Вводный вебинар по проекту СКБР БиоМост
Introductory Webinar on the BioBridge Project

«Расширение сотрудничества между Центрами передового опыта ЦВЕ и Центральной Азии для устранения основных причин утраты биоразнообразия и поддержания здоровья людей, сельскохозяйственных культур и домашнего скота»

"Enhancing Collaboration between the CEE and Central Asia's Centres of Excellence to Address the Key Drivers of Biodiversity Loss and Maintain Human, Crop and Livestock Health".

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Лабораторные технологии и инновационные методы обнаружения в области сохранения биоразнообразия — эффективные подходы, которые включают, **но не ограничиваются** следующими методами:

• Видовая идентификация мясных и растительных компонентов, что может эффективно использоваться как для борьбы с браконьерством, так и для оценки фальсифицированных пищевых продуктов, кормов с целью контроля их качества и безопасности.

• Обнаружение неразрешенных ЖИО, в том числе новых ЖИО, созданных с помощью методов CRISPR-Cas и других современных технологий.

• Выявление патогенов в партиях семян, фруктов и рассаде ценных сельскохозяйственных культур, распространение которых вызывает появление стерильных семян или может привести к распространению болезней и нанести вред сельскохозяйственным растениям и дикой природе.

Laboratory technologies and innovative detection methods for biodiversity conservation are effective approaches which include **but are not limited** to the following methods:

• Species-specific identification of meat and plant components, which is useful to both prevent poaching and assess food and feed products' adulteration with a view to controlling their quality and safety.

• LMO detection of unauthorized LMOs, including novel LMOs developed by CRISPR-Cas methods and other modern technologies.

• Detection of pathogens the spread of which causes the emergence of sterile seeds or could cause the spread of diseases and harm crops and wildlife, in the batches of seeds, fruit, and seedlings of valuable agricultural crops.

Такие методы используются для выявления ключевых взаимосвязанных факторов утраты биоразнообразия, которые в конечном итоге оказывают воздействие на здоровье человека.

Such methods are used to identify key interrelated drivers of biodiversity loss that ultimately impact human health.

Методы лабораторной детекции разрабатываются в научных учреждениях – Центрах передового опыта. Однако существуют ограничения в виде финансовых и человеческих ресурсов, что в конечном итоге сильно осложняет комплексный мониторинг, контроль и надзор за ключевыми факторами утраты биоразнообразия как на национальном, так и региональном уровнях.

Methods of laboratory detection are being developed in Centres of Excellence. However, constraints in terms of limited financial and human resources do not allow one single centre to fully develop and implement all of these, which ultimately renders comprehensive monitoring, control and supervision of such drivers at both the national and the regional levels very difficult.

Какие есть проблемы?	**What are the problems?**
ограниченные финансовые и человеческие ресурсы;	limited financial and human resources;
разный уровень потенциала и опыта в области лабораторной детекции;	varying levels of capacity and experience in in-lab detection;
не достаточность знаний и навыков в отношении конкретных методов и лабораторных процедур в соответствии с международными требованиями к отбору проб, обнаружению и идентификации;	insufficient knowledge and skills regarding specific methods and laboratory procedures in accordance with international sampling, detection and identification requirements;
отсутствие технических нормативных правовых актов (например, технические регламенты, технические кодексы установившейся практики, гигиенические стандарты);	lack of technical normative legal acts (for example, technical regulations, technical codes of established practice, hygiene standards);
недостаточный потенциал для обмена опытом и информацией;	insufficient capacity for exchange of experience and information;
несогласованность методологических подходов;	inconsistency of methodological approaches;
отсутствие обучения;	lack of training;
отсутствие технической поддержки, предоставляемые опытными специалистами	lack of technical support provided by experienced specialists

Цель проекта — развитие долгосрочного сотрудничества между Институтом сельского хозяйства Республики Сербской, Баня-Лука (Босния и Герцеговина), Департаментом профилактики заболеваний и государственного санитарно-эпидемиологического надзора (Кыргызская Республика), отделом по реализации экологических проектов Министерства окружающей среды (Молдова), Институтом биологических исследований «Синиша Станкович» Национального института Республики Сербия Белградского университета (Сербия), кафедрой биотехнологии и системной биологии Национального института биологии (Словения) и Институтом ботаники, физиологии растений и генетики Национальной академии наук Таджикистана (Таджикистан) и ИГЦ (Беларусь) **через создание активной сети Центров передового опыта в Центральной и Восточной Европе (ЦВЕ) и Центральной Азии, которые будут осуществлять сотрудничество для проведения совместных мероприятий по выявлению основных факторов утраты биоразнообразия и защите здоровья людей, животных и растений.**

The goal of Project is to foster cooperation between the Agricultural Institute of Republic of Srpska, Banja Luka (Bosnia and Herzegovina), the Department of Disease Prevention and State Sanitary and Epidemiological Surveillance (Kyrgyz Republic), the P.I. Environmental Projects Implementation Unit of the Ministry of Environment (Moldova), the Institute for Biological Research "Siniša Stanković" of the National Institute of the Republic of Serbia of the University of Belgrade (Serbia), the Department of Biotechnology and Systems Biology of the National Institute of Biology (Slovenia), and the Institute of Botany, Plant Physiology and Genetics of the Tajikistan National Academy of Sciences (Tajikistan) and IGC (Belarus), **through the creation of an active network of Central and Eastern European (CEE) and Central Asian centres of excellence that will cooperate to carry out joint activities to identify key drivers of biodiversity loss, and protect human, animal and plant health.**

Мероприятие 1 / Activity 1	Описание/ Description	Итог/ Output
Организация и проведение ряда совещаний в онлайн-формате	Совещания будут посвящены обсуждению и сравнению правовых и технических норм, а также существующих лабораторных методов и подходов в области идентификации видов, обнаружения и идентификации живых измененных организмов (ЖИО), обнаружения патогенов растений, а также другим ключевым областям, которые могут содействовать сохранению биоразнообразия.	Отчет с подробным описанием и сравнением правовых и технических норм, действующих в странах-партнерах с точки зрения лабораторного обнаружения ключевых факторов утраты биоразнообразия, цель которых заключается в выявлении сходства и различия и внесении вклада в гармонизацию подходов в регионе.
Organize and conduct a series of online meetings	The meetings will serve to discuss and compare legal and technical regulations, as well as existing laboratory methods and approaches in the fields of species identification, detection and identification of Living Modified Organisms (LMO), detection of plant pathogens, and other key areas that may help biodiversity conservation.	Report detailing and comparing the legal and technical regulations in place in the partner countries in terms of in-laboratory detection of key drivers of biodiversity loss, the objective of which is to clarify similarities and differences and contribute to the harmonization of approaches in the region.

Мероприятие 2 / Activity 2	Описание/ Description	Итог/ Output
Организация и проведение учебного семинара	Пятидневный теоретический и практический учебный семинар под руководством экспертов ИГЦ и Национального института биологии Словении. Цель семинара: a) обеспечение теоретической подготовки по лабораторной идентификации видов, скринингу ЖИО и обнаружению патогенов, которые могут оказывать влияние на здоровье людей, животных и растений; а также b) содействии дальнейшему обсуждению наиболее эффективных подходов, методов и протоколов для обнаружения таких факторов.	Обзор методов, используемых в странах-партнерах для внутрилабораторного обнаружения и идентификации ключевых факторов утраты биоразнообразия, информация о которых будет распространена среди Центров передового опыта стран-участниц и использована для развития потенциала.
Organize and conduct a training workshop	A five-day theoretical and practical training workshop, under the leadership of experts from IGC and the National Institute of Biology of Slovenia The goals of on-site seminar: a) Provide theoretical training on in-laboratory species identification, LMO screening, and pathogen detection that may affect human, animal, and plant health; and b) Facilitate further discussion of the most effective approaches, methods and protocols for the detection of such drivers.	Overview of the methods used in the partner countries for in-laboratory detection and identification of key drivers of biodiversity loss, to be shared among the participating centres of excellence and used for capacity development.

Мероприятие 3 / Activity 3	Описание/ Description	Итог/ Output
Инициирование создания региональной сети Центров передового опыта в ЦВЕ и Центральной Азии	На основе обсуждений, начатых во время учебного семинара (мероприятие 2), будет подготовлено подробное предложение по долгосрочному сотрудничеству в форме дорожной карты и плана устойчивого развития для региональной сети Центров передового опыта ЦВЕ и Центральной Азии в сотрудничестве со всеми вовлеченными заинтересованными сторонами.	Соглашение о создании региональной сети Центров передового опыта (ЦВЕ и Центральная Азия). Соглашение будет включать в себя структуру дорожной карты и план устойчивого развития с целью содействия долгосрочному сотрудничеству после реализации проекта. Особое внимание будет уделено участию женщин и молодых ученых как в мероприятиях дорожной карты, так и в мероприятиях плана устойчивого развития.
Initiate the establishment of the Regional CEE and Central Asia's Network of Centres of Excellence	Building on discussions initiated during the training workshop (Activity 2), a detailed proposal for long-term collaboration activities in the form of a roadmap and sustainability plan for the Regional CEE and Central Asia's Network of Centres of Excellence will be prepared in collaboration with all stakeholders involved.	Agreement on the establishment of a Regional Network of Centres of Excellence (CEE and Central Asia). The agreement will include the structure of a roadmap and sustainability plan to facilitate long-term cooperation beyond the implementation of the Project. Special consideration for the participation of women and young scientists will be given in both the roadmap and the sustainability plan.

ПЛАНИРУЕМЫЕ ИТОГИ	EXPECTED OUTPUTS
Итог 1: Отчет с подробным описанием и сравнением правовых и технических норм, действующих в странах-партнерах с точки зрения внутрилабораторного обнаружения ключевых факторов утраты биоразнообразия (идентификация видов, обнаружение и идентификация ЖИО, обнаружение патогенов растений и т.д., что было определено в ходе онлайн-консультаций), цель которых заключается в выявлении сходства и различия и внесении вклада в гармонизацию подходов в регионе.	Output 1: Report detailing and comparing the legal and technical regulations in place in the partner countries in terms of in-laboratory detection of key drivers of biodiversity loss (species identification, LMO detection and identification, detection of plant pathogens, and others as identified during the online consultations), the objective of which is to clarify similarities and differences and contribute to the harmonization of approaches in the region.
Итог 2: Обзор методов, используемых в странах-партнерах для внутрилабораторного обнаружения и идентификации ключевых факторов утраты биоразнообразия, информация о которых будет распространена среди Центров передового опыта стран-участниц и использована для развития потенциала.	*Output 2:* Overview of the methods used in the partner countries for in-laboratory detection and identification of key drivers of biodiversity loss, to be shared among the participating centres of excellence and used for capacity development.

Роли и обязанности технических партнеров и партнеров в области научного сотрудничества/ **Roles and Responsibilities of the Technical and Scientific Cooperation Partners**

Институт генетики и цитологии, Национальный координационный центр биобезопасности*	**Institute of Genetics and Cytology, National Coordination Biosafety Centre**	организация всех мероприятий в рамках проекта, включая проведение онлайн-дискуссий и подготовку учебного семинара, а также реализация других мер, необходимых для расширения сотрудничества между партнерами по проекту;	organize all project activities, including the delivery of online discussions and the preparation of the training workshop, as well as other steps needed to facilitate collaboration among project partners
Кафедра биотехнологии и системной биологии Национального института биологии Словении	**Department of Biotechnology and Systems Biology of the National Institute of Biology, Slovenia**	обеспечение экспертного руководства во время онлайн-дискуссий и обучающего семинара (теория и практика);	provide expert leadership during the online discussions and the training workshop (theory and practice)

*При участии НКЦГР с целью максимально эффективного обсуждения вопросов, связанных с генетическими ресурсами, и выработки совместных рекомендаций по всем сопутствующим вопросам

*With participation of the ABS National Coordination Centre in order to maximize the effective discussion on the issues related to genetic resources and the development of joint recommendations for all related issues.

5 Партнерских Институтов / Организаций	**Five Partner Institutes / Institutions**	**активное участие в обсуждениях регламентов, лабораторных методов и подходов во время совещаний в онлайн-формате**	**actively participate in discussions on regulations and laboratory methods and approaches during online meetings**
		активное участие в обучающем семинаре (теория и практика)	actively participate in the training workshop (theory and practice)
		содействие в выявлении дополнительных факторов утраты биоразнообразия	help identify additional drivers of biodiversity loss
		разработка дорожной карты и плана устойчивого развития для региональной сети Центров передового опыта.	development of a roadmap and sustainability plan for the Regional Network of Centers of Excellence

Comparison of key legal and technical regulations in the area of laboratory detection of key drivers of biodiversity loss in countries – partners to the Project

An initial discussion and comparison of the national and the commonwealths of states (European Union, EU, and Eurasian Economic Union, EAEU) legal regulation, as well as multilateral environment agreements (hereinafter – MEAs) that affect area of laboratory detection of key drivers of biodiversity loss during the Second Workshop on December 11, revealed the following:

1. All participating countries are parties to the Convention on Biological Diversity and the Cartagena Protocol on Biosafety therefore the norms of these MEAs influence decisions on the conservation of biological diversity and area of laboratory detection in them.
2. With regard to the Nagoya Protocol on Access to Genetic Resources and Benefit-sharing, which may significantly affect the areas of monitoring and control of genetic resources, and therefore the areas of laboratory detection, all countries except Bosnia and Herzegovina are parties to this multilateral agreement.
3. In all countries except Kyrgyzstan, a biosafety law has been approved and is in force. Starting from 2022, Kyrgyzstan is actively developing a law on biosafety with support from FAO. The areas of regulation covered by the biosafety law may differ in different countries, from regulation of the safety of LMOs[1] (Belarus) to regulation of both LMOs and GMOs[2] (Tajikistan). In countries where GMOs are not covered by biosafety law, there are other regulations to regulate them. At the same time, in Belarus and Tajikistan National Biosafety Frameworks were developed for ensuring biosafety, which cover the concept of biosafety more broadly than LMOs and GMOs and are aimed at the coordinated activities of state regulatory bodies, monitoring and control in the field of other factors of loss of biological diversity.
4. The scope of legal regulation of laboratory detection of plant damage caused by phytopathogens (viruses, bacteria, phytoplasma) is regulated in Slovenia, Bosnia and Herzegovina, Belarus, and Tajikistan. In Serbia, regulatory clarification is needed. Examples of national regulations are presented in Annex 1.

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[1] According to the CPB, LMO - "Living modified organism" means any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology;
"Living organism" means any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids;

[2] Despite the fact that in many countries GMO can simultaneously mean both a living modified organism and products made from it, in this context we will mean use of genetically modified organisms, activity aimed at production and release at the market of GMOs and its products, including investigation, testing and industrial production.

1. Apparently, the least regulated area of laboratory detection by national legislation is the area of laboratory identification of species in order to prevent the loss of biodiversity and prevent poaching activities. This issue will be further clarified during the 5-day training seminar, however, the presentations submitted from countries seem to indicate that in countries regulation applies only to food products, food raw materials of plant and animal origin, and feed. At the same time, Slovenia, Bosnia and Herzegovina and Belarus are developing their own methods, standard operational procedures, instructions which are introduced into the field of laboratory certification and allow for a species-specific identification of a wide variety of species and subspecies. Examples are given in the Annex 2.
2. With regard to the legislation of the Commonwealth of States in Slovenia, Bosnia and Herzegovina and partly in Serbia, national legislation is harmonized with the legislation of the EU regulating the field of laboratory detection. In the countries – Parties to the Eurasian Economic Union, EAEU (Kyrgyzstan and Belarus) along with national legislation, the laws of the EAEU, the so-called technical regulations of the Customs Union (hereinafter - TRCU), apply. In Belarus, national laws in the field of laboratory regulation are fully harmonized with the TRCU. In Kyrgyzstan, partially, since national laws have not been adopted in all areas of laboratory detection. However, in Kyrgyzstan TRCU operates, and, therefore, the legislation of the these countries is harmonized and the same laboratory detection rules apply on their territory.
3. In Tajikistan, national legislation in the field of LMO regulation including laboratory detection is well developed, including several laws, technical code and technical regulations.
4. In order to assess how similar or different the regulation is in countries that are part of the EU and those that are part of the EAEU, a comparison of legislation has been carrying out using the example of the country of Belarus (EAEU country) and Slovenia (EU country). Conclusions were drawn on the similarities/differences between legislation at the level of commonwealths of states and separately on national legislation. The preliminary results are given in tabular form in the Annex 3. Countries – partners to the BioBridge Project will then be invited to make the same comparisons for areas of laboratory detection covered by the project.
5. The draft study will be presented to countries prior to the 5-day theoretical and practical workshop to make additions and will be finalized during the face-to-face workshop, after which a final comparison of legislation on laboratory detection of key drivers of biodiversity loss will be provided.

1. LMO/ GMO detection and identification.

Testing of LMOs/GMOs[1] is mainly based on the detection of recombinant DNA introduced during the transformation process and differentiates LMOs/GMOs from their non-LMO/GMO counterparts (1,2). For the enforcement laboratories in GMO routine analysis the quantitative PCR (qPCR) is the method of choice (3). Methods for detection, identification and quantification of many genetic elements and event-specific targets of commercially available GMOs and reference genes are available in public databases, like the GMO detection database (GMDD; https://gmdd.sjtu.edu.cn/) and GMOMETHODS (http://gmo-crl.jrc.ec.europa.eu/gmomethods/). These databases have been established for the collection and exchange of developed and validated methods. Potential detection approaches for gene edited organisms or products are the same as those currently used for detection/identification/quantification of genetically modified organisms (GMOs).

Where possible control laboratories are using validated methods and are accredited in line with ISO 17025:2019. In addition, other standards and guidelines are followed (the list of standards and guidelines applied in Belarusian GMO detection laboratories accredited by the national accreditation body is in Annex). For example, the National Coordination Biosafety Centre, IGC (Belarus) is accredited by the State enterprise "Belarusian State Accreditation Center" for compliance with the requirements of GOST ISO / IEC 17025-2019 (Accreditation certificate No. BY / 112 1.1599, dated 07.12.2009, valid until December 07, 2024) for the qualitative and quantitative testing of genetically modified organisms. A comprehensive list of laboratory methods in the area of GMO detection of the IGC is given in the Annex. Department of Biotechnology and Systems Biology is accredited by Slovenian accreditation for qualitative and quantitative testing of genetically modified organisms, Reg. No. LP-028 (the scope of accreditation is partially flexible). Both laboratories would be able to test also for new gene edited plants. Decision on granting authorization to test laboratories in Bosnia and Herzegovina for examination, control and monitoring of the presence of genetically modified organisms in food and animal feed is published in Official Gazette of Bosnia and Herzegovina, No. 15/10, followed by publishing the renewing of authorization. Authorization for LMO/ GMO detection and identification is granted to the Laboratory for Biotechnology, Agricultural Institute of Republic Srpska, Banja Luka

[1] According to the CPB, LMO - "Living modified organism" means any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology;
"Living organism" means any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids;

Despite the fact that in many countries GMO can simultaneously mean both a living modified organism and products ma[illegible] it, in this context we will mean use of genetically modified organisms – activity aimed at production an[illegible] at the market of GMOs and its products, including investigation, testing and industrial production.

Laboratory for Genetically Modified Organisms, Federal Agro-Mediterranean Institute Mostar, Laboratory for Genetically Modified Organisms and Food Biosafety, Institute for Genetic Engineering and Biotechnology Sarajevo, Laboratory for Genetically Modified Organisms, Federal Institute of Agriculture, Sarajevo. By the Decree of the Presidium of the Academy of Sciences of the Republic of Tajikistan No. 108 dated 30.11.2015 the Laboratory of Biological Safety was established at the Institute of Botany, Plant Physiology and Genetics of Tajikistan National Academy of Science, the main tasks of which are the development and application of modern methods of analysis for the detection of biological agents and toxins, chemical contaminants in food products and crops, and analysis of GMO products. In Kyrgyzstan two laboratories accredited at this moment in the national system of accreditation, and one in Serbia.

While some countries use complex screening schemes aimed at covering all GM lines on the market (Slovenia, Belarus) and the main method is quantitative real-time PCR, in other countries it is used as qualitative PCR using gel electrophoresis, and quantitative PCR in real time (Bosnia and Herzegovina, Tajikistan Kyrgyzstan).

Besides qPCR, other approaches like digital PCR (dPCR) (4) and next generation sequencing (NGS) (5,6) have been used for the detection of GMOs in some countries. Short description of the approaches including reflections to detection of gene edited organisms and products is provided below.

Quantitative PCR

Current and new approaches in GMO detection are described in a review by Fraiture et al (3). qPCR approach allows detection, identification and quantification of GMO via the SYBR Green or TaqMan chemistries. Due to the wide use of this approach, qPCR analysis tools were also developed in order to facilitate the interpretation of results. Due to the increasing number of GMOs multiplex qPCR strategies were introduced, however, the development of optimal multiplex assays could be more challenging compared to simplex qPCR. Some other alternative multiplex strategies were also considered, such as combination of PCR and capillary gel electrophoresis or PCR and microarrays, Luminex technology (biotinylated targets amplified by single or multiplex PCR assays and analysed by flow cytometry). For improved specificity of qPCR in the detection of single nucleotid variants (SNVs) qPCR can be adapted, for example, by the use of locked nucleic acids (LNAs) or performed as RNaseH-dependent qPCR (7).

LNA probe approach combined with an internal reference probe (drop-off-assay) has already been used as for measurements of gene editing rates in blastocytes and for accurate detection of mutations in mice (8).

Some other adaptations of qPCR were done and are already available also commercially. An example is KASP, where KASP primers (typically three) are custom-designed to target the SNP or InDel of interest (9).

Some qPCR platforms and qPCR master mixes enable also on-site testing.

Digital PCR

Due to the absolute quantification and resilience to inhibitors dPCR could become a key tool in the field of GMO identification/quantification, while this approach is currently, due to the limitations of technology (low throughput and limited multiplexing), less suitable for the screening step (3).

Nevertheless, dPCR has been proposed as a method fit-for-purpose in the development and characterisation of gene-edited organisms (mainly in human and animals). It has been used for assessing the gene-editing frequencies mediated by site directed nucleases already in 2016. Mock et al (10) described the approach for simultaneous detection of wild-type and nonhomologous end-joining (NHEJ)-affected alleles which enables concurrent quantification of edited and wild-type alleles in a given sample. Similarly, Findlay et al (11) described dPCR as a tool for deciphering homozygous from heterozygous mutations in stem cells with superior levels of precision and sensitivity, while Fallabela et al (8) used it in measurements of editing rates in blastocytes and for accurate detection of mutations in mice. Miyaoka et al (12) have employed dPCR for monitoring of genome-editing outcomes in HEK293T and HeLa cells and showed that the HDR/NHEJ ratios were highly dependent on gene locus, nuclease platform, and cell type. It has been shown that dPCR can also be used for precise quantification of large DNA excisions and inversions, and has been modified to measure precise repair of excision junctions and allele-specific excision, which has important implications for disease modelling and therapeutic gene editing (13).

Peng et al (14) have developed and evaluated duplexed dPCR-based method for the detection and evaluation of gene-editing frequencies in plants (rice and canola). They have shown that the method is applicable also to polyploid plants and processed food samples with low concentrations of DNA.

Until now, the dPCR approach for the gene editing has advanced and also commercial solutions are available (e.g. Bio-Rad).

Next generation sequencing

NGS, allowing a massive parallel DNA sequencing, has been suggested for GMO detection as it does not require the prior knowledge of at least a part of the GMO sequences and could overcome laborious and intricate optimisations of multiplex approaches (3). Two main strategies of sequencing exist: targeted sequencing approach (samples are earlier enriched with sequences of interest) and whole genome sequencing (WGS) approach. With targeted sequencing approach two substrategies are possible (i) amplicon sequencing (sequencing of DNA library of PCR products, which depends on PCR strategy) and (ii) target enrichment sequencing (sequencing of selected DNA fragments from a whole genome library, which depends on hybridization methods for capturing selected fragments). Whit the WGS approach the entire DNA library is sequenced, which enables, using bioinformatics tools, characterization of samples without any prior knowledge. Currently, these approaches are not implemented in testing laboratories due to high costs, compared to PCR based approaches, and the need for adequate computer infrastructure and bioinformatic expertise.

Wang et al (15) have determined the copy number, insertion site, and host genome flanking sequence and detected vector backbone insertion and unintended integration using WGS and showed that this can be effective strategy for the molecular characterization of GMOs.

Sequencing methods are suitable also for detection of small DNA modifications like SNVs and short sequence insertions or deletions (InDels), which are frequent results of genome-editing and (7).

Isothermal approaches

Loop-Mediated Isothermal Amplification (LAMP) was proposed for GMO detection due to its rapidity, specificity, sensitivity, and simplicity, but the design of four primers per target, which guarantee the high specificity and sensitivity of the LAMP, could be difficult. In addition, the identification of several GM targets using a multiplex assay is not applicable (3). As the instrumentation for LAMP is portable and easy-to-use, LAMP is applicable for on-site GMO testing (16).

Comparison of approaches

Costa et al (17) were comparing performance of qPCR, dPCR and NGS for molecular characterization of grapevine edited lines. These lines contained a knock-out mutation, obtained via CRISPR/Cas9 technology, in genes involved in plant susceptibility to two important mildew diseases of grapevine. The results showed good agreement in integration copy number of a transgene. NGS was less appropriate as it was not able to discriminate the integration points in three out of ten lines. Still, NGS method could positively identify T-DNA truncations or the presence of tandem/inverted repeats. Based on the results of Costa et al (17) the integrated use of all the three proposed approaches would enable the characterization of transgenic plants already at an early stage.

Conclusion

All listed approaches were used in detection and/or identification and/or quantification of GMOs. All except LAMP, were also employed in the characterisation of gene edited organisms as well as in evaluation of gene editing approaches. All these approaches could be used also in testing of gene edited organisms as they are enabling detection of large and small changes of the genome. In addition, laboratories that are currently testing for GMOs would be capable of testing for gene edited organisms. However, there is a limitation in the use of these methods, especially for small changes in the genome. While detection is possible for such changes, they could also appear naturally or by conventional breeding and thus the identification of the regulated gene edited organism or product may be hampered (7,18).

1. Detection of plant pathogens

Conventional (traditional) diagnostic methods

- Direct examination of dry seeds – inspection;
- Seed washing test;
- Examination of the embryo - Embryo count method;
- Staining Methods;
- Incubation of seeds (Incubation tests);
- Incubation of seeds on nutrient medium (Agar plates);
- Incubation of seeds on moist filter paper (Blotter test);
- Seedling symptom tests (Biotest).

Immunodiagnostic (conventional) methods

Enzyme immunoadsorption test - ELISA test;
Immunofluorescence test - IF Test;
Seed immunoblot binding assay – SIBA;
Dyed latex bead agglutination test - LA test;
Immunodipstick assay.

Molecular methods

Methods based on PCR:
(Conventional PCR, Nested PCR, Multiplex PCR, RT-PCR, Bio-PCR, etc.);
Genetic imprint - fingerprinting;
Methods based on DNA hybridization;
DNA sequencing.

European and Mediterranean Plant Protection Organization contains compendium of methods to detect plant pathogens (19). Database of the National Institute of Biology also contains methodologies for bacteria, phytoplasmas, viruses and viroids detection (20) These databases can contribute capacity building in CEE-CA Network of Centres of Excellence

Conclusions

Since there are a lot of diseases and they differ in the causative agent that causes them (various bacteria, viruses and phytoplasma), the choice of the most suitable method is based on case by case. At the same time, PCR method and its variations are increasingly being used, as one of the most accurate methods. PCR is very often used along with ELISA to detect seed planting material for virus infection.

Since pathogens may differ from region to region, from country to country, it will be relevant to create a comprehensive list of pathogens for cultivated crops and wild plants in the country, jointly develop methodologies for common types of phytopathogens by the CEE-CA Network and, based on the established capacity, each country will be able to develop DNA or RNA markers and protocols for the detection of specific phytopathogens relevant for the country.

The existing databases of international organizations and individual institutions can greatly help the CEE-CA network of Centers of Excellence in building capacity both to develop screening complex schemes and reliable methods for the presence of phytopathogens in crop seeds and in wildlife.

1. Species-specific identification

Since there are a lot of species of plant and animal origin that need to be identified in a country or region in order to conserve biodiversity, the choice of the most suitable method is based on case by case.

This is probably why there is no clear legislation in countries regarding their laboratory control, especially for wild species. At the same time, as necessary, methods for detecting species are being developed in countries, a number of which are included in the scope of accreditation, and therefore constitute a regulatory legal act of laboratory regulation for a given species.

Currently, GOST 31719-2012 “Food products and feed. Express method for determining raw material composition (molecular)” has been introduced into the scope of accreditation of a number of countries. The standard is intended for the accelerated identification of species-specific DNA of cattle (Bos taurus), pig (Sus scrofa), chicken (Gallus gallus), soybean (Glycine max), corn (Zea mays), potato (Solanum tuberosum), etc. in feed compositions, raw materials, semi-finished products, finished food products using polymerase chain reaction (PCR) in order to prevent product counterfeiting and poaching. GOST is also used by laboratories involved in the analysis of falsification of one type of fish and seafood by another.

Because species identification needs may differ between countries, proprietary techniques are being developed, primarily based on PCR and its variations.

Examples include developed methods introduced into the scope of accreditation of **the Institute of Genetics and Cytology of the National Academy of Sciences of Belarus** (21):

- Identification and certification of varieties of agricultural crops (soft wheat, potatoes, tomatoes, flax and beets) based on DNA markers.
- Methodological recommendations for identification and certification of apple and pear varieties based on DNA markers.
- Guidelines for the use of DNA testing in livestock farming in Belarus.
- Instructions for the use of molecular genetic analysis to establish the species (population) identity of fish of the sturgeon family and products made from them.
- Technology for genetic identification of salmon species in fish raw materials and food products (salmon, rainbow trout, pink salmon, coho salmon, chum salmon, sockeye salmon).
- Technology for genetic identification of fish and seafood species in fish raw materials and food products.

Bosnia and Herzegovina:

- Test of the quality of the mother flock and fry of fish;
- Selective breeding of fish;
- Hybridization test for salmonids and cyprinids;
- Genetic screening (mitochondrial DNA and lactate dehydrogenase, LDH) of the mother flock and juvenile fish;
- Genetic characterization of Bosnia and Herzegovina autochthonous fruit and vegetable varieties;
- Genetic characterization of Bosnia and Herzegovina autochthonous cattle breeds;
- Genetic characterization of Bosnia and Herzegovina autochthonous varieties of forest trees.

At the same time, as in the case of phytopathogens it will be relevant to create in each country a comprehensive list of interest of plant, animal and fungi origin, jointly develop methodologies of species-specific identification for common species of interest and, based on the established capacity, each country will be able to develop PCR markers and protocols for the detection of specific species for the country.

Conclusion

For species-specific assessment, there are regulations in the field of methodological detection of species that describe sequences and primers for a number of plant and animal species. Some partner countries, such as Slovenia, Bosnia and Herzegovina, and Belarus, are developing their own methods for detecting the species of interest and detection protocols, mainly based on PCR and variations of this method.

Since there are a lot of species of plants, animals and fungi on earth, and since the purposes of laboratory identification of species in the field of conservation of biological diversity may vary from region to region and from country to country, for example, to prevent poaching and extermination of species, analysis after an incident of poaching in forensic science, analysis of adulterations in food, food products, and stern, etc., it is advisable to create a comprehensive list of interest of plant, animal and fungi origin in the country, jointly develop methodologies of the species-specific identification for common species of interest and, based on the established capacity, each country will be able to develop PCR markers and protocols for the detection of specific species for the country.

"Enhancing Collaboration between the CEE and Central Asia's Centres of Excellence to Address the Key Drivers of Biodiversity Loss and Maintain Human, Crop and Livestock Health"

Networking of 7 CEE and Central Asisn countries and improvement of laboratory detection with the application of molecular genetic methods *for identification of key drivers of biodiversity loss*

- *Species-specific identification*
- *LMO detection*
- *Pathogen detection…. others*

Reliable methods – the Fine Art of Global Collaboration

Көңүл бурганыңыздарга рахмат!

Hvala vam na pažnji!

Дзякуй за ўвагу!

Хвала на пажњи!

Ташаккур барои таваҷҷӯҳ!

Hvala za pozornost!

Thank you for your attention!

Благодарю за внимание!