I.A., Arzamasov A.A., Butenko I.O., Altukhov I.A., Nikitina A.S., Govorun V.M., Ribosome profiling reveals an adaptation strategy of reduced bacterium to acute stress. Biochimie. 2016.

SCREENING THE COLLECTION OF RUSSIAN SOFT WHEATS TO DEVELOP NOVEL TECHNOLOGY OF THEIR CONVERSION TO HARD-GRAINED CULTIVARS

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Key words: *Grain hardness, Pina, Pinb, Triticum aestivum, sgRNA, CRISPR/Cas9*

Motivation and Aim: Bread wheat (*Triticum aestivum* L.) is one of the most important food crops in the world. Grain texture is a primary marketing characteristic and a main determinant in end product quality of bread wheat and has profound effects on milling, baking and noodle-making. The *Pina* and *Pinb* genes have been confirmed as the major genetic factors contributing to grain hardness. Knocking out of these genes can help to yield hard grain wheat from the soft one's with distinguished agricultural attributes. The aim of the current study was to select Russian soft wheat cultivars suitable for modification and to design sgRNA for CRISPR\Cas9 modification of their *Pina* and *Pinb* genes.

Methods and Algorithms: Sixteen Russian soft wheat cultivars were checked for ability to form callus and to regenerate according protocols described in [1]. Wheat plants were grown in ICG greenhouses. *Pina* and *Pinb* diagnostic markers [2] were used to identify their allelic variants in the cultivars tested. PCR was performed as described in [2]. Partial sequences of *Pina* and *Pinb* of selected cultivars were obtained using ABI 3130XL analyser. CRISPR RGEN and CRISPRdirect software were exploited for sgRNA design.

Results: Cultivars (Zhnitsa, GDS_11, Krasa, Selenginskaya, Alenkaya, Albidum_3700, Balaganka, Krasnoyarskaya_1103, Omskaya_2078, Lutescens_62, Irkutskaya_49, Pobeda, Tarskaya_2, Rodina, Golubka, Pirotrix_28) were tested for capability to form callus and regenerate. Krasa, Alenkaya, Albidum_3700, Lutescens_62, Rodina, Golubka and Pirotrix_28 showed high regeneration ability. Genotyping and sequencing confirmed the presence of functional *Pina* alleles in these cultivars. sgRNA was designed for further *Pina* knocking out.

Conclusion: The data obtained will be useful for CRISPR\Cas9-based conversion of Russian soft wheat cultivars to hard-grained ones.

References

1. Transgenic Wheat, Barley and Oats: Production and Characterization Protocols, H.D. Jones, P.R. Shewry (Eds). Springer. 2009;345.
2. Huang X.-Q., Brûlé-Babel A. Development of simple and co-dominant PCR markers to genotype puroindoline a and b alleles for grain hardness in bread wheat (*Triticum aestivum* L.). Journal of Cereal Science. 2011;53:277-284.

IDENTIFICATION OF BACTERIA TRANSCRIPTION FACTOR TARGETS BY MACHINE LEARNING

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Key words: *mollicutes, genome reduction, transcription, promoters*

Motivation and Aim: Reconstruction of transcription regulation networks for bacteria is the important problem that still have not resolved. Current methods rely on information about known transcription factors of coregulation networks. In this work we aimed to develop a new method for prediction of regulated genes by using machine learning methods.

Methods and Algorithms: We used random forest algorithm of machine learning to predict strength of bacterial promoters by sequence. As experimental data for learning we used published and obtained by our laboratory data on coverage and position of transcription start sites. We used these features of promoter sequence to build model: sequences of -10 box, extension of -10 box, -35 box, nucleotides on transcription start site, length of spacers between -10 and -35 boxes, GC content of spacers between -10 and -35 boxes and between -10 box and transcription start site, up element upstream of -35 box, sequence upstream and downstream -10 box.

Results: We propose new algorithm that calculates probability of gene to be regulated. Our model predicts the bacterial promoters` power on the basis of its sequence. We applied this model for three bacteria with different genome size and number of regulators in genome. Comparing promoter power (theoretical prediction) with the data on promoters' activity (experimental data) we predicted promoters which activity is deviant from their power. We confirmed our approach on well-studied bacteria with big number of known regulators and then applied it to genome reduced bacteria Mycoplasma gallisepticum with a few number of known transcription factors. About 60 promoters of Mycoplasma gallisepticum are affected by repressors, which is 10 times higher than number of transcription factors identified by conservation. These repressed promoters don't have any sequences near promoters similar to known for Mycoplasma transcription factor binding sites. So, probably, these repressed genes represent a hidden layer of regulation that work only in specific conditions or we faced with new type of regulation in bacteria.

Conclusion: New method for prediction of repressed genes in bacterial genomes was developed and tested on three bacteria species. We identified many new potential targets for regulation of Mycoplasma gallisepticum. Our study thus provides insight into transcriptional regulation of bacteria.

RUNT AND *VASA* GENE EXPRESSION IN REGENERATION OF HOLOTHURIAN *EUPENTACTA FRAUDATRIX*

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Key words: *Runt, Vasa, Eupentacta fraudatrix, cell proliferation, gene expression, regeneration*

Motivation and Aim: Regeneration is the morphological process of replacing different structures (from parts of cells to large parts of the body) after natural wear or accidental loss, the result of which is the preservation of the integrity of the organism and the recovery of the lost function. This ability is widely distributed among representatives of flora and fauna. Among the invertebrates, representatives of the phylum Echinodermata are distinguished by pronounced regenerative capacity. Echinoderms are Deuterostomia and have a common ancestor with vertebrates. We chose the holothurian *Eupentacta fraudatrix*, which is a good model object for studying the mechanisms of regeneration. For many holothurians, there is such an interesting typically occuring phenomenon as evisceration. In the studied holothurian species, during evisceration, the whole digestive system is thrown out. It has been shown that the gut of *E. fraudatrix* after its complete loss is formed from the mesodermal cells as a result of their transdifferentiation. The morphology and cellular mechanisms of this process in *E. fraudatrix* have been described in detail, but the molecular mechanisms underlying the restorative morphogenesis and transdifferentiation are not known. Earlier, the genes transcripts of *runt* and *vasa* were found in the regenerating rudiments of *E. fraudatrix*. Runt proteins belong to a family of transcription factors that play an important role in controlling cell proliferation and cell differentiation in various morphogenetic processes. The *vasa* gene is known as a marker of stem cells and is necessary to maintain their undifferentiated state. Despite great attention to the mechanisms of cell transformation, such studies of echinoderms have not previously been conducted. To date, the *runt* and *vasa* genes in holothurians remain little-studied. For this work, it is necessary to study the expression of the *runt* and *vasa* genes in the regeneration process of the digestive system of holothuria *Eupentacta fraudatrix*.

Methods and Algorithms: As an object of the study, adults of holothurians *E. fraudatrix* were used both in normal state and at different stages of regeneration: 3, 5, 7, 10, 14 and 20 days after evisceration. We isolated total RNA from the intact gut and its rudiments at different stages of regeneration. To establish the complete sequence of transcripts of the studied genes, the method 5'- and 3'- Step-Out RACE was used. We obtained several reaction products of different sizes, which were then cut from the gel and used for transformation and sequencing. A study on changing gene expression was conducted using qPCR with the reference genes *ef1α* and *tubulin*. Sequence alignment of the found genes and their homologues in other animals for the construction of phylogenetic trees was also conducted.

Results: As a result of sequencing of the isolated products, complete sequences of the studied genes' coding regions of the transcripts were obtained. Using online service

BlastX it was revealed that the proteins Runt and Vasa have several highly conserved regions that are the same in all animals, from coelenterates to mammals. Gene expression of the *runt* and *vasa* was detected in the gut of holothurian *E. fraudatrix* in normal state. Expression occurs at a low level. When regenerating, on the 3rd day 80-fold increase in the expression of *runt* occurs and a 4-fold decrease in *vasa* expression occurs. On the 5th day, the expression of *runt* reaches its maximum, which is 120 times higher than the norm, while the *vasa* expression is restored to the normal level on the 5th and 7th days. At the same time, the expression of *runt* decreases on the 7th day. On the 10th, 14th and 20th days, the expression of *runt* gradually decreases, but even on the 20th day it is still 18 times higher than norm. The expression of *vasa* on the 10th day has its maximum, and, like *runt* on the 14th and 20th days, gradually decreases its expression, reaching the norm.

Conclusion: As a result of our studies, it has been shown that, both during regeneration and in normal state of holothurian *E. fraudatrix*, expression of the genes *runt* and *vasa* takes place. The study showed that the activity of the gene *runt* reaches its maximum on the 5th day after the eversion, during the active stage of morphogenesis, when the rudiment of the intestinal tube is formed and the cells migrate. In our opinion, during regeneration in the holothurians, *runt* plays a key role in providing a coordinated transition from proliferation to differentiation. The *vasa* gene has a maximum of expression on the 10th day of regeneration. At that time, the basic structures of the anterior end of the holothurian are already formed, they start to grow, and the differentiation of the cells that form them begins. At the same time, high proliferative activity of cells is maintained. We assume that *vasa* in *E. fraudatrix* is involved in the regulation of proliferation during the regeneration of internal organs.

Acknowledgements: This study was supported by the Russian Foundation for Basic Research (Grant No. 17-04-01334).

ORGANIZATION AND EVOLUTION CHALCONE SYNTHASE GENE FAMILY IN BREAD WHEAT AND RELATIVE SPECIES

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Key words: *Chs gene, chalcone synthase, gene duplication, flavonoid biosynthesis*

Motivation and Aim: Gene duplication followed by sub- or neofunctionalization is of substantial evolutionary importance. Homoeologous gene copies can appear due to polyploidization, while paralogous copies can be generated by segmental duplication. In allohexaploid wheat *Triticum aestivum* both homoeologs and paralogues of the same gene can be found. Multiple *Chs* gene copies, encoding one of the key enzyme of flavonoid biosynthesis, chalcone synthase, have been not characterized in wheat yet. The aim of the current study was to investigate structural and functional organization of *Chs* gene family and its evolution in wheat species.

Methods and Algorithms: Homologous sequences search was performed using BLAST algorithm in three databases (https://urgi.versailles.inra.fr/blast/blast.php, http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/search_reads.php, www.ncbi.nlm.nih.gov/Database/) within genomic sequences of *T.aestivum* and its tetraploid (*T.durum*) and diploid (*T.monococcum*, *T.urartu, Aegilops speltoides*, *Ae. sharonensis*, and *Ae. tauschii)* relatives. Missing part of sequence of one copy was sequenced using ABI 3130XL analyser. Multiple sequence alignment was done with MULTALIN 5.4.1. Cluster analysis was performed with MEGA v6.06 software using Neighbor-Joining algorithm. PrimerQuest Tool (https://eu.idtdna.com/Primerquest/Home/Index) was used to design a set of copy-specific primer pairs, which were exploited for PCR from DNA of nulli-tetrasomic and deletion lines and RT-PCR from cDNA for pericarp, coleoptile and root.

Results: The nucleotide sequences of the five *Chs* copies in *T.aestivum* were identified Among them two homologous gene copies in A- and D-genomes (*Chs-A1* and *Chs-D1*) and three paralogous gene copies in B-genome (*Chs-B1*, *-B2*, *-B3*). It was shown that all *Chs* gene copies are located on the distal regions of 2AS, 2BS and 2DS chromosomes. All copies with the exception of *Chs-B2* transcribed in colored pericarp and coleoptile, but they were not transcriptionally active in colorless pericarp and root. *Chs-B2* was transcribed in colored coleoptile only. To clarify the origin of paralogous *Chs* duplications in the B genome, we compared sequences of *Chs* genes in different *Triticum* and *Aegilops* species and calculated the time of divergence of the paralogues.

Conclusion: First *Chs* duplication event took place in common ancestor of *Triticum* and *Aegilops* about 10 MYA, then one of the copies was again duplicated 6-7 MYA in the ancestor of the B-genome, while other copy likely pseudogenized in *T.aestivum* 2A and 2D chromosomes. All 5 *T.aestivum* were transcriptionaly active, however one of the B-genomic copies demonstrated functional divergence from others.

The study was partially supported by RFBR (16-04-00912).

PREDICTION OF ANTIMICROBIAL PEPTIDES BASED ON FEATURE SELECTION METHODS

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Key words: *antimicrobial peptides (AMPs), classification, prediction*

Motivation and Aim: Due to the microorganisms antibiotic resistance increase to existing drugs, discovery for natural antibacterial agents is getting high-priority issue in various researches. Antimicrobial peptides (AMPs) appear to be one of the most appropriate candidates for solving this problem because of their target specificity and speed of action. To design a new mathematical-computational algorithm, oriented to identify compounds that is resistant against bacteria due to mechanism of action, is the aim of this work.

Methods and Algorithms: A database containing sequences of antimicrobial peptides has been created out of 5 different databases: UniProtKB/Swiss-Prot DB [1], APD3 [2], ADAM [3], CAMPR3 [4], and DADP [5]. Collected AMPs were screened for physicochemical properties such as molecular weight, a number of residues, isoelectric points, net charge under different pH and hydrophobicity based on the GRAVY scale were predicted using R packages seqinr [6], protr [7] and Peptides [8]. *In vitro* and *in vivo* aggregation properties were defined with the default option using algorithm TANGO [9] and AGGRESCAN server [10], respectively. For cluster analysis such clusterization like k-means, Fuzzy c-means and Model-based clustering were used. To construct prediction tool was used active learning strategy. The training set was taken from known sequences, the test set included real and synthesized data.

Results: Based on the results of analysis AMPs' sequences were separated by several clusters. Prediction strategy was created by machine learning mining methods.

Conclusion: Antimicrobial prediction method was developed. Predicted antibacterial peptides are subject to broad research due to their potential application and the benefit they can provide for a wide range of diseases.

Acknowledgements: The authors thank the laboratory of genetic engineering of FRCC PCM of FMBA for an invaluable contribution to the work. The reported study was funded by the Russian Foundation for Basic Research (RFBR) according to the research project № 16-34-00936 mol_a.

References

1. Bateman A., Martin M.J., O'Donovan C. et al. UniProt: A hub for protein information. Nucleic Acids Res. 2015;43(D1):D204-D212. doi:10.1093/nar/gku989.
2. Wang G., Li X., Wang Z. APD3: the antimicrobial peptide database as a tool for research and education. Nucleic Acids Res. 2015;44(November 2015):gkv1278. doi:10.1093/nar/gkv1278.
3. Lee H.-T., Lee C.-C., Yang J.-R., Lai J.Z.C., Chang K.Y. A Large-Scale Structural Classification of Antimicrobial Peptides. Biomed Res Int. 2015;2015:475062. doi:10.1155/2015/475062.
4. Waghu F.H., Barai R.S., Gurung P., Idicula-Thomas S. CAMP R3 : a database on sequences, structures and signatures of antimicrobial peptides. Nucleic Acids Res. 2015;44(D1):gkv1051. doi:10.1093/nar/gkv1051.

5. Novković M., Simunić J., Bojović V., Tossi A., Juretić D. DADP: The database of anuran defense peptides. Bioinformatics. 2012;28(10):1406-1407. doi:10.1093/bioinformatics/bts141.
6. Biological T., Retrieval S. Package "seqinr." 2016.
7. Xiao N., Xu Q., Cao D. protr : R package for generating various numerical representation schemes of protein sequence. 2014.
8. Indices T.C., Osorio A.D., Rondon-villarreal P. Package "Peptides." 2015.
9. Fernandez-Escamilla A.-M., Rousseau F., Schymkowitz J., Serrano L. Prediction of sequence-dependent and mutational effects on the aggregation of peptides and proteins. Nat Biotechnol. 2004;22(10):1302-1306. doi:10.1038/nbt1012.
10. Conchillo-Solé O., de Groot N.S., Avilés F.X., Vendrell J., Daura X., Ventura S. AGGRESCAN: a server for the prediction and evaluation of "hot spots" of aggregation in polypeptides. BMC Bioinformatics. 2007;8(1):65. doi:10.1186/1471-2105-8-65.

MYCOBIONT AND PHOTOBIONT OF LICHEN *CLADONIA ARBUSCULA* GETTING *IN VITRO*

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Key words: *lichens, mycobiont, photobiont, cultivation*

Motivation and Aim: Today a complete list of lichens components is not yet fully revealed, despite the fact that in the 19th century Simon Schwendener discovered the dualistic nature of the lichen. Yeast-like basidiomycetes were found in most species of lichens in 2016 [1]. The theoretical significance of the work is that with the help of pure cultures of the lichen phototubes and mycobionts, it is possible to determine the necessary conditions for obtaining a lichen *in vitro*. The pure cultures of the mycobiont obtained by us can be cultivated with the aim of producing an image of biologically active additives in biotechnology. Aims: getting mycobiont and photobiont of lichen *Cladonia arbuscula* for the resynthesis of lichen *in vitro*.

Methods and Algorithms: According to I. Yoshimura, Y. Yamamoto, T. Nakano, J. Finnie [2], we are getting lichen's components, but with some modifications:

1. We weigh the mass of dry lichen.
2. Rinse the lichen under running water for 10 minutes.
3. We place the lichen in a sterile pounder and homogenize with a sterile water.
4. We filter the homogenizate through a nylon net.
5. We add 3 ml of the homogenizate to Bold's Basal Medium (on photobiont with 1400 Lx lighting) and Malt / Yeast extract medium (on mycobiont) and place its in the thermostat with the necessary temperature (10–15 °C).

Results: The results of a photobiont culture study. We added 3 ml of sterile water and increased the lighting to 3780 Lx after a week of incubation in a thermostat. Algae began to grow by the end of the second week. We were able to visually colony the photobiont *Trebouxia* under a binocular microscope by the end of the third week. *Trebouxia* is characterized by large globular cells of light green color with stellate chromatophores. Some algae growing out of pieces of a lichen thallus.

The results of a mycobiont culture study. Mycobiont *Cladonia arbuscula* colony is a white reliefic with a characteristic wavy edge. Mycobiont forms dense structures on the surface by the second week of incubation, which are the rudiments of a granular lichen thallus.

Conclusion: The mycobiont and photobiont of *Cladonia arbuscula* can be obtained by cutting thallus and incubating the homogenizate for various conditions. It was shown that it is not necessary to grow on wet sterile filters, followed by isolation of hyphae growing from the core. Experiments on the synthesis of lichens *in vitro* are planned with pure cultures,.

Acknowledgements: We thank Leading Researcher of Department of Mycology and Algology Pankratov T.A. (MSU) for leadership and financial support.

Work was carried out under the grant RFFI № 16-04-00966.

References

1. Glagolev S. (2016) The third is not excess: in most lichens there are two fungi and algae, Elements. http://elementy.ru/novosti_nauki/432835/Tretiy_ne_lishniy_v_bolshinstve_lishaynikov_prisutstvuyut_dva_griba_i_vodorosl.
2. Yoshimura I., Yamamoto Y., Nakano T., Finnie J. Isolation and culture of lichen photobionts and mycobionts (2002), In: Protocols in lichenology culturing, biochemistry, ecophysiology and use in biomonitoring. I. Kranner, R. Beckett A., Varma (Eds), 3-33 (Publisher).

APPLICATION OF INTERNAL TRANSCRIBED SPACER SEQUENCES FOR THE IDENTIFICATION AND PHYLOGENETIC RELATIONSHIP STUDY OF *WALDSTEINIA TERNATA* (ROSACEAE) POPULATIONS

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Key words: *DNA barcoding, Waldsteinia, internal transcribed spacers, ITS1, ITS2, Baikal Siberia, nemoral relict species, endangered species*

Motivation and Aim: At the present time, bioinformatic analysis of DNA sequences is irreplaceable technology, which provides series of new data for molecular phylogeny and DNA barcoding of different taxonomic groups. From the first report of the utility of the internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA in plants, they have been extensively used to distinguish closely related species and even populations.

The present study was aimed to estimation of the possibility of ITS sequences effectively use for the identification and phylogenetic relationship of *Waldsteinia ternata* (Steph.) Fritsch populations. *W. ternata* is a nemoral relict species, which characterized by strongly disjunctive areal. European, Siberian and East-Asian isolated ranges of *W. ternata* are distinguished. Originally *W. ternata* were described from the population on the Khamar-Daban ridge (the Baikal Siberia) and until now there is no common opinion of the systematic status and historical relationship of the distant populations from the different regions.

Methods and Algorithms: Comparative analysis of ITS regions sequences of several *W. ternata* populations from Siberian (the Eastern and Western Sayan mountings, the Khamar-Daban ridge) and East Asian (the Far Eastern) parts of its disjunctive areal was carried out. As a reference, European close related species *W. geoides* Willd. was sampled from the Botanic Garden of Irkutsk State University. Total gDNA was isolated from silica-dried leaf tissue following CTAB method [1] with modifications [2]. The complete ITS region was amplified using the forward ITS1-P2 [3] and reverse ITS4 [4] primers. DNA sequencing by the Sanger method using 3500 Genetic Analyzer (Applied Biosystems) was applied. Nucleotide sequences alignment by MUSCLE, the phylogenetic analysis by maximum likelihood method in consideration of the best-fit models of nucleotide substitution were carried out in MEGA v. 7.0.16. Haplotype net constructions were carried out in Haplotype viewer software (Center for Integrative Bioinformatics, Vienna).

Results: Our results showed intra- and interspecific variability of ITS1 region which allow using this molecular marker for identification of *Waldsteinia* species and study of the populations phylogenetic relationship. A high intraspecific and intrapopulation variation of ITS2 region, together with a low number of systematic substitutions do not allow to distinguish closely related species and populations. That is why, we used only the ITS1 region for analysis of phylogenetic relationship of *Waldsteinia* populations.

The haplotype analysis of ITS1 found, that on long-range areas within the Khamar-Daban ridge gene flows between *W. ternata* populations are limited. At least the western populations could have relatively independent history from the other Khamar-Daban populations. The levels of genetic distance between the Khamar-Daban, the Eastern and Western Sayan populations indicate relatively short-term isolation from each other. The results indicate that the genetic distance between Siberian and East Asian populations of *W. ternata* is much lower than previously assumed on the basis of morphological analysis and their geographical distribution. Results also confirm the hypothesis of the East Asian origin of the Siberian populations.

Conclusion: The detected levels of intra- and interspecific ITS1 variability allow using this molecular marker for phylogenetic studies and identification of *Waldsteinia* species. The low copy number variants of ITS1, revealed by molecular cloning approach, can be used for identification of distinct populations of W. ternata. A high intraspecific and intrapopulation variation of ITS2 region, together with a low number of systematic substitutions do not allow to distinguish closely related species and populations of *W. ternata*.

Acknowledgements: The reported study was partially supported by RFBR, research projects No. 16-34-60135mol_a_dk, 16-05-00783. The authors thank Prof. N.S. Probatova and Dr. E.A. Pimenova for the help with sampling of the Far Eastern populations of *W. ternata*.

References

1. Doyle J.J., Doyle J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue, Phytochem. Bull. 19:11-15.
2. Protopopova M.V. et al. (2016) The perspectives for the internal transcribed spacer (ITS1 and ITS2) Application for the endangered plant species identification with Waldsteinia (Rosaceae) as an example, The bulletin of Irkutsk State University. Series «Biology, Ecology». 17:5-11.
3. Utelli A. et al. (2000) Molecular and morphological analyses of European Aconitum species (Ranunculaceae). Plant Systematics and Evolution. 224:195-212.
4. White T.J. et al. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications. M.A. Innis et al. (Eds.). 315-322 (Academic Press.).

MOLECULAR EVOLUTION ANALYSIS OF GENETIC NETWORK RELATED TO PLANT TRICHOME DEVELOPMENT

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Key words: *molecular evolution, gene network, epidermal cells, trichomes*

Motivation and Aim: Trichomes are involved in many significant functions such as the transpiration, thermoregulation and protection from insect attacks. On the other hand specialized cell formation is an fruitful model system for analyzing the molecular mechanisms of plant cell differentiation, including cell fate choices, cell cycle control, and cell morphogenesis. In plants, epidermal cells are easily accessible and allow *in vivo* study. Unicellular trichome formation is a classical experimental model for identification of the activator–inhibitor and the activator–depletion pattern formation models, studying the interplay between cell cycle and cell differentiation and numerous of genes involved in these processes were found. However, the evolution of specialized epidermal cell formation genetic network remains unclear. In this study, we analyze the phylogenetic relationships of genes associated with the formation of trichomes and root hairs from various species of flowering plants.

Methods and Algorithms: Using the text mining technology we reconstructed the network of interactions between known leaf pubescence genes using *A. thaliana* as a model organism. The genetic network model was clarified by the iterative improvement based on gene co-expression data. For each node was performed extraction of sets of homological sequences presets from databases was carried out using the reciprocal BLAST search. Multiple sequence alignment was conducted with MAFFT algorithm. The PhyML maximum likelihood algorithm was used to reconstruct the phylogeny and bootstrap resampling technique was used for testing the topology. Genetic networks containing target genes were reconstructed using Cytoscape and Pathway Studio software.

Results: We reconstructed a model of the trichomes development gene network in *A. thaliana*. The main stages of gene network evolution has been traced. A time of appearance of its components has been predicted. We estimated number of orthologous genes in plant genomes.

Conclusion: Genetic network of trichome formation contain highly connected region, responsible for the cell fate specification. This part of the network activates a series of genes that regulate various processes during the trichome cell differentiation. Key genes of "trichome initiation complex" (EGL1, GL3, GL2, TTG1) are present in the common ancestor of flowering plants (monocotyledonous and dicotyledonous). Main genes controlling trichome cell differentiation processes also appeared about 500 years ago.

SCREENING OF THE RUSSIAN SPRING BARLEY COLLECTION TO SELECT TARGET CULTIVARS AND TARGET GENES FOR FURTHER CRISPR/CAS9-EDITING

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Key words: *CRISPR/Cas9, barley, genome editing, Hordeum vulgare*

Motivation and Aim: Discovered in 2013 as the gene editing tool the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 nuclease (Cas9) system continues to actively develop. It is possible to obtain nontransgenic plants carrying stably inherited, specifically determined mutations using the CRISPR/Cas9 system. One of the further directions of the development of CRISPR/Cas9 editing system on plants is the involvement of more cultivars in the editing process. Just a few genotypes have been used for CRISPR/Cas9-based gene modifications thus far. In our study, we focused on the use of CRISPR/Cas9 system on local cultivars of Russian spring barley collection which consists of 116 cultivars.

Methods and Algorithms: To estimate susceptibility for transformation it was decided to screen local cultivars for ability to callus induction, and regeneration processes *in vitro*, both important properties for successful transformation. For this purpose, ten most prospective cultivars were selected. Model cultivar "Golden promise", known as most amenable for embryos transformation, was taken as control. Primary target genes selected for modification in barley are *Nud* and *Vrs1* genes. Knockout of these genes is expected to transform hulled grains to naked and two-rowed spikes to six-rowed, respectively. Target genotypes are hulled two-rowed spring barley cultivars from the local collection of spring barley. All 116 cultivars from the collection were subjected to PCR screening of genes *Nud* and *Vrs1*. The nucleotide sequence of the genes *Nud* and *Vrs1* was confirmed by sequencing on selected cultivars. This data were used for constructs development for CRISPR/Cas9-based knockout.

Results: The current results of ongoing large-scale screening of ten cultivars ("Biom" (28,5), "Talan" (54,4), "Vorsinskiy 2" (44,3), "Aley" (63,2), "Acha" (28,4), "Signal" (46,25), "L-421" (20,7), "Kolchan" (16,5), "V-1" (15,25), "Krasnoyarskiy 91" (68,75)) for regeneration ability are presented and discussed (the percentage of regeneration is indicated in parentheses). PCR screening of the *Nud* gene has been shown that naked Russian cultivars have a deletion in the region. PCR markers were developed for the *Nud* gene. Selection of sgRNA has been performed using the programs "CRISPRdirect" and "E-crisp". Selected sgRNA has been inserted into the vector pBUN421. Experiments of the CRISPR/Cas9 constructs transformation to immature barley embryos are still in progress.

Conclusion: Spring barley "Aley" has been selected as primary candidate for CRISPR/Cas-based knockout of the *Nud* gene to produce naked analogue of this cultivar.

Acknowledgements: The study was supported by the Russian Science Foundation (Project No 16-14-00086).

THE USE OF POSTGENOME TECHNOLOGIES IN INVESTIGATION OF PLAGUE NATURAL FOCI OF NORTH CAUCASUS

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Key words: *proteomic profiling, natural foci, Yersinia pestis, species-specific proteins*

Plague is a zoonotic bacterial infection with natural foci. In the area of plague natural foci human infections may occur by transmission, through contacts or via droplets. There are 11 plague natural foci in the area of the Russian Federation. Plague epizooties were registered in 8 of them. Since 2014 human cases are annually reported in Russia. A steady tendency of activation which is observed in a number of natural foci of plague points to the necessity of strengthening of epidemiological monitoring to prevent human cases.The two *Yersinia pestis* subspecies circulating in the area of North Caucasus and Transcaucasis – the main subspecies *Y. pestis subsp. pestis* and the caucasian subspecies *Y. pestis subsp. caucasica* are differentiated by their virulence, biochemical properties and the presence of genetic determinants of pathogenicity. Differentiation and typing of strains of the plague microbe isolated in the area of natural foci is of great importance for the estimation of the degree of intensity of epizootic process and for revealing the ways of spread of the causative agent.

The aim of the work was to study *Y. pestis* strains circulating in the area of North Caucasus and Transcaucasia by MALDI-TOF mass spectrometry and proteomic analysis.

Object of study: 50 *Y. pestis* strains isolated during the period 1950–2012 in the area of 7 natural foci of North Caucasus and Transcaucasia. Preliminarily all the strains were identified to subspecies. The most part of strains (36) belonged to the main subspecies *Y. pestis pestis*, the remaining strains – to the non-main subspecies typical of some foci of the Caucasus and Transcaucasia – *Y. pestis caucasica*.

Materials and Methods: Mass spectrometry profiles of *Y. pestis* cell extracts were obtained using mass-spectrometer Microflex LT (Bruker Daltonics). A saturated solution of α-hydroxycinnamylic acid (CHCA) was used as a matrix. The Bacterial Test Standard (Bruker Daltonics) was used as a calibrator. Mass spectra were obtained over the range 2,000-20,000 Da. Spectra of each strain were processed and analyzed using the programme FlexAnalysis. A super-spectrum was created out of 20 individual mass spectra using the standard algorithm of the programme MALDI Biotyper v. 3.0. To control the quality of MSP-dendrograms of *Y. pestis* strains MLVA genotyping of the investigated strains using 25 variable loci (Le Flèche et al., 2001; Li et al. 2009) was carried out concurrently. Plasmid profiling of *Y. pestis* strains was carried out as described previously (Woron et al., 2006). To present evolutionary relations between the investigated strains of *Y. pestis* the UPGMA algorithm of the programme START2 (www.pubmlst.org) was used. For identification of specific proteins a set of equipment for 2-D gel electrophoresis (Bio-Rad) and a tandem time-of-flight mass-spectrometer UltraflexTreme (Bruker Daltonics) were used. A kit of reagents for fluorescent protein labeling CyDye DIGE Fluor labeling

kit (GE Healthcare) was used for differentiating gel electrophoresis. Proteins were identified using the interactive programme Mascote.

Results and discussion: A database of mass spectra of 50 *Y. pestis* strains ssp. *pestis* and *caucasica* isolated in the area of plague natural foci of North Caucasus and Transcaucasia we created is designed for identification of cultures of the causative agent of plague using the programme Biotyper v3.0 which enables reliable identification of cultures of the plague microbe with SV $\geq$ 2.3–2.4 and differentiation of them from cultures of closely related species (*Y. pseudotuberculosis*). On the basis of analysis of spectra a MSP-dendrogram of spectra was constructed, which reflects phylogenetic relations between the investigated strains by the presence of fragments of genus and species specific proteins within the range of detected masses of 2-20 kDa. The two main branches are distinguished in the structure of MSP-dendrograms. The big branch includes strains belonging to the main subspecies, the little one - strains of the Caucasian subspecies of the plague microbe. The topology of the MSP-dendrogram was confirmed by the results of MLVA genotyping and by the dendrogram constructed according the results of molecular and genetic analysis. As the analysis of peak-lists showed, species identification of the causative agent of plague is carried out by peptide fragments, most of which are classified as fragments of ribosomal (50S and 30S) and structural proteins. When analyzing peak-lists and mass spectra of most strains the peak 3065 Da previously described as species specific for *Y. pestis* strains was identified. This peak is a 30 aa peptide which in the database UniprotKB is identified as a fragment of plasminogen-activator protein localized on the plasmid of pesticinogenicity pPla (pPst). The high intensity of the specific peak 3065 Da is caused by the fact that the gene *pla* encoding the synthesis of plasminogen-activator protein is multicopious. As our results showed this differentiating marker is typical only of the main subspecies of *Y. pestis* strains having the plasmid pPla (pPst). Strains not belonging to the main subspecies have neither the 3065 Da peak nor the plasmid pPla (pPst) which was confirmed by the results of plasmid screening. Besides, we showed the possibility of differentiation of strains of the plague microbe strains belonging to the main subspecies – *Y. pestis pestis* to the biovar level on the basis of their differences in mass spectrum profiles. While analyzing the protein profiles of *Y. pestis pestis* strains, belonging to biovars *medievalis, orientalis, antiqua* we isolated 3 differentiating regions: Diff 1 with the range of 3533-3682 Da, Diff 2 with the range of 6560-6700 Da, and Diff 3 with the range of 7190–7785 Da and 3 separate peaks: 5623, 5891, 7452 Da, various combinations of which permit differentiation of biovars of the main subspecies of *Y. pestis*. Using the method of 2-D differentiating gel electrophoresis we analyzed acid-soluble protein extracts of 2 *Y. pestis* strains, belonging to the main and caucasian subspecies. The comparative analysis of the intensity of specific fluorescence of separate protein points revealed peptide fragments of acid hydrolysis of protein extracts which were identified by the method of tandem time-of-flight mass spectrometry. As the analysis of the results showed the fragments of ribosomal (50S and 30S) and structural proteins as well as virulence factors (capsular antigen, plasminogen) are dominating in protein fractions of *Y. pestis* strains of both subspecies and enable not only effective differentiation of *Y. pestis* from closely related species (*Y. pseudotuberculosis, Y. enterocolitica*), but also intraspecific discrimination of strains.

CHARACTERISATION OF PATHOGENIC AGROBACTERIA IN VINEYARDS OF KRASNODAR TERRITORY USING PCR METHOD

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Key words: *Agrobacterium vitis, grapevine, PCR, Ti-plasmid, test-systems, opine-types, pehA, virD2, virF*

Motivation and Aim: Bacteria of the *Agrobacterium* spp. genus, affecting a large range of fruit, berry plants and grapes, cause a disease called crown gall. Currently the most widely used nomenclature is based on the classification of *Agrobacterium* spp. in biotypes: biotype-1, biotype-2 and biotype-3, respectively, *A. tumefaciens*, *A. rhizogenes* and *A. vitis*. Grapevines are mostly infected by agrobacteria *A. vitis* and less often *A. tumefaciens*. Recently, it became important to classify agrobacteria by the type of Ti-plasmid contained in them. The most common types of *A. vitis* Ti plasmids opine-types are octopine, nopaline and vitopine. The purpose of the study is identification the pathogenic species and diversity of agrobacteria in the vineyards of the Krasnodar territory.

Methods and Algorithms: Tissues from grape plants of different varieties collected in different vine growing areas of the Krasnodar territory were used as a material. DNA from tumors was isolated by the CTAB method with some additions [1]. Polymerase chain reaction method was used to identify agrobacteria by using the "Tertzik" device. Evaluation of the results was done by the agarose gel electrophoresis method. The DNA markers of the genes *pehA*, *virF* and *virD2* were used, the sequences of primer pairs PGF/PGR [2], $VIRFF_1$/$VIRFR_2$ and $VIRD2S4F_{716}$/$VIRD2S4R_{1036}$ [3], VIRD2A/VIRD2C [4] were taken from literature.

Results: At the beginning, all samples were studied by universal primers to the conservative part of the *virD2* gene, specific for most pathogenic agrobacteria. However, only 31 % of the analyzed samples showed the presence of the target fragment. Similar results have been noted in some other works on the study of pathogenic agrobacteria [5]. The reason of this may be a high genetic diversity of the studied samples. By using species-specific PGF/PGR primers to the polygalacturonase gene (*pehA*) of *A. vitis*, it was possible to amplify the target fragment in all the analyzed samples, which indicates that the agrobacterium species under study belong to the species *A. vitis*. Test-system of $VIRFF_1$/$VIRFR_2$ for the *virF* gene of the octopine and nopaline Ti plasmid of *A. vitis* gave the positive results in 79 % of the researched samples, which indicates the probability of presence of agrobacteria with octopine or nopaline type of Ti plasmids in these samples. The test-system $VIRD2S4F_{716}$/ $VIRD2S4R_{1036}$ for the *virD2* gene of the vitopine plasmid *A. vitis* gave a positive result in 27 % of the samples, suggesting the presence of the vitopine Ti plasmid in these samples. Combining the $VIRFF_1$/$VIRFR_2$, $VIRD2S4F_{716}$/ $VIRD2S4R_{1036}$ and PGF/PGR test-systems into a multiplex allows detecting the presence or absence of any of the investigated genes simultaneously.

Conclusion: Thus, in the vineyards of the Krasnodar territory, *A. vitis* agrobacteria parasite, differing in the Ti-plasmid type. The obtained results are intermediate. Information that is more complete will be obtained by increasing the number of samples and using the new test-systems of others agrobacteria genes for identification of agrobacteria diversity.

Acknowledgments: The work was carried out with the financial support of the RFBR grant № 16-34-00827 mol_a.

References

1. Doyle J.J., Doyle J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh tissue. Phytochem. Bull. 19:11-15.
2. Herlache T.C., Hotchkiss A.T., Burr T.J., Collmer A. (1997). Characterization of the *Agrobacterium vitis pehA* gene and comparison of the encoded polygalacturonase with the homologous enzymes from *Erwinia carotovora* and *Ralstonia solanacearum*. Applied and Environmental Microbiology, 63(1):338-346.
3. Bini F., Kuczmog A., Putnoky P., Otten L., Bazzi C., Burr T.J., Szegedi E. (2008). Novel pathogen-specific primers for the detection of *Agrobacterium vitis* and *Agrobacterium tumefaciens*. Vitis-Journal of Grapevine Research. 47(3):181-189.
4. Haas J.H., Moore L.W., Ream W., Manulis S. (1995). Universal PCR primers for detection of phytopathogenic *Agrobacterium strains*. Applied and Environmental Microbiology. 61(8):2879-2884.
5. Kuzmanović N., Gašić K., Ivanović M., Prokić A., Obradović A. (2012). Identification of *Agrobacterium vitis* as a causal agent of grapevine crown gall in Serbia. Archives of Biological Sciences. 64(4):1487-1494.

THE EXPRESSION OF GRIA1, CACNA2D3, POMC AND MAPK1 GENES AND THEIR ROLE IN AGGRESSIVE BEHAVIOR IN RATS

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Key words: *aggressiveness, behavior, gene expression, gray rat*

Motivation and Aim: The study of genetic determinants of different behavior models (including aggression) remains relevant in the modern world because of a large practical contribution to working with animals, as well as fur farming. Aggressive behavior is one of the significant problems having biological roots. The purpose of our investigation was to study the molecular mechanisms of hereditary-mediated aggressive behavior on laboratory animal models. We used unique experimental model of grey rats (*Rattus norvegicus*) developed at the Institute of Cytology and Genetics SB RAS. Rats have been subjected to selection in two directions – tolerant and aggressive behavior towards human and other rats. We evaluated the expression of several genes in rat brain areas, presumably associated with the manifestation of aggressiveness: Gria1, Cacna2d3, POMC and MAPK1.

Gria1 encodes the glutamate ionotropic receptor AMPA type subunit 1, which is one of the predominant receptors of the excitatory neurotransmitter in the mammalian brain and participates in various normal neurophysiological processes.

Calcium voltage-gated channel subunit alpha1 B encoded by the Cacna2d3 gene is a protein that is the pore-forming subunit of an N-type voltage-dependent calcium channel, which controls neurotransmitter release from neurons.

POMC gene encodes a preproprotein, which after processing may yield as many as ten biologically active peptides associated with a number of cellular functions.

Mitogen-activated protein kinase 1encoded by MAPK1gene is a member of the MAP kinase family, that involved in a wide variety of cellular processes.

Methods and Algorithms: RNA-seq sequencing of rat brain areas samples was done using Illumina HiSeq. The set of computer tools and data processing pipelines helped to find genes and gene regulation patterns applied to behavior models. RNA-profiling experiments revealed the lists of differentially expressed genes in the brain samples and differentially spliced isoforms. Gene expression level of the genes under study was tested by RT-PCR.

Results: Transcriptome profiling, as well as RT-PCR confirmed differential expression of Gria1, Cacna2d3, POMC and MAPK1 genes in rat brain differing by behavior. A lot of synapse associated genes have statistically significant deviation in splicing depending on brain regions and behavioral models (aggressive/tame) of rats. It is a new phenomenon of the transcriptome data in studying of aggressive behavior. The results of the work can be widely used in agriculture, medicine and biology.

Conclusion: Heredity has been found to significantly contribute to aggressiveness in studies of various animal species. Consequently, the genetic factors exert a strong influence to the phenotypic variation of aggressive behavior in populations. This is confirmed by inter-strain differences in the manifestation of aggression in laboratory animals and by the fast progress of selection for aggressiveness traits.

Acknowledgements: Authors acknowledge ICG SB RAS budget project (0324-2016-0008) and RSF.

LEAF LIPIDS AND PIGMENTS OF RELICT SPECIES *GLOBULARIA PUNCTATA*

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Key words: *Globularia punctata, relict, lipids, pigments*

Motivation and Aim: In nature, wild plants are adapted to the environment and at the cellular level they have a specific composition of membrane components. This composition is formed in the process of evolution and corresponds to abiotic conditions. Relict plant species of limited distribution can be especially adapting. For example, the calcefil Globularia punctata Lapeyr (Globulariaceae) is a rare species, the Pliocene mountain-steppe relic, which is listed in the Red Books of the USSR, the RSFSR and the Russian Federation. Cellular membranes are actively involved in signal interactions between the cell and the external environment, which take and transmit incoming impulses from the outside world. The aim of the work was to investigate the peculiarities of the composition and content of lipids and pigments in leaves of this species growing in the steppe zone of the Middle Volga region.

Methods and Algorithms: The analysis of lipids, fatty acids and pigments is made in accordance with the methodical recommendations of M. Keits.

Results: The total amount of pigments is in the range of 0.27–0.32 mg/g, which is a small amount compared to other relic species. The total lipid content was 37.0 mg/g weight. Of these, 42% is for membrane glycolipids (GL), 10% for membrane phospholipids (PL) and 27% for spare neutral lipids (NL). A distinctive feature of PL composition was a relatively high content of phosphatidylcholine – 87% and a low content of phosphatidylethanolamine – 1%. Their content in the leaves of higher plants is usually 40–60% and 12–20%, respectively. A peculiarity of the GL composition was a high relative content of dihalactosyldiacylglycerol – 46% and sulfoquinose-diacyl glycerol sulfolipid – 19%, with a relatively low value of monogalactosyldiacylglycerol – 35%. Three- and diacylglycerols (31 and 15%), sterols and their ethers (15 and 16%) prevail in the composition of NL. The share of hydrocarbons, waxes, free acids, alcohols accounted for 2–8% of NL. In the fatty acids (FA) of lipids, 15 components were identified. The composition of the FA is 70% represented by unsaturated FA, of which oleic acid is 9%, linoleic acid is 22% and linolenic is 42%.

Conclusion: Thus, the specificity of the composition of pigments and lipids of the leaves of the relic species of *Globularia punctata* has been established.

Acknowledgements: I would like to thank my scientific adviser Professor Olga N. Makurina, Professor Olga A. Rozentsvet and researchers at Institute of Ecology of the Volga River Basin of Russian Academy of Sciences for their help and support.

EXPRESSION OF HOLOTHURIA *EUPENTACTA FRAUDATRIX* ARROWHEAD GENES DURING REGENERATION

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Key words: *regeneration, Eupentacta fraudatrix, lim homeobox, arrowhead, LHX, gene expression*

Motivation and Aims: Regeneration is a morphogenetic process of replacement of various structures after natural wear or situational loss, which results in preserving of integrity of an organism and restoration of lost function. Echinoderms are known as animals with good regenerative abilities. Among echinoderms holothurians have most diverse morphogenetic reactions. They can regenerate external appendages and internal organs as well as reproduce asexually. Holothurians are animals with soft worm-like body. Unlike the other Echinodermata, their skeleton is greatly reduced and is represented with only small spicules, situated inside body wall and a calcareous pharyngeal ring. The body is prolonged in oral-aboral direction and has both pentactinal and bilateral symmetry.

Morphogenetic processes tightly connected to regeneration such as asexual reproduction and autotomy are widely spread among holothurians, which makes them convenient model organism for studying those processes. In addition, holothurians have a unique form of autotomy inherent, perhaps, only to them, called evisceration which is especially interesting.

The object of our study, holothurian *E. fraudatrix*, can eject its oral complex of organs (its "head") and all digestive system. Regeneration following evisceration in *E. fraudatrix* occurs by transdifferentiation of mesodermal cells into endodermal. Morphology and cellular mechanisms of this process have been studied well earlier, which is not the case with molecular basis.

Earlier transcriptome analysis of regenerating organs anlages in *E. fraudatrix* revealed many differentially expressing genes. One of them is *arrowhead* (*LHX 6-8*). *Arrowhead* genes are members of LIM homeobox class. Genes of the LIM homeobox (Lhx) family perform fundamental roles in tissue-specific differentiation and body patterning during development in both vertebrates and invertebrates. These genes comprise a family of DNA-binding proteins with six subfamilies. LIM homeobox (Lhx) transcription factors are unique to the animal lineage and have patterning roles during embryonic development with a conserved role in specifying neuronal identity. Lhx proteins are composed of two N-terminal LIM domains (named after the founding members LIN-11, Islet-1, and MEC-3) and a helix-turn-helix forming homeodomain that binds regulatory DNA surrounding target genes. The zinc-finger forming LIM domains are essential for protein function in several subfamilies and are thought to regulate DNA binding by the homeodomain by interacting with other nuclear proteins.

Methods and Algorithms: We made analysis of gene sequences using Blast X. We constructed phylogenetic tree using "Neighbor-Joining" method. Alignment was made

via Clustal X method. Sequenation of E.fraudatrix transcriptome during intestine regeneration via transdifferentiation after evisceration had been performed.

Results: We found that expression of *arrowhead* gene increased during regeneration of *E. fraudatrix*. We compare *arrowhead* from the holothurian with that of *Homo sapiens, Mus musculus, Danio rerio, Nematostella sp., Saccoglocuss kowalewski, Strongylocentrotus purpuratus, Acropora digitifera*. A sequence of the *arrowhead* gene 825 nucleotides long and corresponding amino acid sequence 275 amino acid residues long have been discovered with computer analysis. There are 54 amino acid residues in highly conserved regions. Arrowhead transcript has most similarity with *S. purpuratus arrowhead*. Conservative region differs by 15 amino acid replacements. Similarity between holothurian arrowhead and sea urchin arrowhead *Paracentrotus lividus* is 63%. Similarity with *Saccoglossus kowalevskii* arrowhead is lower – 59%. Similarity with vertebrate *LHX6* and *LHX8* is even lower – 55%. We suppose, that amino acids replacements did not influenced crucially on arrowhead activity and it functions in organism preserved. So we expect *arrowhead* involved in tissue-specific differentiation and body patterning during regeneration processes.

Acknowledgements: This study was supported by the Russian Foundation for Basic Research (Grant No. 17-04-01334).

MACHINE LEARNING TECHNIQUES FOR ANALYSIS OF PHYSICAL PROPERTIES PROFILES OF *E. COLI* PROMOTERS

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Key words: *promoter, prokaryote genetics, genome annotation, DNA physics, machine learning*

Motivation and Aim: An astonishing amount of DNA primary structure data provided by recent sequencing techniques gives opportunity to study multiple genomes and metagenomes at a time. However there are obstacles such as low accuracy of regulatory sites prediction. Commonly used annotation algorithms that employ nucleotide sequence analysis fail to provide acceptable accuracy rates for regulation sites, especially promoters. It is suggested that structural, physical as well as textual characteristics of regulatory regions are to be considered together in the task of the sequences prediction [1]. Here we suggest to divide the totality of DNA physical properties into several groups in order to choose most distinctive and less correlated ones. Firstly, *dynamical* and *steady-state* properties are different in whether they take into consideration kinetics of processes. On the other hand, depending how structure can influence on a property profile one can distinguish *local* (affecting nearby DNA context only), *global smooth* (affecting extended intervals relatively equally), and *global burst* (profile is affected by extended DNA context but the influence is localized only on a small part of the sequences).

Methods and Algorithms: In the work electrostatic potential (EP – global smooth, steady-state property) [2], activation energy (AE) and size (S) of open states (local dynamic) [3], and 200 b.p.-long sliding GC-content (textual characteristic) were used. Considered global burst characteristic – stress-induced duplex destabilization (SIDD, [1]) – was shown to be less informative for promoter prediction, so it was not used hereinafter. Fitness of the properties as promoter determinants was evaluated using genome of *E. coli* (strain K-12) obtained from RegulonDB 8.5. We took 699 experimentally found promoters, 1880 non-promoters (regions that located more than 300 bp from TSS), and 3427 genes sequences. Additionally using PlatProm algorithm [4] designed for promoter prediction 2228 promoter islands and 2000 “antipromoters” (sequences with lowest score) were obtained. For all the sequences 200 nts intervals [–150; 50] in the vicinity TSS or pseudo-TSS were used; in case of EP the interval was [–180, 540] Å due to the algorithm properties. Principal components analysis (PCA) was performed allows to reduce the number of variables more than 10 times. For all 5 datasets (4 initial and reduced ones) unsupervised machine learning (Ward’s method clusterization) was carried out. Later in the work binary classifier for ‘Promoters’ – ‘Sequences of other type’ pairs were trained; two methods (Naive Bayes and Random Forest) were used. Models for 50, 100, 150 principal components cases and training proportions 0.7, 0.8, and 0.9 were obtained with 10-fold repetition.

Results: As a result of clusterization certain groups of promoters have fallen together into same clusters according to different partitions. GeneOnthology enrichment analysis has shown presence of clusters with significant enrichment in promoters corresponding

to genes with certain functions. The clusterization has shown that using reduced (after PCA) data set produces better result. This implies that supervised machine learning (classifiers training) is applicable as well. Indeed, binary classifiers were shown to perform well. Overall accuracy rates of Random Forest models was higher than Naive Bayes rates. Since promoter islands are predicted by analyzing DNA primary structure only, it is worth noticing that promoters were better distinguished from promoter islands in comparison to other sequence types.

Conclusion: Machine learning techniques (both unsupervised and supervised) were used successfully to distinguish promoter sequences based on a variety of their physical properties. Such an approach takes into consideration multi-step process of transcription initiation as well as promoters variability. It could also be combined with traditional sequence-based algorithms. In the work surprisingly high performance for 'promoters' – 'promoters islands' subset was obtained, which highlighted that the two types that have high textual similarity differ significantly by physical properties profiles.

Acknowledgements: The work was supported by RFBR grant №16-37-00303 mol_a.

References

1. Wang H.Q., Benham C.J. Promoter prediction and annotation of microbial genomes based on DNA sequence and structural responses to superhelical stress. BMC Bioinformatics. 2006;7:248-262.
2. Polozov R.V., Dzhelyadin T.R, Sorokin A.A., Ivanova N.N., Sivozhelezov V.S., Kamzolova S.G. Electrostatic potentials of DNA. Comparative analysis of promoter and nonpromoter nucleotide sequences. J. Biomol. Struct. Dyn. 1999;16:1135-1143.
3. Grinevich A.A., Ryasik A.A., Yakushevich L.V. Trajectories of DNA bubbles. Chaos, Solitons & Fractals. 2015;75:62.
4. Shavkunov K.S. et al. Gains and unexpected lessons from genome-scale promoter mapping. Nucleic acids research. 2009;37(15):4919-4931.

ZP-DOMAIN PROTEINS IN THE *AURELIA AURITA* JELLYFISH TRANSCRIPTOME

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Key words: *Aurelia aurita, mesoglein, ZP-domain, proteins, zona pellucida*

Motivation and Aim: A protein mesoglein was described in the mesoglea of Scyphomedusa *Aurelia aurita.* After definition of its amino acid sequence, it became clear that this protein relates to ZP-domain protein superfamily [1]. Many of the members of this superfamily play important role in oogenesis processes, fertilization and prenatal development of invertebrate and vertebrate animals [2]. Also, two major proteins have been found in female *A. aurita* gonads before. Its molecular weights are 180 and 210 kDa and these proteins are also thought to contain ZP-domains [3]. Until this moment, no ZP-proteins, which can participate in these processes, were described within Cnidaria. This is why finding ZP-domain-carrying transcripts, which encode high-molecular proteins of the *zona pellucida* inside the *Aurelia aurita* jellyfish, became the main aim of this research (transcriptome ID: 252562).

Methods and Algorithms: A database PRJNA252562 (ID: 252562) was used for this research. It is a transcriptome of different life stages of *Aurelia aurita* (Red sea population) [4]. Several programs were used in this work: BLAST, NCBI Conserved Domain Search online service, SMART, Molbiol, PI/Mw, ProtParam, NetOglyc, NetNglyc, Primer-BLAST, IBS.

Results: After alignment of nucleic acid sequence of ZP-domain in mesoglein on the total *A. aurita* trancriptome using tblastx, 80 sequences were found. These sequences contained areas similar to primary structure of ZP-domain in mesoglein. After several steps, this number was reduced to 17. In these 17 sequences there were other domains also found besides ZP: in 2 transcripts – DSL domain, in 2 – FGB, in 1 – CLECT, in 1 – MNNL, there are 2 EGF_CA domains in transcript comp188549, 3 EGF domains in comp191187, 2 ZP-domains in comp197876, there is a transmembrane region in 10 transcripts out of 17 and 9 transcripts with signal peptides in the sequence. From 17 investigated sequences, only one transcript has the highest similarity to mesoglein ZP-domain. It is the comp186661 transcript. After the alignment of amino acid on the investigated and described *A. aurita* mesoglein sequence, it became clear that they match, except for a few amino acid replacements. Besides that, the isoelectric point in the investigated (8,72) and described (8,75) transcripts, as well as molecular mass (46646,06 Da in investigated and 46889,33 Da in described mesoglein), are almost the same. The distinctive feature of comp186661 from the described mesoglein is that the new one contains a transmembrane domain on the C-terminus of the sequence. The presence of this domain is typical for the majority of excreted ZP-proteins among vertebrates and invertebrates [2]. One of the major tasks in this research was looking for potential candidates for the roles of novel peptides with molecular weight of 180 and 210 kDa, which were described previously in the group of noncoding DNA in Institute of Cytology RAS. After analysing *A. aurelia* transcriptome, such proteins have not been found. Probably, these proteins are present in

a dimer form. Transcripts, pretending to be monomers in dimer proteins, were found for the protein with mw of 180 kDa (transcripts comp199379, comp200405, comp190987 (88210.38, 86750.32, 91169.22 Da, respectively)) and 210 kDa (transcript comp197876 with molecular weight of 102222.18 Da). Each of these transcripts contain one ZP-domain in its sequence, and transcript comp197876 contains two ZP-domains. Besides that, each of these 3 transcripts contain signal peptide of the N-terminus of the protein, which is typical for extracellular ZP-domain proteins [5]. Histochemical methods show high levels of glycosylation within protein components of *zona pellucida* of *A. aurita* [3]. This is also typical for many of ZP-protein family [6]. For testing potential sites of N- and O-glycosylation within investigated hypothetical proteins, amino acid sequences, received from transcripts comp199379, comp200405, comp190987 and comp197876, were analyzed with programs NetOglyc and NetNglyc. All hypothetical amino acid sequences possess high levels of glycosylation. To test the presence of these transcripts in female *A. aurita* reproductive gland's cDNA, it is necessary to set PCR analysis with gen specific primers. Using program Primer-BLAST, 4 pairs of gene specific primers were made for each transcript: comp199379, comp200405, comp190987, comp197876.

Thus, in this work, 4 prospective transcripts were selected from database PRJNA252562 (ID: 252562) of *Aurelia aurita,* Red Sea inhabitant. We suppose that these transcripts are localized in the *zona pellucida* of the jellyfish.

Conclusion:

1. 17 sequences, containing ZP-domain, were found in the transcriptome of Aurelia aurita;

2. Reading frame was defined for each of analyzed sequences, after analyzing domain architecture, such domains as DSL, transmembrane region, FBG, signal peptide, CLECT, MNNL, EGF_CA, EGF were found in these hypothetical amino acid sequences;

3. Molecular weight of these sequences ranges from 37 kDa to 102 kDa, isoelectric point – from 7, 84 to 9,65;

4. Potential candidates for the role of zona pellucida proteins with molecular weights of 180 kDa and 210 kDa are 4 sequences (comp200405, comp199379, comp197876, comp190987);

5. 16 pairs of gene specific primers are made for selected sequences.

References

1. Matveev I.V. et al. (2007) A novel *Aurelia aurita* protein mesoglein contains DSL and ZP domains. Gene. 399:20-25.
2. Jovine L. et al. (2005) Zona pellucida domain proteins. Annu. Rev. Biochem. 74:83-114.
3. Adonin L.S. et al. (2012) *Aurelia aurita* (Cnidaria) oocytes' contact plate structure and development. PLoS ONE, 7(11):e46542. doi:10.1371/journal.pone.0046542.
4. Brekhman V. et al. (2015) Transcriptome profiling of the dynamic life cycle of the scypohozoan jellyfish *Aurelia aurita*. BMC Genomics. 16(1):74.
5. Kapp K. et al. (2009) Post-Targeting Functions of Signal Peptides, In: Protein Transport Into the Endoplasmic Reticulum. R. Zimmermann (Ed.). 913-918 (Landes Bioscience).
6. Wassarman P.M. et al. (2001) A profile of fertilization in mammals. Nat. Cell. Biol. 3:59-64.

MEMBRANE POTENTIAL AS A NEW FACTOR, MODULATING PERIPLASMIC NITRITE REDUCTASE ACTIVITY

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Key words: *nitrite respiration, E.coli, membrane potential, mathematical model*

Motivation and Aim: Under anaerobic conditions the most energetically favorable for *E. coli* substrate is nitrate. Nitrite is the side-product of nitrate utilization that can be an electron acceptor by itself. In contrast to nitrate respiratory chain, composition of nitrite-associated one is not completely understood. During cultivation of cells on glucose, formate donate electrons and respiratory chain may be formed by FDH-N and FDH-O formate dehydrogenases. Although, these dehydrogenases are lowly expressed at stationary *E.coli* culture growth in the chemostat. FDH-H dehydrogenase shows the highest activity under these conditions [1], but in contrast to FDH-N and FDH-O dehydrogenases, not directly binds to menaquinones, that mediate electron transition. It was assumed [2] that, the membrane potential (MP) plays a critical role in periplasmic NrfA nitrite reductase activity regulation, the second component of respiratory chain, by activating subunits transport to the periplasm and formation of active enzyme. This fact rises the question on MP formation mechanisms during nitrite respiration. This investigation is dedicated to reconstruction of MP formation by FDH-H dehydrogenase and analysis of its contribution in NrfA activity regulation while cultivation of *E. coli* cells in the chemostat.

Methods and Algorithms: Generalized Hill functions [3] were used to describe gene expression mechanisms, involved in nitrite electron transport chain and nitrite metabolism (*fdh*, *nrf* and *nir*) in *E. coli* cells. Rate of enzyme reactions were described by Michaelis-Menten equations. Parameters of the model were evaluated from the published data or were estimated during model's adaptation to experimental data. STEP+ [4] was used for numerical calculations of the model.

Results: The model was created, describing hypothetic respiratory chain formation during anaerobic respiration of *E.coli* in the chemostat with formate hydrogenlyase complex (composed of FDH-H dehydrogenase and HYD-3 hydrogenase), one of two membrane hydrogenases (HYD) and NrfA reductase. Realization of this respiratory chain occur at nitrite level ~ 1mM, when level of *fdhF* gene, coding FDH-H dehydrogenase is maximal [1]. Hydrogen is an intermediate source of electrons, which is synthesized by HYD-3 hydrogenase, diffuses in the periplasm and serves as a substrate for HYD hydrogenase. In the model MP described as periplasmic to cytoplasmic proton concentration ratio. The model describes MP pattern in accordance to experimental data, and MP mechanism, incorporated in the model [2] of nitrite utilization by *E. coli* cells, allows adequately describe experimental nitrite accumulation dynamic in the chemostat. The results of the model do not exclude the possibility of MP formation via FDH-H and HYD-4 hydrogenase without involvement of other hydrogenases [5].

Conclusion: Suggested scheme of MP formation allows to simulate positive effect of membrane potential on NrfA reductase activity in the periplasm and does not contradict molecular-genetic nature of possible fomate hydrogenlyase-mediated MP formation mechanisms at low nitrite level in the medium.

References

1. Wang H. et al. (2003) Coordinate regulation of the Escherichia coli formate dehydrogenase fdnGHI and fdhF genes in response to nitrate, nitrite, and formate: roles for NarL and NarP. J. Bacteriol., 185: 5076-85.
2. Khlebodarova T.M. et al. (2016) On the control mechanisms of the nitrite level in Escherichia coli cells: the mathematical model, BMC Microbiol., 16: Suppl 1:7.
3. Likhoshvai V., Ratushny A. (2007) Generalized hill function method for modeling molecular processes, J. Bioinform. Comput. Biol. 5:521-531.
4. Fadeev S.I. et al. (2001) Software automated package STEP for numerical investigation of autonomous systems of general type. Mathematical modeling of the catalytic processes. Proceedings of the XV International Conference on Chemical Reactors. 71-74.
5. Bagramyan K. et al. (2002) The roles of hydrogenases 3 and 4, and the F0F1-ATPase, in H2 production by Escherichia coli at alkaline and acidic pH. FEBS Lett. 516:172-178.

MARKING OF MISCANTHUS C4-PHOTOSYNTHESIS, METHOD DEVELOPMENT

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Key words: *Miscanthus, C4 photosynthesis, pyruvate phosphate dikinase (PPDK), antibody, ELISA, isotope shift of ^{13}C*

Motivation and Aim: The prospect of using plants with rapid growth as cellulose sources is widely investigated all over the world. However, there have been few attempts to grow them in cold climates. The plants with high biomass yields in short vegetation period can allow to use huge areas of marginal lands ineffective for traditional agriculture. Miscanthus (mostly M. × giganteus) is widely used as a plant biomass source. However, most cultivars of miscanthus cannot be efficiently cultivated in cold climate [1, 2]. We developed the Soranovskii cultivar of miscanthus, with high cold tolerance and high biomass yield. It was added to the State register of selection achievements In 2013 (certificate of authorship no 58540). And now it is regarded as new cellulose source for Russia.

Miscanthus, as well as many other efficient agricultural cultures (corn, sugar cane), has the C4 photosynthesis pathway [3]. In C4 grasses, mesophyll cells fix atmospheric CO2 via cytosolic phosphoenolpyruvate carboxylase (PEPc; E.C.4.1.1.31) using PEP regenerated via chloroplastic pyruvate phosphate dikinase (PPDK; E.C.2.7.9.1). The C4 product is transported to bundle sheath cells, where CO2 is released by decarboxylation of this product and refixed via Rubisco (E.C.4.1.1.39). The process activity directly depends on the PPDK activity, whom regulation depends on illumination. The effective studding of proteins and metabolism need to have antibodies allowing quantitative analysis and visualization of protein positions in experiments. The main aim of the work was development of monoclonal antibodies to PPDK of miscanthus.

Methods and Algorithms: A known sequence of M. giganteus ppdk gene from GenBank (AAP34175) was used to obtain recombinant PPDK [4]. Since this gene is long, we selected the conserved PPDK_N domain (amino acid positions 106–425). This sequence was optimized for *Escherichia coli* expression and synthesized by ATG Service Gene (Saint-Petersburg, Russia). Monoclonal antibodies were obtained by us in collaboration with RusBioLink (Moscow, Russia). Miscanthus plants were grown in the ICiG SB RAS hydroponic hothouse at the average temperature of 20 °C. Eight circles (four from the 2nd leaf and four from the 3rd) were excised using a metal tube 8 mm in diameter, which yields 4 cm2 of leaf surface. Proteins were used for Western blot and ELISA.

Results: We cloned the gene fragment encoding PPDK [4], purified the resulting protein by affinity chromatography, identified it using MALDI mass spectrometry, and obtained monoclonal antibodies by immunizing BALB/c mice. Selectivity of monoclonal antibodies were assessed by Western blot using the protein extracts of

Miscanthus sp. Soranovskii. Protein bands from SDS-PAGE were excised, lyzed by trypsin, and analyzed by mass spectrometry. These protein bands were found to contain protein closest to PPDK (C4-specific pyruvate orthophosphate dikinase [Miscanthus × giganteus]). Therefore, we developed and tested the method for determining PPDK quantity in miscanthus using ELISA. In finally, the obtained monoclonal antibodies are highly specific for miscanthus PPDK.

We used specific monoclonal antibodies to recombinant PPDK to quantify PPDK content in photosynthesizing leaves of a collection of miscanthus specimens from the Far East and of the Soranovskii cultivar. PPDK content ranged from 0.318 (clones D36 from the Far East) to 0.534 μg cm^2 (Soranovskii cultivar). We confirmed that in the studied varieties photosynthesis goes through 4C by determining the isotope shift of ^{13}C in biomass.

Conclusion: The report represents a reliable confirmation that the miscanthus actually has C4 photosynthesis. It becomes interesting to study as it is capable of vegetation at low temperatures. This is not typical for plants with C4 photosynthesis, and it is important for cultivation in countries with a cold climate. The data obtained allow to improve the characteristics of important agricultural plant in future.

References

1. Farrell A.D., Clifton-Brown J.C., Lewandowski I., Jones M.B. (2006) Genotypic variation in cold tolerance influences the yield of Miscanthus. Annals of Applied Biology. 149:337-345.
2. Anderson E., Arundale R., Maughan M., Oladeinde A., Wycislo A., Voigt T. (2011) Growth and agronomy of Miscanthus × giganteus for biomass production. Biofuels. 2:167-183.
3. Ehleringer J.R., Cerling T.E., Helliker B.R. (1997) C4 photosynthesis, atmospheric CO2, and climate. Oecologia. 112:285-299.
4. Naidu S.L., Moose SP., Al-Shoaibi A.K, Raines C.A., Long S.P. (2003) Cold tolerance of C4 photosynthesis in Miscanthus × giganteus: adaptation in amounts and sequence of C4 photosynthetic enzymes. Plant Physiology. 132:1688-1697.

EVALUATION OF THE RATE OF CHANGE OF THE STRUCTURAL GENES RESPONSIBLE FOR BIOSYNTHESIS AND MODIFICATION OF LIPIDS BASING ON ANALYSIS OF THE FULL GENOMES PROVIDED BY THE NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION

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Key words: *genome, genes classification, genes responsible for biosynthesis and modification of lipids, I and M groups of genes*

Motivation: Interpreting natural phenomena / biological processes in living organisms through their genome composition is of particular scientific interest today. Studies of the genome have been going on for quite some time and will continue to be relevant for a long period of time in future. However, their directions, objectives and so on are going to change: the decoding of genomes becomes less popular. The next step in research that is now gaining recognition is making conclusions from the genome sequencing data and its comparison.

The working hypothesis of the study is based on certain features of the processes running inside living organisms: taking into account the diversity of lipids which is deemed to us relatively unlimited, we can assume that the number of genes is directly proportional to the evolutionary advancement of the species. It is also assumed that the growth rate of the number of the "lipid group" genes is stepwise, which corresponds to the uneven rate of evolution that is observed in all sorts of excavated organisms from bacteria to animals.

The theoretical and methodological basis of the study are articles and data provided by the National Center for Biotechnological Information: NCBI. Instrumental and methodological framework of the research is a set of scientific methods: categorical, structural and comparative analysis, graphical and tabular methods of data interpretation.

To solve the objectives, the following algorithm of actions was used: search of the necessary data, their subsequent processing (writing a program, if necessary), drawing up tableson the basis of the processed information, and further presentation of the tabular data in the form of a graph.

Making the analysis that is structured similarly to the described above algorithm, we have come to certain conclusions. First, the structural genes of the "protein groups" are demonstrate higher growth. Secondly, the number of genes of the "lipid group" consistently increase with the advancement of the organism in the evolutionary hierarchy. However, we cannot unequivocally confirm (refute) the working hypothesis, since the line corresponding to the change in the number of genes in accordance with the evolutionary advancement of the species has some kind of heterogeneity: jumps. Thirdly, we have observed that the lines corresponding to the change in the number of the genes of the groups responsible for various metabolic processes are similar in structure.

NUMERICAL ANALYSIS OF THE DIAGNOSTIC PROPERTIES OF TUMOR MARKERS

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Key words: *tumor marker*, *p53, miRNA, delay differential equations, numerical simulation*

Motivation and Aim: One of the priorities of modern biomedical research is the search for effective biomarkers for early cancer detection and other serious diseases related with dysfunction of processes of cell death. The p53 protein (tumor necrosis factor), involved in many life and death processes, including the formation of tumors and aging, is expressed in all the cells of the organism. Mdm2 protein is considered to be the key negative p53 regulator [1–3]. It is known that p53 regulates the class of microRNAs, which are characterized as the most important intermediates of p53 in tumor control [4]. Thus, the investigation of the function of the p53 protein and updating of the diagnostic properties of microRNA is paramount both for developing new approaches to cancer treatment and determining the prevention strategy for many diseases, including measures to slow the aging processes.

Methods and Algorithms: We consider in this work two mathematical models of the dynamics of the tumor markers network p53–Mdm2–microRNA for microRNA class with a direct positive connection with p53. These models include the systems of three nonlinear equations with the two retarded arguments. The time delay parameters determine in the Mdm2 and microRNA reactions to a change in the state of the p53 protein. The solution of system equations is based on the widely known method of steps. From considerations of the simplicity of the numerical implementation, in all the calculations the delay values were taken as a multiple of the step of the computational grid (on condition that the grid step is a rather small value, this limitation avoids having a substantial effect on the character of the solution). At each time step, the problem's solution is found by numerical methods for solution of Cauchy problem. In addition we implement a limiting passage from delay equations to two related ODE systems of large dimension. We show numerically that in the passage to the limit in which the ODE systems has infinitely many equations we obtain model based equation with retarded argument [5].

We also constructed a sufficiently simple mathematical model of p53–Mdm2–microRNA network, in which the microRNA is a negative regulator of Mdm2. This type of microRNA is capable of disrupting the mechanisms of negative feedback and stimulating the activation of tumor markers.

Results: All obtained numerical solutions have a sufficiently clear biomedical meaning. The adopted mathematical model describes the functioning of the p53–Mdm2 network in normal conditions and predicts possible dangerous situations for the organism. Stress situations associated with the emergence of an imbalance in the rates of p53 and Mdm2 generation and degradation and also with disturbances in the mechanism of in implementation interaction of proteins regulated within the considered models

through the constants of dissociation have been studied. Numerical investigation of the microRNAs functioning in conditions of the deregulation of p53 and p53–Mdm2-network is carried out. The deregulation of microRNA in detail is studied. The situations in which p53, its inhibitor Mdm2 and microRNAs exhibit critical properties for the patient's status and can be identified as diagnostic markers of cancer and neurodegenerative disease are studied. The results of numerical analysis are in good agreement with the data of clinical and laboratory studies of known microRNAs.

Conclusion: According to the results of these investigations, p53-responsive microRNA of given class can be used to clarify function of p53 as a biomarker of cancer and neurodegenerative diseases [6]. If the network p53–microRNA functions normally, then microRNA duplicates diagnostic properties of p53. The variants of microRNAs deregulation are found, where microRNAs are an even better marker for disease than p53. There is the most probable situations in which extreme levels of miRNAs are not consistent with the state of p53 once deregulation of microRNA. This indicates a possible independent role of miRNAs as factors in diagnostics, prognosis or therapeutic. Therefore clarification of the native functions of miRNAs is required.

References

1. Lane D., Levine A. (2010) p53 research: The past thirty years and the next thirty years. Cold Spring Harb. Perspect. Biol. 2:a000893.
2. Loewer A., Batchelor E., Gaglia G., Lahav G. (2010). Basal dynamics of p53 reveals transcriptionally attenuated pulses in cycling cells. Cell. 142(1):89-100.
3. Batchelor E., Loewer A., Mock C., Lahav G. (2011). Stimulus-dependent dynamics of p53 in single cells. Molecular Systems Biology. 7(488):8.
4. Hermeking H. (2012). MicroRNAs in the p53 network: micromanagement of tumor suppression, Nature reviews cancer. 12(9):613-626. doi: 10.1038/nrc3318.
5. Voropaeva O.F., Kozlova A.O., Senotrusova S.D. (2016) Passage from delay equation to ODE system in the mathematical model of the tumor markers network. Computational Technologies. 21(2):12-25.
6. Voropaeva O.F., Senotrusova S.D., Shokin Yu.I. (2017). Deregulation of p53-dependent microRNAs: the results of mathematical modeling. Mathematical Biology and Bioinformatics. 12(1).

IDENTIFICATION AND ANALYSIS OF THE *MYC* GENE FAMILY IN TRITICEAE

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Key words: *bHLH, flavonoid biosynthesis, gene divergence, gene duplication, Hordeum, MYC, transcription factor, Triticum*

Motivation and Aim: MYC transcription factors with the MYB and WD40 proteins are forming the MBW regulatory complex, which is necessary for activation of the structural flavonoid biosynthesis genes expression. There are *MYC*-encoding genes in wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) genomes, which are involved in synthesis of the flavonoid pigments – *TaMyc1* and *HvAnt2*, respectively. The aim of this research was the identification, comparison and analysis of full-length sequences of duplicated copies of these genes.

Methods and Algorithms: The search of homologous sequences was made in databases for not annotated wheat and barley sequences using BLAST. Cluster analysis using MEGA software was based on the UPGMA algorithm. Promoters of the genes were analyzed with PLACE and PlantCARE. Primers design for PCR, RT-PCR, qRT-PCR and sequencing was performed using OLIGO. Analysis of methylation patterns of nucleotide sequences has been done using EpiTect Bisulfite Kit (QIAGEN) and further sequencing of the amplified products. Genetic mapping of the *HvAnt2copy*-specific CAPS marker was performed in DOMxREC mapping population using MAPMAKER program.

Results: Eleven *MYC* genes were identified in the second and fourth homeological groups of wheat chromosomes and compared with 22 homologues present in genomes of wheat progenitors (also identified in this study). For barley, two *MYC* genes were found in chromosomes 2H and 4H. The latter was mapped precisely (tightly linked to marker *XBmac186-4H*). Exon-intron organization of all identified genes is similar to the structure of *TaMyc1* and *HvAnt2*. The transcription activity of the detected genes in various parts of plant varied among the copies.

Conclusion: Two *MYC* genes in the barley genome and eleven *MYC* genes in the wheat genome are present. Analysis of genetic similarity has shown that the first duplication of the *MYC* gene was in the common diploid ancestor of the Triticeae tribe. We have discovered that the duplications of these genes occurred several times in the course of evolution of diploid wheat progenitors. In general, the obtained results are showing us that the duplicated genes maintaining is caused by their functional specialization.

Acknowledgements: This study was partially supported by the RAS MCB Programme (0324-2016-0026) and by RFBR (16-04-00912).

DIVERSITY AND EVOLUTION OF TC1/MARINER DNA TRANSPOSONS IN ORTHOPTERA SPECIES

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Key words: *Tc1/mariner, transposons, Orthoptera, Insecta, HMMER search, evolution*

Motivation and Aim: Tc1/mariner elements are the mobile genetic elements of class of DNA transposons. These elements are wide spread in insects and significantly affect the size, function and evolution of the host genome. Studying of the Tc1/mariner elements is an important part of genome survey and giving additional information about their structure and evolution, also providing insights about interactions between genomes of reproductively isolated species.

Insects of the Orthoptera order are the important part of natural ecosystems, including several dangerous pests of agricultural crops, such as *Locusta migratoria* and *Schistocerca gregaria*. Orthoptera species are interesting organisms to study the diversity and evolution of Tc1/mariner elements. For example, the genome of *L. migratoria* is the largest among sequenced animal genomes for today. Genomic studies revealed that the distribution of mobile elements is the major cause of such a large genome size in locusts. Thus, approximately 60% of *L. migratoria* genome consists of repetitive elements, about 24% of which are DNA transposons. Despite this fact, DNA transposons are poorly investigated for *L. migratoria*. For *S. gregaria* EST database is available, but information about Tc1/mariner elements in there is very limited.

Methods and Algorithms: To search and analyze the distribution of Tc1/Mariner elements in genomes of *L. migratoria* and *S. gregaria* the specific algorithm was developed. The algorithm include: 1) initial search for Tc1/Mariner transposase sequences using specific HMM profile [1] in the HMMER3 suite; 2) identification of the sequences of terminal inverted repeats near the HMM signals using the custom Python script designed by authors; 3) selection of the Tc1/Mariner transposon sequences with the complete/(potentially intact) transposase open reading frame; 4) preliminary classification (grouping) of identified Tc1/Mariner transposons into subfamilies of Tc1/Mariners (mellifera, cecropia, etc.) using custom HMM profiles specific to their transposase sequences with hmmerscan tool from the HMMER3 suite; 5) rough clusterization within split subfamilies to identify typical representatives using CD-HIT suite.

In order to add more species into analysis, experimental search was performed for Tc1/mariner transposons in genomes of 10 Orthoptera species using standard molecular genetic methods (DNA extraction, PCR, cloning and sequencing).

Phylogenetic analysis of combined data was performed using maximum likelihood method in PhyML v 3.0.

Results: In the present study, we analyzed complete genome of *L. migratoria* for the presence of Tc1/mariner transposons, using HMM method. 27139 sequences of Tc1/mariner transposase domain were found and phylogenetic analysis was performed in

order to establish their belonging to different families of Tc1/mariner superfamily. These sequences divided into 6 known families of Tc1/mariner subfamily (Gambol, Ludens, Tc1, Pogo, mariner and Mori). Sequences from mariner family divided into 7 known subfamilies (Capitata, Cecropia, Irritans, Mariner, Mauritana, Mellifera and Vertumana). Mention should be made of the fact that 5 new possible subfamilies of the mariner family were established during this study.

We conducted an analysis of the genome of *S. gregaria* from EST database [2] for the presence of Tc1/mariner transposons, using same algorithm we used for *L. migratoria*. We found 30 sequences of Tc1/mariner transposase, following phylogenetic analysis divide them into 4 families of Tc1/mariner superfamily (mariner, Pogo, Tc1 and Ludens).

To analyze the presence of Tc1/mariner elements in wider range of Orthoptera species, we obtained experimentally 98 sequences from 10 different Orthoptera species.

Conclusion: We performed the phylogenetic analysis of the complete genomes of *L. migratoria* and *S. gregaria* and 98 sequences from 10 Orthoptera species. 5 new possible subfamilies of mariner family were found.

Availability: Sequences of Tc1/mariner's transposases obtained experimentally were deposited to GenBank.

Acknowledgements: The project was supported by the Russian Foundation for Basic Research (Grant No. 16-34-00893).

References

1. Eddy S.R. (1998) Profile hidden Markov models. Bioinformatics (Oxford, England). 14:755-763.
2. Badisco L., Huybrechts J., Simonet G., Verlinden H., Marchal E., Huybrechts R., Schoofs L., De Loof A., Vanden Broeck J. (2011) Transcriptome analysis of the desert locust central nervous system: production and annotation of a *Schistocerca gregaria* EST database. PLoS One. 6:72-74.

METABOLIC AND TRANSCRIPTIONAL CHANGES IN GRAPE VARIETIES WITH CONTRASTING SUSCEPTIBILITY DURING INFECTION OF DOWNY MILDEW

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Key words: *grapes, downy mildew, contrasting susceptibility, metabolites, gene expression*

Motivation and Aim: One of the ways to intensify viticulture is the use of agrotechnologies, based on the analysis and application of biological properties of plant genotypes, such as their natural resistance. Diseases caused by fungal phytopathogens cause significant damage to viticulture. There are no cultivars of grapes belonging to the species Vitis vinifera L. or to its interspecific hybrids immune to common fungal diseases [1]. The identification of the essence of the prevailing physiological patterns of the formation of plant resistance to stressors is the main step in the management of quantitative and qualitative indicators of grapes production. The aim of the study was to identify the specific features of the immune response of grape plants with different susceptibility to fungal pathogens during infection with downy mildew.

Methods and Algorithms: The objects of the study were plants of two varieties of grapes: Vostorg and Muskat belyy, contrasting in resistance to mildew (*Plasmopara viticola* Berl. et Toni). Analysis of the content of phenol carboxylic acids was carried out by capillary electrophoresis on a Kapel 105 [2]. RT-PCR was performed on a Bio-Rad CFX C1000, the results were processed using the BioRad CFX Manager software. The calculation of the relative expression of genes was carried out by the method of Livak. Reference genes are actin and tubulin. Proteins were extracted from the grated vine leaves in liquid nitrogen [3]. The peroxidase activity was determined by the method of A.N. Boyarkin. Visualization isoforms antioxidant enzymes was performed as previously described methods [4]. The level of lipid peroxidation was estimated from the change in the optical density of the solution during the reaction of malonic dialdehyde (MDA) with thiobarbituric acid (TBA) [5].

Results: MDA formation serves as a marker of oxidative stress. Both studied varieties show a decrease in the level of MDA relative to the control plants 48 hours post-infection (hpi), followed by a pronounced increase. Infected plants of the Vostorg variety are generally characterized by a lower MDA content in the leaves than the Muscat belyy variety. The MDA content in leaves of Vostorg variety remains about the same level after 72 hpi, and at the leaves of Muscat belyy variety it increases during the analyzed period. The activity of peroxidase significantly changed during the experiment. The isozyme spectrum of peroxidase changes in the resistant Vostorg variety at 72 hpi, in the unstable Muscat belyy variety there are no changes. Phenolic components play an important role in resistance against phytopathogens, which is associated with the antifungal activity of products of their oxidation with peroxidases [6]. The greatest differences between varieties appear in the content of coumaric, benzoic, caffeic and chlorogenic acids. In general, in the leaves of the resistant Vostorg variety contained less of phenolic compounds, than grade in leaves of Muscat belyy variety. This may

indicate a nonspecific adaptive response of this variety to the effect of the pathogen due to the accumulation of a large number of phenolic compounds, but not their further transformation with low peroxidase activity. We analyzed the expression of various genes associated with the reaction of plants to the effect of the pathogen. In a stable Vostorg variety expression of resveratrol synthase and PR10 genes is at a high level already 4 hpi. The lipoxygenase gene is expressed after 8 and 96 hpi in the Vostorg variety. A slight increase in expression of the phenylalanine ammonia lyase gene is observed 4 hpi, then this gene is not expressed. In the Muscat belyy variety, the expression of these genes is at a relatively lower level than in the Vostorg variety, characterized by a peak expression of lipoxygenase and phenylalanine ammonia lyase at 96 hpi. This confirms that the sensitive variety reacts later to the effect of the pathogen.

Conclusion: In both grape varieties, the main stress reactions occur in the first 48 hours after infection. Various links of the shikimat pathway are activated at the initial stages of the formation of a stress response to biotic influences in the grape varieties differ in susceptibility. Susceptible genotypes react to the stress-factor effect by a significant increase in the content of phenolic compounds. In contrast, in the tissues of resistant genotypes, the consistency of the processes of the synthesis of phenolic compounds and their rapid metabolization due to the rapid activation of regulatory processes and the rapid formation of substances that effectively resist the pathogen play a main role. The susceptible variety later reacts to the effect of the pathogen, the reactions are less expressed and more nonspecific.

Acknowledgements: The study was carried out with the support of the Russian Foundation for Basic Research, Grant No. 16-34-60-154.

References

1. Boso S., Kassemeyer H.H. Different susceptibility of European grapevine cultivars for downy mildew. 2008;47(1):39-49.
2. Zakharova M.V., Ilina I.A., Lifar G.V. Method for determination of mass concentration of ascorbic, chlorogenic and caffeic acids. Methodical and analytical support of research on horticulture. Krasnodar: NCZRIHV. 2010;279-283.
3. Perez F.J., Lira W. Possible role of catalase in post-dormancy bud break in grapevines. J. Plant Physiol. 2005;162:301-308.
4. Sundyreva M.A., Savchenko T.V. Methods of extraction and analysis of antioxidant enzymes of vegetative organs of grapes. Modern instrumental-analytical methods of research of fruit crops and grapes. Krasnodar, 2015;(19-21):115.
5. Molecular-genetic and biochemical methods in modern plant biology / Ed. Vl.V. Kuznetsov, V.V. Kuznetsov, G.A. Romanov. Moscow: Binom. Laboratory of Knowledge. 2012;487.
6. Latouche G., Bellow S., Poutaraud A., Meyer S., Cerovic Z. Influence of constitutive phenolic com-pounds on the response grapevine (*Vitis vinifera* L.) leaves to infection by Plasmopara viticola. Planta. 2013;237:351-361.

GENETIC RELATIONSHIP OF CRIMEAN INDIGENOUS "SINAP" APPLE CULTIVARS AND CULTIVARS FROM WORLD APPLE GERMPLASM AS REVEALED BY SSR MARKERS

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Key words: *Malus, apple, SSR-analysis, indigenous cultivars*

Apple (Malus × domestica Borkh.) is one of the most important fruit crops worldwide. Cultivation of the apple has a long history and always was connected with development of civilization. Budding technology allowed propagation of apple trees with valuable traits after their selection among wild growing populations of Malus species (e.g. *M. sylvestris*, *M. orientalis* and *M. siversii*). It could be also not only exactly species-related apple trees, but also spontaneous interspecific hybrids. Many autochthonous cultivars were created in different regions of the world on the basis of such selection from local native apple trees. Indigenous cultivars are the valuable source for apple breeding due to high adaptation to stress-factors with respect to local environment conditions. So conservation and complex study of such cultivars are very important. One of the main scientific questions for genetic characterization of indigenous apple germplasm is the study of their genetic diversity and DNA-fingerprinting of germplasm accessions. SSR-analysis is effective method for such tasks in apple genetics [1, 2].

The Crimea has a reach history of apple cultivation which is determined by the history of local nations [1]. Most of Crimean local apple cultivars originated due to Crimean Tatars apple cultivation activity and named with a Crimean Tatars words. One of the most famous Crimean indigenous apple cultivars are Sinap cultivars which were very distributed in the apple orchards in Crimea [1, 3]. As the Sinap cultivars are most famous indigenous apple cultivar and may present unique apple germplasm in relation to world apple genetic resources we conducted study to estimate genetic relatedness between Sinap apple cultivars and word wide distributed apple cultivars from different regions of the apple cultivation by SSR-loci analysis.

Material and Methods: Six different Sinap cultivars were studied – five indigenous Crimean: Sary Sinap, Sinap Sudakskij, Sinap Belyj, Kara Sinap, Kandil' Sinap and one additional Sinap Alma-Atinskij originated from Kazakhstan. Set of worldwide distributed cultivars included the following cultivars: McFree, Liberty, Florina, Idared, Gala, Red Delicious, Prima and Fuji. Additionally Favorit cultivars from Crimean modern breeding was used due to its parental cultivars are the foreign cultivars Trident and RedFree. DNA was extracted from leaf tissue using CTAB protocol. Seven SSR-markers were used for cultivars genotyping: Hi02C07, GD147, CH02c11, CH04c07, CH01d03, CH-Vf, CH02c02a. PCR reaction was carried with standard protocols (ссылка на испанцев) with some modifications. Detection of PCR fragment size was performed using an ABI Prism3130 genetic analyzer. The cluster analysis was performed by the UPGMA method with the use of PAST soft wear.

Results: SSR-markers used in the study showed from 5 to 14 alleles per locus: Hi02C07-5, GD147-10, CH02c11-10, CH04c07-13, CH01d03-9, CH-Vf-7, CH02c02a-14. Analysis of allele's distribution among cultivars revealed some alleles specific for Sinap cultivar group. Allele with fragment size 118 base pairs (bp) for Hi02C07 marker was identified only in Sinap cultivars, allele 161 bp for CH-Vf marker and 135 bp for CH01d03 marker was identified in all Sinap except Kara Sinap and was not presented in any cultivar from studied worldwide apple cultivars set. Cluster UPGMA analysis determined three clusters with following cultivars:

Cluster 1: includes all Sinap cultivars except Kara Sinap;

Cluster 2: Florina, Gala, Red Delicious, Fuji and Kara Sinap;

Cluster 3: McFree, Liberty, Idared, Prima and Favorit.

Such distribution of Sinap cultivars into separate cluster confirms their genetic distantness from worldwide apple cultivars. Otherwise it can be assumed that pedigree of Kara Sinap cultivar, which incorporated into same cluster with Florina, Gala, Red Delicious and Fuji, includes some parental form which is distant from Crimean indigenous apple germplasm and closer to European apple cultivars.

Conclusion: SSR-marker based analysis revealed genetic distantness of most Crimean indigenous cultivars Sinap from cultivars which present worldwide apple germplasm.

References

1. Gharghani A. et al. (2009) Genetic identity and relationships of Iranian apple (Malus domestica Borkh.) cultivars and landraces, wild Malus species and representative old apple cultivars based on simple sequence repeat (SSR) marker analysis. Genet. Resour. Crop. Evol. 56:829-842.
2. Urrestarazu J. et al. (2012) Genetic diversity and structure of local apple cultivars from Northeastern Spain assessed by microsatellite markers. Tree Genetics Genomes. 8:1163-1180.
3. Simirenko L.P. (1996) Mestnye starodavnie sorta plodovykh kul'tur Kryma, Simferopol': Tavriya, 240 p.
4. Simirenko L.P. (1912) Krymskoe promyshlennoe sadovodsto. M., 746 p.

COMPUTER ANALYSIS OF 3D CHROMOSOME CONTACTS

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Key words: *bioinformatics, 3D genome structure, software tools, gene expression regulation, Hi-C, ChIA-PET, review*

Motivation and Aim: Transcription regulation in eukaryotes is a complex process, in which chromatin interactions play a critical role for gene expression regulation as well as to further influence other cellular activities. Series of post-genome technologies have been developed to study the binding of transcription factors for transcription regulation, such as chromatin immunoprecipitation (ChIP) arrays, ChIP-chip, ChIP-Seq [1]. Another challenge is to define whether such binding sites distal from gene regions are functional, i.e. physically contact target gene promoters via chromosome loops or attracting RNA polymerase II complex for gene transcription. Identification of genome-wide distal chromatin interactions provides novel insights into the study of transcription regulation. Chromatin Interaction Analysis with Paired-End-Tag sequencing (ChIA-PET) method for such analysis requires development of specialized software [2]. New software tools for genome 3D structure analysis and gene expression regulation have to be systematically compared by availability, effectiveness and range of sequencing data analysis problems

Methods and Algorithms: The aim of the work was to review existing computer tools for 3D genome structure data analysis and spatial topological domains [3]. Such data have been obtained experimentally by using methods ChIP-seq, Hi-C, ChIA-PET. Gene annotation was obtained from UCSC Genome Browser.

Results: We present review on 3D genome structure and transcription factor binding sites analysis tools. A test example consider analysis of distribution of CTCF binding sites and randomly generated list of such sites in the human genome by topological domains.

Conclusion: With the rapidly increasing resolution of Hi-C datasets, the size of the chromatin contact map will soon exceed the memory capacity of general computers. The same problem related to ChIA-PET and subsequent data integration to be solved by software development.

Acknowledgements: The research has been supported by RFBR and ICG SB RAS budget project (0324-2016-0008). Computing was done at Siberian Supercomputer center SB RAS (SSCC).

References

1. Li G. et al. Chromatin Interaction Analysis with Paired-End Tag (ChIA-PET) sequencing technology and application. BMC Genomics. 2014;15(12):S11.
2. Fullwood M.J. et al. An oestrogen-receptor-alpha-bound human chromatin interactome, Nature. 2009;462(7269):58-64.
3. Li G. et al. (2012) Extensive promoter-centered chromatin interactions provide a topological basis for transcription regulation. Cell.148(1-2):84-98.

POLYMORPHISM OF THE WILD CAUCASIAN PEAR POPULATION IN THE NORTHWEST CAUCASUS REGION

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Key words: *Pyrus caucasica, SSR, genetic resources, biodiversity, fragment analysis, DNA-markers*

Motivation and Aim: the Caucasus region is the center of the diversity of the wild pear – *P. communis* – the Caucasus mountains provide a variety of habitats that support a wide polymorphism of the geneplasm. The modern classification considers caucasian pear as a subspecies within the species *Pyrus communis* L. – *Pyrus communis* L. subsp. *caucasica*, nevertheless the species name of the caucasian pear *Pyrus caucasica* Fed is still common [1]. Taxonomic studies of the genus *Pyrus* members show a close relationship of the phenotype with the region of origin, and morphological and chemical analysis supports the concept of pear separation into Western European and East Asian groups. But these methods are insufficient for an unambiguous division into subspecies of *Pyrus communis*, most closely associated with the cultured pears [2]. The use of DNA marking methods allows the most accurate and reliable assessment of the genetic diversity of the geneplasm being studied and is often used in phylogenetic studies. A study of the biodiversity and genetic structure of the wild pear population will clarify the issues of the systematics and the origin of pear varieties. The aim of the work is to study the genetic structure of the wild population of the Caucasian pear within the North-Western Caucasus.

Methods and Algorithms: During the study, 4 expedition trips were made within the Krasnodar Territory to select samples from the wild populations of the Caucasian pear. Expedition routes for the selection of plant material were carried out with a uniform distribution of location from north to south and from east to west within the range of pear distribution. 1st selection point – Seversky district; 2 – Abinsk district; 3 – Gostagayevskaya village; 4, 5 and 6-th selection point – Absheron district. The DNA was isolated using the CTAB protocol. In the work 20 microsatellite DNA markers (SSR-markers) were involved, which are distributed into 6 multiplexed sets (see table). Fragment analysis was carried out on the genetic analyzer ABIprism 3130, the data was processed in the GeneMapper 4.1 program.

Results: At this stage of the study, DNA samples were obtained for 127 samples of caucasian pear from six selection sites. Based on the results of analysis of 57 samples representing the selection points in the Seversky, Abinsk and Absheron districts, the level of polymorphism from 7 to 20 alleles per locus was revealed. The most polymorphic was the marker EMPc115 – at this locus were detected 20 alleles, the size of 167 to 254 base pairs (bp). Slightly less polymorphic, revealing 19 alleles was the locus EMPc108. The EMPc117 locus also showed a high level of polymorphism, revealing 17 alleles in the sample studied. Also, markers CH01f07a and CH01d09 were detected on 17 alleles. It should be noted that the first three markers - EMPc115, EMPc108 and EMPc117 – were specially developed for the genotyping of *Pyrus* genus, while CH01f07a and CH01d09

are for genotyping *Malus*. This confirms the high transferability of apple markers on the gene of the *Pyrus* genus. Seven markers revealed from 10 to 14 alleles per locus (NH004a, CH03g07, GD96, CH03d12, EMPc11, CH01d08, CH01h01), and the markers NH015a and CH04e03 had 8 and 7 alleles, respectively (see table). The maximum number of unique alleles per locus – 9 – was identified by the EMPc108 marker. Four markers revealed three unique alleles, 3 markers – 5, two markers for 2 and 6 unique alleles, and one marker identified 4 and 1 unique alleles.

Used microsatellite markers

Locus	Dye	N. of alleles	Size, bp	Locus	Dye	N. of alleles	Size, bp
NH004a	FAM	10	76-96	CH01d08	FAM	14	241-302
NH015a	R6G	8	101-125	CH02b10	TAMRA	9	121-148
GD96	TAMRA	11	146-180	Nh011b	ROX	8	162-186
EMPc108	R6G	19	80-133	Ch05c06	FAM	10	80-109
EMPc117	TAMRA	17	96-128	GD142	ROX	8	151-171
EMPc115	ROX	20	167-254	BGT23b	TAMRA	8	179-241
CH04e03	TAMRA	7	183, 209	CH05e03	ROX	7	159-212
CH01h01	R6G	12	153-174	CH03d12	R6G	13	105-136
CH01d09	ROX	17	134-170	EMPc11	TAMRA	10	146-160
CH01f07a	TAMRA	17	182-216	CH03g07	ROX	13	210-255

Conclusion: Used markers showed a fairly high level of polymorphism, on average – higher than in the works that we did to study the cultural gene pool of the pear [3]. The results of statistical data processing – analysis by the method of principal coordinates and cluster analysis – showed that the level of intrapopulation variability turned out to be higher than the interpopulation level, which may be due to insufficient isolation of wild pear populations in the foothill zone, while the interpopulation variability of samples taken in mountains – higher. It is obvious that the contribution of spatial isolation to biodiversity significantly increases with a change in the altitude of the habitat. Also, it should be noted that triploid samples are most often found in high-altitude sampling locations.

Acknowledgements: Work is supported by a grant RFFI 16-44-230333.

References

1. Volk G.M. et al. (2006) Diversity of Wild Pyrus communis Based on Microsatellite Analyses. J. Amer. Soc. Hort. Sci. 131:408-417.
2. Challice J.S., Westwood M.N. (1973) Numerical taxonomic studies of the genus Pyrus using both chemical and botanical characters. Bot. J. Linnean Soc. 67:121-148.
3. Suprun I.I. et al. (2016) SSR polymorphism of modern cultivars and autochthonous forms of the pear tree from North Caucasus. Russian Journal of Genetics. 52:1149-1156.

ECOLOGICAL ESTIMATION OF THE NEW FALSE FLAX (*CAMELINA SATIVA* (L.)) VARIETIES DEVELOPED AT VNIIMK

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Key words: *winter false flax, yield, oil content, variety, breeding*

Motivation and Aim: In the context of the enlargement of a crops range produced for oil seeds, some oil crops of cruciferous (Brassicaceae) family can very interesting, such as rapeseed, mustard, turnip rape, and false flax.

One of the perspective crop from this family, being less susceptible to the cultivation conditions and more attractive for agricultural producers recently, is false flax (*Camelina sativa* (L.)). It is characterized with frost resistance (seeds are able to germinate at +1 °C, seedlings can live frosts up to –10 °C), winter resistance (isn't inferior to winter rye), and decentish drought resistance. Winter and spring forms of false flax possess high environmental plasticity that contributed to its cultivation in the different soil and climatic conditions. Sowing areas under this crop was 141 thousand hectares in the Russian Federation in 2016. The main regions of its production are Penza, Saratov, Rostov and Volgograd regions [1, 2].

Potential seed yield of winter false flax is 3.0 t per ha, of spring form – 2.5 t per ha, oil content varies from 39 to 44%. Oil meal obtained after oil extraction is a highly protein forage for animals which contains 36–40% of digestible protein [1].

Oil from false flax is a source of unsaturated fatty acids including oleic acid – 15–19% (ω–9), linoleic acid – 16–20% (ω–6) and linolenic acid – 36–40 % (ω–3) and this allows using it for food purposes. One of the perspective directions of false flax oil usage is its processing for biofuel of the second generation, as well as for production of bio-kerosene, greasing substances, paint and coatings, in pharmaceutical, cosmetic and perfume industries [1, 3]. Except those, the false flax oil is characterized with high tocopherol content with a unique stability to oxidation.

At VNIIMK works with false flax were renewed in 2008, and in present time the main research directions are breeding for: yield, oil content, optimization of oil-and-fatty composition of oil, resistance to diseases and lodging.

The purpose of our work was estimation of yield qualities of the new winter false flax variety Karat and spring one Kristal in the different agroclimatic conditions.

Methods and Algorithms: Tests of winter false flax were conducted in the conditions of Krasnodar and Penza regions, and spring false flax was tested in the conditions of Krasnodar, Rostov and Omsk regions. The materials of the researches were known and promising varieties of winter and spring false flax bred at All-Russia research institute of oil crops (VNIIMK), Penza agricultural research institute, the Siberian experimental station of VNIIMK. The experiments were done in accordance the methods developed by these research institutions.

The weather conditions in 2014–2016 can be characterized as relatively favorable for all testing plots.

Results: The results of the researches showed that in the condition of the central zone of Krasnodar region the new winter false flax variety Karat exceeded the standard variety Penzyak on seed yield on 0.60 t per ha, oil content in seeds – on 1.0%, and oil yield – on 0.23 t per ha under competitive trials. The variety Karat was differed with an increased tolerance to the major pathogens, uniformity on plants height, flowering and maturing compared to the standard variety. In condition of Penza region seed yield of the new variety Karat was 1.91 t per ha that exceeds the yield of the standard variety Penzyak on 0.29 t per ha.

The new spring false flax variety Kristal shows seed yield 1.5–1.6 t per ha, oil content – 41.5%, that exceeds the meaning of the standard variety VNIIMK 520 on 0.25 t per ha and on 2.2%, respectively, in ecological trial in the condition of the central zone of Krasnodar. The variety Kristal is differed with an increased resistance to diseases, higher adaptability for mechanical cultivation compared to the standard variety. During ecological trials in conditions of Rostov and Omsk regions among all rested varieties and samples, the new variety Kristal also showed maximal yield at the level 1.39 t per ha, that exceeded the standard variety in Omsk region on 0.16 t per ha and in Rostov region on 0.05 t per ha.

Conclusion: Thus, the ecological testing of the new winter and spring false flax varieties Karat and Kristal bred at VNIIMK showed that they possess high adaptability and environmental plasticity, and are perspective for production in the wide range of the soil and climatic conditions.

References

1. Gorlov S.L., Trubina V.S., Serdyuk O.A. Winter false flax variety Karat. Oil crops: Sci.-tech. bul. VNIIMK. 2015;2(162):127-128.
2. Semyonova E.F., Buyankin V.I., Tarasov A.S. Oil false flax: biology, technology, efficiency. Novocherkassk, 2005;1-87.
3. Prakhova T.Ya. Productivity of winter false flax depending on the cultivation methods. Young scientists. 2013;6:783-784.

ACRIDIDAE FAMILY: ESTABLISHING OF PHYLOGENETIC RELATIONSHIPS BASED ON MITOCHONDRIAL AND NUCLEAR DNA MARKERS

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Key words: *Acrididae, phylogeny, mitochondrial DNA, ribosomal DNA*

Motivation and Aim: Acrididae is the largest family in the superfamily Acridoidea (Insecta: Orthoptera: Caelifera). The family consists of 6787 species out of 12186 species in the Caelifera suborder. For a long time reconstruction of phylogenetic relationships, systematic and evolution of grasshoppers were based mostly on the morphological differences. As the result, multiply models of their evolution were developed for the high rank taxa. However, such approach can not precisely establish positions of some taxa on the family and subfamily levels.

One of the most effective methods of phylogenetic relationship establishing is the analysis of various DNA markers. Thus, our main aim was to investigate phylogenetic relationships among the subfamilies of Acrididae, as well as within the individual subfamilies, based on the sequences of *COI*, *COII* and *Cytb* mitochondrial genes and ribosomal *ITS2* and *28S RNA* sequences.

Methods and Algorithms: We conducted the search in NCBI database in order to obtain sequences of five DNA markers both mitochondrial (*COI*, *COII*, *Cytb*) and nuclear (*ITS2*, *28S rRNA*), as well as complete mitochondrial sequences. To complete the data, *COI*, *COII* and *ITS2* sequences of 66 species of Acrididae family were obtained experimentally.

Phylogenetic analysis was performed for different combinations of these markers by maximum likelihood and Bayesian methods (PhyML v. 3.0 and Mr. Bayes v. 3.2.6 program packages, respectively).

Results: We performed bioinformatic search in NCBI database for different sequences of Acrididae family and obtained 51 complete mitochondrial sequences; 288, 180 and 102 sequences of *COI*, *COII* and *Cytb* mitochondrial markers; 85 and 67 sequences of *ITS2* and *28S rRNA* nuclear markers respectively. We obtained experimentally *COI*, *COII* and *ITS2* sequences for 66 species of Acrididae family. As the result of the phylogenetic analysis of 369 species of Acrididae family, 8 clusters were defined and phylogenetic relationships between species were established, mostly supporting the current systematic. However, several subfamilies appear to be polyphyletic, which contradicts with the current systematic.

Conclusion: Thus, we investigated phylogenetic relationships between 369 species of Acrididae family. The obtained data allow us to clarify the Acrididae species classification.

Availability: All experimentally obtained sequences of *COI*, *COII*, *Cytb ITS2* and *28S rRNA* markers were deposited to GenBank.

Acknowledgements: The project was supported by the Russian Foundation for Basic Research (Grant No. 17-04-01615).

ANALYSIS OF ABORIGINAL VARIETIES EKIM KARA, KEFESSIA, GEVAT KARA AND KRONA BY MORPHOMETRIC PARAMETERS OF THE LEAF AND MICROSATELLITE LOCI

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Key words: *grapes, wine, leaf morphometry, SSR PCR, microsatellite loci*

Motivation and Objectives: Crimean autochthonous grape varieties are of great value for breeding programs and for preservation of the national grape gene pool. Over 60% of the varieties belong to wine grapes. The most famous is the legendary brand of vintage red dessert wine "The Black Doctor" awarded with gold and silver medals. This wine is made from Ekim Kara, Kefessia, Krona and Gevat Kara varieties that constitute a valuable gene pool of the Crimean native varieties. These varieties were in the focus of our research. Previously, in the process of ampelographic research it was hypothesized that varieties Kefessia and Ekim Kara were synonymous, while Krona was possibly a clone of Ekim kara. However, this assumption remains controversial. The opinion has been supported by agronomists-practitioners who currently cultivate these varieties. There is still an unresolved issue of the relations across Gevat Kara, Kefessiya and Krona genotypes. Thus, the issue of assessing the genetic relations across the varieties included into our study is relevant for wine growers and winemakers both from scientific and practical points of view. One of the traditional methods of phenotyping grape varieties is description of the leaf morphometric parameters, as they constitute the most taxonomically significant phenotypic feature [1]. Effective for studying the genetic diversity and identifying inter-linkages are the methods based on polymorphism of the primary DNA structure analysis [2]. Thus, the purpose of our research is to study the genetic relationship across Ekim Kara, Kefessia, Gevat kara and Krona grapes based on the genotype analysis by morphometric parameters of the leaf and SSR PCR method for nuclear (nSSR) microsatellite loci.

Methods and Algorithms: An ampelometric method for variety description by morphometric parameters of the leaf and polymerase chain reaction (PCR) method were used in accordance with the methodologies and recommendations for European projects. The genotype analysis was performed on 22 nuclear (nSSR) microsatellite loci. Assessment of the genetic relationship across the varieties based on SSR PCR analysis results was performed by Weighted Neighbor-Joining method, DARwin 6.0 program.

Results: The varieties were described by 12 morphometric parameters of the leaf. PCR analysis produced microsatellite profiles of the varieties on 22 microsatellite loci. Comparative analysis of phenotypic characteristics and DNA profiles based on analysis of 22 microsatellite loci allowed us to establish the genetic relationships among the varieties. Cluster analysis based on the obtained distance matrix allowed evaluating the genetic relationships across the investigated grapes varieties.

Conclusion: Due to extensive variation as to the ampelometric parameters of the leaf in the grape samples under investigation their distribution regions overlapped and no clear differentiation was observed among the varieties. The DNA profiles of the varieties on SSR loci and the genetic distances matrix allow us to conclude that Ekim kara, Kefessia and Gevat kara grapes are different but quite related to each other. The hypothesis that Krona variety is a clone of Ekim Kara was confirmed, since their genotypes on 22 nSSR are identical.

References

1. Troshin L.P., Risovannaya V.I., Polulyakh A.A. (1996) Variability of leaf characteristics for grapes belonging to Vitis vinifera pontica balcanica Negr., Viniculture and winemaking. 1:15-20.
2. Goryslavets S., Risovannaya V. et al. (2010) Parentage Study of Closely Related Ukrainian Wine Grape Varieties using Microsatellite Markers. Cytology and Genetics. 44(2):95-102.

BREEDING OF OIL FLAX FOR RESISTANCE TO FLAX SICKNESS OF SOIL

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Key words: *oil flax, flax sickness of soil, resistance to Fusarium wilt, brief-field crop rotation, arid regions*

Motivation and Aim: The problem of the flax oppression with a premature return to the same field is known for a long time and was called "flax sickness of soil" or "auto-intolerance" [1–5]. The main causes of flax sickness of soil at various historical stages were: the accumulation of microorganisms, harmful for flax into the soil; unilateral depletion and change in soil structure; accumulation of specific weeds, autotoxic allelopathy [3–5]. As a rule, symptoms of flax sickness of soil, regardless of the reasons that cause it, begin to develop already in the second generation of flax on the same field [1, 2, 5]. However, despite the long history of research, this phenomenon has not been fully understood. As a result, both at the end of the 19th century and at the beginning of the 21st century, the problem of flax sickness of soil can be solved so far only in two ways: either by observing crop rotations, or by breeding autotolerant varieties of flax [5].

Methods and Algorithms: The studies were conducted in 2012–2016 at the central experimental base of the FGBNU VNIIMK, Krasnodar. In the field of flax in the experimental brief-field crop rotation the Fusarium wilt foci was detected. In the epicenter of these foci, the single surviving flax plants without symptoms of lesion were identified and selected. At the same time, an experimental field was established for flax growing in a monoculture in order to rapidly accumulate in the soil all possible factors of flax sickness of soil. During 2013–2016 the flax was continued to be sown in this area, to enhance the effect of flax sickness of soil and the natural accumulation in the soil of fungi of the genus *Fusarium spp*. Since 2013, at this nursery the progeny of plants isolated in the epicenters of Fusarium wilt foci was annually estimated for field resistance to flax sickness of soil and Fusarium wilt. Variety of oil flax VNIIMK 620 served as a control. Seed reserves were sown in field where flax was not grown for 8 years, for proliferation and selection assessment. In 2015–2016, the best varieties were evaluated on plots of 24 m^2 in four repetitions.

Results: In some studies, such as the article of Y. M. Stam (1952), it was observed that the symptoms of oppression of flax plants from Fusariosis are similar to the inhibition of flax sickness of soil [2]. We formulated a hypothesis that plants do not distinguish between the effects of fungal and "flax-sickness" toxins. The reason for this may be the same physiological or biochemical processes in the flax tissues, which is exerted by the oppressive effects of Fusariosis and flax-sickness toxins, or both toxins have similar chemical formulas and a similar molecular structure [7]. Therefore, based on the assumed uniform response of flax to these factors, fusarium-resistant biotypes may have increased resistance to flax sickness of soil and vice versa.

In 2012, in field of oil flax in the epicenter of the Fusarium wilt focus, several surviving plants without symptoms of fusarium damage were detected. In 2013 progeny of these plants were individually sown on the field where in the previous year flax plants

were grown and smelt. Visible symptoms of oppression of individual progenies of flax varieties it was not found. In 2014, flax lines, most resistant to flax sickness of soil, re-sown on a field with the soil flax-sickness factors already accumulated for two years. As in the previous year, resistant plants symptoms of oppression completely lacked. The other varieties and lines sown in the same area were depressed or died. Field observations in 2015–2016 confirmed the complete resistance of previously isolated flax samples to flax sickness off soil. The resistant to soil flax-sickness varieties also showed their advantage over conventional flax varieties and lines when growing in the usual 8-rotational crop rotation, with the background of the epiphytotic development of Fusarium wilt in 2015.

Conclusion: Thus, five-year studies have established that non-splitting oil-flax lines are stably high resistant to fungi of the genus *Fusarium* spp. and flax sickness of soil. These lines are suitable for direct propagation and introduction into production as self-tolerant varieties for cultivation; both in brief-field (3–4 fields) crop rotations, and even in monoculture. These lines can also be used as sources of complex resistance to flax sickness of soil and Fusariosis in the breeding of oil and fiber flax varieties.

References

1. Tobler F., Brederman G., Ryabov I.I., Opinz K., Shilling E. (1931) Lyon: pryadil'noe i maslichnoe rastenie. M.; L.: Sel'hozgiz. 131-139.
2. Stam Ya.M. (1952) Nekotorye voprosy ustojchivosti maslichnyh l'nov k fuzariozu. Tr. po prikl. bot., gen. i selekcii. 29 (2):138-148.
3. Gorelik I.I. (1984) Fiziologicheskie osobennosti rastenij l'na-dolgunca v usloviyah substratoutomleniya: avtoref. dis. ... kand. biol. nauk. Minsk: 24.
4. D'yakov A.B. (2006) Fiziologiya i ehkologiya l'na. Krasnodar. 193-196.
5. Zelentsov V.S., Ryabenko L.G., Moshnenko T.V., Olejnik V.I. (2015) Sozdanie ustojchivogo k l'noutomleniyu iskhodnogo materiala dlya selekcii avtotolerantnyh sortov maslichnogo l'na. Materialy VIII Mezhdunarodnoj konferencii molodyh uchyonyh i specialistov 19–20 fevralya 2015, Krasnodar. 58-62.

CONSTRUCTION OF THE PLASMID VECTOR CARRYING *TAWCS120* GENE FOR AGROBACTERIUM MEDIATED PLANT TRANSFORMATION

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Key words: *dehydrin, Triticum aestivum, agrobacterium mediated plant transformation, transgenes, pRI 101-AN, cold resistance, sequencing*

Motivation and Aim: In recent decades, significant progress has been made in developing methods and ways of applying genetic transformation of plants. There are both scientific and practical interests in the usage of genetic engineering achievements in the development of agriculture and obtaining new varieties of plants. The creation of transgenic plants makes it possible to obtain new highly productive crops resistant to adverse environmental conditions. For the growing in the areas with low temperatures plants should have the properties of protection from hypothermia. It is known that low-temperature stress induces the accumulation of specific proteins – dehydrins, which is one of the mechanisms that protect plants from hypothermia. Dehydrins exhibit antifreeze functions, participating in the protection of cells during the formation of extracellular ice. Genetically modified plants transformed by different dehydrins have increased resistance to the adverse low-temperature factors. One of the well-known and studied dehydrins is WCS120 from *Triticum aestivum* L. It is shown, that transgenic plants carrying *TaWCS120* gene have increased resistance to low-temperatures. The aim of this work is to create a genetic construction based on the vector pRI 101-AN with the *TaWCS120* gene for agrobacterium mediated plant transformation.

Methods and Algorithms: Wheat germinates were incubated for 2 days at 4 °C to increase dehydrines mRNA amount in cytoplasm. Total RNA was isolated from 200 mg of wheat germinates using the guanidinium thiocyanate-phenol-chloroform extraction method and MaXtract High Density gel (Qiagen). mRNA was purified using Oligotex mRNA Mini Kit (Qiagen) and reverse transcribed by M-MLV Reverse Transcriptase (Promega). Primers for the amplification of TaWCS120 open reading frame (1176 bp) were design according to the NCBI data base (accession number – M93342.2). Primers were additionally modified by adding of restriction sites needed for cloning in plasmid vectors. Obtain PCR products were cloned in the pTZ57R/T vector and sequenced by the Sanger method using 3500 Genetic Analyzer. Plasmid vector for the Agrobacterium mediated transformation was performed on the basis of the commercial pRI 101-AN binary vector containing the general CaMV35S promoter, selective kanamycin resistance gene – *nptII* and 5'non-coding region (5'-UTR) of *Arabidopsis thaliana* alcohol dehydrogenase (ADH) gene as a translational enhancer. PCP product cloned in pTZ57R/T and pRI 101-AN were restricted by BamHI and SacI restriction enzymes. Then target *TaWCS120* was ligated with the linearized pRI 101-AN vector. The obtained pRI 101-AN vector with *TaWCS120* was cloned in *E. coly* and again sequenced to check the correctness of the genetic construction. All nucleotide sequence alignments were carried out Sequencher 5.1.

Results: In our study, two types of polymerases were used for the amplification of target genes. GoTaq Flexi Polymerase Mix (Promega) was firstly applied for PCR. All obtained clones contained from 2 to 6 mistakes in nucleotide sequences in comparison with NCBI database. The second proof reading polymerase was applied to confirm the sequencing correctness. iProof High-Fidelity DNA Polymerase (Bio-Rad) allowed to obtain only one type of PCR product (16 independent clones). There were only 2 differences in nucleotides in comparison with NCBI database (C replaced to G in the position 115 and G replaced to C in the position 481). Due to the repeatability of replacements in the same nucleotide sites, the detected difference in the *WCS120* gene sequences from GeneBank and studied *Triticum aestivum* variety Irkutskaya most reliably explained by the mutability during selective breeding or by mistake in the GeneBank data base. As a result of the present study the genetic construction based on the pRI 101-AN vector with *TaWCS120* gene was created.

Conclusion: The obtain genetic construction may be used for the agrobacterium mediated plant transformation to obtain transgenic plants resistant to low-tepmeratures.

Acknowledgements: The study was financially supported by Complex program of fundamental researches Siberian Branch of Russian Academy of Sciences (project No 0343-2015-0005) and scholarship of President of Russian Federation for young scientists (SP-3823.2015.1).

WHEAT LEAF TRICHOMES PATTERN FORMATION: FROM IMAGE ANALYSIS TO COMPUTATIONAL MODELING

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Key words: *wheat leaf, trichome patterning, L-systems, mathematical modelling, computer simulation, laser scanning microscopy, ImageJ*

Motivation and Aim: Plant epidermis provides an ideal system in which to study the arrangement of cell types within a tissue [1]. The epidermis of the wheat leaf is a complex tissue consisting of different cell types (stomata, trichomes, epidermal cells) forming a certain pattern from parallel cell rows. Wheat leaf trichomes are unicellular unbranched epidermal outgrowths forming in zone located at the leaf base, while preserving approximately the same number of epidermal cells them. Occurring for a long time a unidirectional growth of wheat leaves enables to observe a series of successive morphogenetic stages of trichomes formation at one time moment. Computational modeling became one of the main methods of theoretical study of the mechanisms underlying the regulation of the dynamics of spatial patterns in developing tissues and/or organs forming from the growth, division and differentiation of cells. Efficiency morphogenetic studies using computer modeling essentially depends on the development of mathematical tools and computer technologies to produce a qualitative description of the growing spatial structures (sufficient for verification and validation of models) based on an analysis of its 3D images. The aim of our work was to study the mechanism of trichomes spatial pattern formation using 3D image processing and computational modeling approaches.
Methods and Algorithms: We used methods of fluorescent staining and laser scanning microscopy to obtain 3D images visualizing cell walls and nuclei. To obtain accurate morphometric data, the most effective was to combine a multi-frame and multi-channel scanning followed by a linear combination of intensity information from different channels during image processing. We used an ImageJ-plugin to automate routine stages of obtaining data process, including frames merging, shifts and overlaps correction, and manual trichomes detection and geometric measurements. We elaborated a mathematical model based on the extension of L-systems approach and its implementation for computational simulation in Mathematica package [2]. In the model, we assumed a unidirectional growing cell ensemble starting from a meristem-like layer of generative cells and then generating parallel cell rows from every cell of the initial layer. We considered the growth zone of the leaf included division and elongation zones; in addition, the division zone included a zone of asymmetric divisions where trichomes formed. In the work, hypotheses are tested that the trichome spacing pattern is established in asymmetric divisions zone by the following mechanism selecting single trichome cell from otherwise equivalent epidermal cells. We proposed that trichome produce a morphogene that diffuses along the cell row and inhibits the formation of other trichomes (Such a mechanism was used for Arabidopsis [3]). We assumed that the postulated inhibitor is destroyed by epidermal cells, so that a gradient is set up around a

trichome cell, and that there is a threshold level of inhibitor below which development of the trichome cell begins (the above-threshold region defining an inhibitory zone). It is supposed that an epidermal cell starts a non-refundable course of differentiation into trichome as soon as the concentration of inhibitor falls below threshold.

Results: For a Chinese spring wheat, we got a series of 3D images containing information about the structure of a large fragment of cell row containing trichomes. For this fragment, we measured cells lengths and identified trichomes described the formation of trichomes pattern in the leaf growth zone. The model parameters was fitted to experimental data on quantitative characteristics describing the trichomes pattern obtained by 3D image analysis.

Conclusion: The proposed approach of constructing mathematical models for describing the growth of plant tissue, taking into account the results of image analysis of specific plant organs, is an efficient method for studying the influence of various, including stressful, factors on epidermal pattern.

Availability: Image data, program code and model parameters are available on request from the authors.

Acknowledgements: This study was supported by the project 0324-2016-0008 from the Russian State Budget.

References:

1. Robinson D.O., Roeder A.H. (2015). Themes and variations in cell type patterning in the plant epidermis. Current Opinion in Genetics Development. 32:55-65.
2. Zubairova U., Nikolaev S., Penenko A., Podkolodnyy N., Golushko S., Afonnikov D., Kolchanov N. (2016). Mechanical behavior of cells within a cell-based model of wheat leaf growth. Frontiers in Plant Science. 7:1878.
3. Dupuy L., Mackenzie J., Rudge T., Haseloff J. (2008). A system for modelling cell–cell interactions during plant morphogenesis. Annals of Botany. 101(8):1255-1265.

FOUNDER EFFECT IN PREVALENCE OF HEREDITARY DEAFNESS CAUSED BY MUTATIONS IN GENE *GJB2* (13Q11-Q12) AMONG SIBERIAN POPULATIONS: COMMON ANCESTRAL HAPLOTYPES FOR MAJOR *GJB2* MUTATIONS AND ESTIMATION OF THEIR AGE

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Key words: *genetic diversity, founder effect, hereditary deafness, gene GJB2, Siberian populations*

Motivation and Aim: Prevalence of many monogenic diseases can be determined by a number of factors forming the specifics of population structure (ethnic composition, migration, isolation, founder and bottleneck effects, proportion of consanguineous and assortative marriages). Nonsyndromic deafness is one of the most common sensorineural genetic disorders and several dozen genes contribute to its pathogenesis. Mutations in the gene *GJB2* (MIM 121011, 13q11-q12) encoding connexin 26 (Cx26) account for a significant portion (up to 50%) of hereditary deafness. Spectrum of the *GJB2* mutations and their prevalence are highly specific for various populations. Identification of major *GJB2* mutations and estimation of their frequency are important both for medical genetic studies as well as for understanding of evolutionary history of populations of different ethnic origins. We investigated the molecular basis of deafness by screening of the *GJB2* mutations in some regions of Siberia and evaluated the mutational spectrum and contribution of the *GJB2* gene to hearing loss of patients. We found high prevalence of recessive *GJB2* mutations p.W172C, IVS1+1G>A, c.235delC in indigenous Turkic-speaking Siberian peoples (the Tuvinians and the Altaians) and predominance of recessive mutation c.35delG among individuals of European origin. High frequency of recessive mutations p.W172C, IVS1+1G>A, and c.235delC in indigenous peoples of the Tuva and the Altai (the Tuvinians and the Altaians) assumes the key role of the founder effect in their prevalence in studied Siberian regions.

This study aims to evaluate the role of founder effect in prevalence of major *GJB2* mutations among indigenous populations of Siberia and to estimate their age and regions of origin.

Methods and Algorithms: Data on genotyping of 7 STRs (D13S1316, D13S141, D13S175, D13S1853, D13S143, D13S1275, and D13S292) flanking the *GJB2* gene and encompassing ~ 3.5Mb (by GeneScan) and 9 intragenic and flanking *GJB2* SNPs (rs747931, rs5030700, rs3751385, rs2274083, rs2274084, SNP13:20767153C>T, rs9552101, rs117685390, and rs877098) (length of region ~ 79kb) (by restriction analysis and Sanger sequencing) were used for the reconstruction of common haplotypes for major *GJB2* mutations p.W172C, IVS1+1G>A, and c.235delC. The data on allelic frequencies

of D13S141, D13S175, D13S1853, and rs3751385 were used for reconstruction of haplotypes with mutation c.35delG. Reconstruction of STR- and SNP-haplotypes was implemented by using software package "Arlequin" (algorithm EM). Age of mutations was calculated according to [1].

Results: The data on allelic diversity of STR- and SNP-markers were obtained for the samples of homozygotes for each of recessive mutations p.W172C, IVS1+1G>A, and c.235delC and among control individuals without of these mutations. We revealed the common haplotypes for each of these mutations with significantly higher frequencies compared to control samples. Reconstructed STR-haplotype for p.W172C (~1.59 Mb) was 269-121-**p.W172C**-105-202-121 (D13S1316-D13S141-*GJB2*-D13S175-D13S1853-D13S143) and corresponding SNP-haplotype (including all studied SNPs in linear order) was T-C-C-**p.W172C**-A-G-T-G-T-C. The corresponding haplotypes for IVS1+1G>A were 121-**IVS1+1G>A**-105-202-121-208-209 (D13S141-*GJB2*-…-D13S292) (~3.46 Mb) and C-C-C-A-G-**IVS1+1G>A**-C-G-T-C (all studied SNPs); for c.235delC: 267-121- **c.235delC**-105-202-121-210 (D13S1316-…-*GJB2*-…-D13S1275) (~1.69 Mb) and T-C-C-A-**c.235delC**-G-C-G-T-T (all studied SNPs). Thus, the specific conserved haplotypes were found for each of mutations p.W172C, IVS1+1G>A, and c.235delC (*GJB2*) suggesting their single origin from a common ancestor. It is known that the prevalence of p.W172C is restricted only by the Tuva, Altai and Mongolia territories. The essential accumulation of p.W172C on the Tuva territory assumes this region as the place of origin of p.W172C. We obtained a rough estimation of age of p.W172C - ~1000–680 years. The age of mutation IVS1+1G>A in the Tuva has been estimated as ~4800–2180 years, while the age of expansion of IVS1+1G>A in Yakutia has been earlier estimated as ~800 years [2]. These data corresponds to the hypothesis of introduction of IVS1+1G>A by migration flows of the Turkic-speaking peoples from the southern regions of Siberia to Yakutia.

We revealed two specific haplotypes (D13S141-rs3751385-**c.35delG**-D13S175-D13S1853) (~316 kb) in patients homozygous for specific "European" mutation c.35delG: 126-T-**c.35delG**-105-202 and 124-T-**c.35delG**-105-202 and estimated the age of the c.35delG expansion in Siberia as ~8800–6800 years.

Conclusion: We revealed the allelic diversity of STR- and SNP-markers flanking the *GJB2* gene (13q11-q12), and the common haplotypes specific for each of the major *GJB2* mutations p.W172C, IVS1+1G>A, and c.235delC. These data confirmed the hypothesis on the main role of the founder effect in their prevalence among the indigenous populations of Siberia. Approximate estimations of age of p.W172C and IVS1+1G>A are in good agreement with available ethnohistorical data on the ethnogenesis of indigenous Siberian peoples. The data on common haplotypes for mutation c.35delG and estimation of its expansion in Siberia fits to corresponding data for European populations.

Acknowledgements: This study was supported by the Project # II.2П/VI.58-6 (# 0324-2016-0031) of the Federal Research Center Institute of Cytology and Genetics and by the RFBR grant #15-04-04860_a.

References

1. Rich N. et al. (1995) Genetic analysis of idiopathic torsion dystonia in Ashkenazi Jews and their recent descent from a small founder population. Nat. Genet. 9:152-159.
2. Barashkov N. et al. (2011) Autosomal recessive deafness 1A (DFNB1A) in Yakut population isolate in Eastern Siberia: extensive accumulation of the splice site mutation IVS1+1G>A in GJB2 gene as a result of founder effect. J. Hum. Genet. 56:631-639.

COMPARATIVE ANALYSIS REVEALS DRAMATACALY RESHAPING CHICKEN CHROMATIN ACHITECTURE DURING ERYTHROCYTE MATURING

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Key words: *Hi-C, spatial chromatin structure, topologically domain, evolution, chicken.*

Motivation and Aim: Using the last decade developed techniques such as the Hi-C method allow to discover the new layer of spatial chromatin organization being formed by the closely interacting chromosomal regions, in other words, topologically associated domains (TADs). The TAD architecture appears to play important roles in gene regulation, transcription and replication. The domain boundaries are shown to mark the transition between domain-like chromosomal compartments, early and late replicating chromatin, to be enriched by insulator elements, house-keeping genes and histone modifications [1]. Furthermore, investigations of mammalian genomes reveal high conservation of chromosomal domain structure across both cell types within one organism and evolution lineages[1,2].

In contrast to mammals, TADs had not been described yet in other vertebrate subgroups including birds to be the most diverse group of terrestrial warm-blooded animals. Notably, avian genomes are the smallest among amniotes mainly because of the loss of repetitive sequences, large segmental deletion and gene shortening. Most of birds are known to have remarkably stable karyotype consisting of about 40 chromosome pair, with interchromosomal rearrangement events occuring rare during the avian evolution.

To bridge the gap, we profiled the chicken chromosome topology and examined it to determine whether there are the patterns obtained from mammal genome in the avian genome. This could give fresh insights to molecular mechanisms to form chromosomal domain architecture.

Methods and Algorithms: To study chromatin structure in avian somatic cells, we determined genome-wide chromatin interaction frequencies, having perform the Hi-C experiment in chicken embryonic fibroblasts (CEF) and chicken mature erythrocytes (CME). Hi-C data were processed to identify corresponding domain sets, using the standard algorithm[1,3].

To characterize domains, we examined the distribution of genomic elements in relation to the topological domain, analyzing the high and low expressed genes, CTCF-binding sites, H3K4me3 and H3K27ac histone modifications, salt-soluble chromatin domains, conservative non-coding and repetitive elements. With avian mature erythrocytes being transcriptionally inactive, we used the data obtained from immature cells. To evolutionary analyze, we compared mouse, human and chicken domain structure profiles through the consideration of the ortholog disposition around domain boundaries.

The data evaluation based on bootstrapping with an empirical confidence interval defined as three standard deviation.

Results: In contrast to the mammals, the domain structure was not conserved between examined chicken cell types. The simularity of CME and CEF domains are not statistically significant as compared to permutated domain sets. Futher investigations show to be the radically difference in the genomic element distribution. The CME domain boundaries are depleted by histone modifications and salt-soluble chromatin domains. In addition, the distribution other genomic elements do not differ from random. On the contrary, the CEF domain boundaries are enriched by the tested genomic elements except the conservative non-coding elements. Moreover, the CEF and mammalian domains share this patterns

The evolutionary analysis showed the high degree of chicken, mouse and human domain structure similarity. Ortholog genes conserve their location near domain boudaries across evolution in spite of the difference in a gene density, a domain length and a boundary quantity. Around 45% of chicken boundaries are boundaries in mouse and human, that is close to the result of mouse-human comparing (around 50%).

Conclusion: Ours results suggest that there are common mechanisms forming chromosomal domain structure across the avian and mammal lineages. The same patterns of genomic element distribution show the topological domains to play a same roles in genome function across evolution.

The topological associated domains show the strong evolution conservation. Ortholog genes tend to conserve their location around domain boundaries in spite of radically different chromosomal evolution within avian and mammalian lineages.

However, the dramatically difference between the topological domain features of chicken mature erythrocytes and other cell types (both chicken, mouse and human) suggest that the chromatin architecture of the avian mature erythrocytes is likely to be defined by yet unknown mechanisms.

Availability: All Hi-C data obtained in this study have been deposited to GEO under the accession number GSE96037.

Acknowledgements: The research was supported by Russian Science Foundation (grant #14-14-00131). The work was partially performed using experimental equipment of the Research Resource Center 'Biobank' of St Petersburg State University. Cell culture and Hi-C libraries preparation were performed in Resource Center of the Institute of Cytology and Genetics (state project No. 0324-2016-0002 of Russian Academy of Sciences).

Reference:

1. Dixon, J. R. et al. (2012) Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature,* **485:** 376–380.
2. Battulin, N. et al. (2015) Comparison of the 3D organization of sperm and fibroblast genomes using the Hi-C approach. *Genome Biol.,* **16:** 77.
3. Filippova, D. et al. (2014) Identification of alternative topological domains in chromatin. *Algorithms Mol. Biol.,* **9:** 14.

Научное издание

SYSTEMS BIOLOGY AND BIOINFORMATICS
THE NINTH INTERNATIONAL YOUNG SCIENTISTS SCHOOL SBB-2017

Abstracts

Printed without editing

СИСТЕМНАЯ БИОЛОГИЯ И БИОИНФОРМАТИКА
ДЕВЯТАЯ МЕЖДУНАРОДНАЯ ШКОЛА МОЛОДЫХ УЧЕНЫХ SBB-2017

Тезисы докладов

Издается в авторской редакции

Выпуск подготовлен информационно-издательским отделом ИЦиГ СО РАН
Начальник отдела: Т.Ф. Чалкова
Дизайн и компьютерная верстка: А.В. Харкевич

Подписано в печать 31.05.2017. Формат 70 × 108 $^{1}/_{16}$. Усл. печ. л. 7,70.
Тираж 120 экз. Заказ № 142

Федеральное государственное бюджетное научное учреждение
«Федеральный исследовательский центр Институт цитологии и генетики
Сибирского отделения Российской академии наук» (ИЦиГ СО РАН)
630090, Новосибирск, проспект Академика Лаврентьева, 10

Отпечатано в типографии ФГУП «Издательство СО РАН»
630090, Новосибирск, Морской проспект, 2