Contribution ID: c37e13c9-9918-4463-a80f-dcb3925bcecf
Date: 30/04/2020 16:06:38

Member State questionnaire on new genomic techniques to contribute to a Commission study requested by the Council

Fields marked with * are mandatory.

Questionnaire on new genomic techniques to contribute to the study requested by the Council

endorsed in the Joint Working Group of GMO competent authorities on new genomic techniques on 15 January 2020

Introduction

With this questionnaire the Commission is collecting contributions from Member States competent authorities to respond to the Council's request[1] for "a study in light of the Court of Justice's judgment in Case C-528/16 regarding the status of novel genomic techniques under Union law" (i.e. Directive 2001/18 /EC, Regulation (EC) 1829/2003, Regulation (EC) 1830/2003 and Directive 2009/41/EC). The scope of the study goes beyond new mutagenesis techniques, as there are other new techniques, for which the Council seeks clarification. Therefore, the study covers all new genomic techniques, which have been developed after 2001.

For the purpose of the study, the following definition for new genomic techniques (NGTs) is used: techniques, which are capable to alter the genetic material of an organism and which have emerged or have been developed since 2001[2].

Unless specified otherwise, the term "NGT-products" used in the questionnaire covers plants, animals, micro-organims and derived food and feed products obtained by NGTs for agri-food, medicinal and industrial applications and for research. GMO competent authorities are invited to seek input from other competent authorities when appropriate.

The questionnaire is meant to provide information primarily, but not exclusively, at national level. Please substantiate your replies with explanations, data and source of information as well as with practical examples, whenever possible. If a reply to a specific question only applies to a specific NGT, please indicate this in the reply. With regard to agri-food applications, replies may include considerations on specific sectors, such as the organic sector.

Please indicate which information should be treated as confidential in order to protect the commercial

interests of a natural or legal person. Personal data, if any, will be protected pursuant to Regulation (EU) 2018/1725 [3].

[1] Council Decision (EU) 2019/1904, OJ L 293 14.11.2019, p. 103-104, https://eur-lex.europa.eu/eli/dec/2019/1904/oj
[2] Examples of techniques include: 1) Genome editing techniques such as CRISPR, TALEN, Zinc-finger nucleases, mega nucleases techniques, prime editing etc. These techniques can lead to mutagenesis and some of them also to cisgenesis, intragenesis or transgenesis. 2) Mutagenesis techniques such as oligonucleotide directed mutagenesis (ODM). 3) Epigenetic techniques such RdDM. Conversely, techniques already in use prior to 2001, such as Agrobacterium mediated techniques or gene gun, are not considered NGTs.
[3] Regulation (EU) 2018/1725 of the European Parliament and of the Council of 23 October 2018 on the protection of natural persons with regard to the processing of personal data by the Union institutions, bodies, offices and agencies and on the free movement of such data, and repealing Regulation (EC) No 45/2001 and Decision No 1247/2002/EC, OJ L 295, 21.11.2018, p. 39–98

Instructions

Please note that the survey accepts a maximum of 5000 characters (with spaces) per reply field. You might be able to type more than 5000 characters, but then the text will not be accepted when you submit the questionnaire. You will also receive a warning message in red colour below the affected field.

You have the option to upload supporting documentation in the end of each section. You can upload multiple files, up to the size of 1 MB. However, note that any uploaded document cannot substitute your replies, which must still be given in a complete manner within the reply fields allocated for each question.

You can share the link from the invitation email with another colleague if you want to split the filling-out process or contribute from different locations; however, remember that all contributions feed into the same single questionnaire.

You can save the draft questionnaire and edit it before the final submission.

You can find additional information and help here: https://ec.europa.eu/eusurvey/home/helpparticipants

Participants have until 30 April 2020 (closure of business) to submit the questionnaire via EUsurvey.

QUESTIONNAIRE

* Which Member State are you representing?

Hungary

A - Implementation and enforcement of the GMO legislation with regard to new genomic techniques

* **1. Have you been consulted by companies/organisations/research institutes for regulatory advice or another issue on products developed or to be developed by NGTs ?**

- ○ Yes
- ◉ No

* **2. Have you taken specific measures (other than inspection) related to the application of the GMO legislation to NGT-products?**

- ◉ Yes
- ○ No

* Please describe the measures and, if possible, their effectiveness

According to Act No. XXVII of 1998 on gene technology activities, the responsibilities of authorisation of gene technology activities in Hungary are divided between two competent ministries, depending on the field of the respective activity. In case of gene technology activities in the agricultural and food sector (including processed additives used in food production) and in contained use, as well as in case of other industrial gene technology activities, the Ministry of Agriculture as the environmental, agricultural and industrial gene technology competent authority is responsible (EAI-CA). In case of gene technology activities related to human health, to the production of human pharmaceutical products and to cosmetics in direct contact with the human body, the competent healthcare gene technology authority is the National Institute for Pharmacy and Nutrition (HH-CA).
After the ruling of the Court of Justice of the European Union on new mutagenesis techniques, in March 2019 the EAI-CA requested the users to report about all gene technology activities including the activities with regard to new mutagenesis techniques that were carried out. According to the data gathered through the reporting, new mutagenesis techniques were applied in contained use only, mostly for basic research. At that time the EAI-CA also informed all stakeholders about the outcome of the ruling and called upon them to apply for the necessary authorisations with regard to class 2, 3 and 4, if applicable and also with regard to field and clinical trials.
In February 2020 the EAI-CA authority requested the users, who are currently carrying out researches in contained uses to report about all the gene technology activities relating to new genomic techniques (NGTs) according to the definition and examples determined in the introduction of this questionnaire. The results of this reporting can be found in section "Information on research and innovation", questions 8-12, particularly question 8.
Both data gathering exercises were very useful, as now the EAI-CA has all the necessary information about the ongoing research activities relating to NGTs carried out in Hungary. The EAI-CA is now informed not only about the results, but also about the founding formations, the purposes of the respective researches and the concerns of the applicants/users. Secondly the data request was a good opportunity for the EAI-CA to communicate with the users in order to inform them about the situation on NGTs. The users also welcomed the update provided by the EAI-CA on the ongoing activities on EU level and also information on the legal status and on the possible consequences.

* What best practices can you share?

See the details in the previous sub-reply.

* 2 bis. Have you encountered any challenges or limitations, including administrative burden or costs?

- ○ Yes
- ◉ No

* Please explain why not

According to Decree No 138/2004. (IX. 23.) of the Ministry of Agriculture and Rural Development on the authorization fees for the authorization of the gene technology activity, an administrative service fee shall be paid to the EAI-CA for each procedure except for public institutes, universities, and institutes of the National Academy of Sciences. The major part of the gene technology activities relating to NGTs in Hungary are carried out by these institutions, therefore these applicants are not charged by administrative fees. The rest of the activities carried out by companies, already have their permissions for conducting these types of researches.

* **3. Have you adapted your inspection practices to cover all NGT-products and to ensure the enforcement of traceability requirements?**

- ○ Yes
- ◉ No

* Please explain why not

Government Decree No. 22 of 2012 (II. 29.) on the National Food-Chain Safety Office entered into force on 15 March 2012, created the National Food Chain Safety Office (NFCSO) under the direction of the Minister of Agriculture. The NFCSO is the central governmental competent authority responsible for carrying out official controls in relation to food safety and food quality requirements.

1. Food and Feed samples:

Within the NFCSO, Presidential Decree No 5/2013 introduces an integrated approach to improve the Food and Feed Chain Monitoring Plan. A coordinating working group (headed by NFCSO-SMSD: Directorate for System Management and Supervision) has been set up to ensure that the planning is in line with objectives, to carry out risk assessment, to identify the priorities in the control system, to follow up the execution of the plan.

NFCSO- Food Chain Safety Laboratory Directorate – NFCSO-FSLD GMO analysis team of Microbiological National Reference Laboratory (MNRL), is the only official control laboratory performing GMO analyses of food, feed in Hungary and performs confirmatory tests of plant propagating materials.

The GMO analysis team is accredited to detect and quantify all the EU-authorised GMO events for maize, soy, rapeseed and sugar beet using real-time PCR method. In addition to the above mentioned accredited tests the laboratory performs tests for LLP (low level presents) GMOs according to the 619/2011 Regulation. Furthermore the laboratory is able to detect some unauthorized GMOs for which the detection method is available at the EU Database of Reference Methods for GMO Analysis webpage https://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx

The GMO analysis team uses validated methods available at the EU Database https://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx

The laboratory at the moment is not able to detect GMOs, which were produced by NGTs, because there are no validated methods and no reference materials available yet on the above-mentioned database.

The laboratory follows up other databases as well. Some GM plants (none of them are approved in the EU), produced by new genomic techniques can be found in the EUginius (https://www.euginius.eu/euginius/pages

/gmo_fulltext_searchresults.jsf) database, but there are no detection methods and no reference materials available yet in this webpage either (only taxon-specific methods can be found as detection method).

If validated real-time PCR methods are available for the detection of NGT products, the laboratory is ready to perform the verification procedure and the method can become accredited in a short time. If the validated detection method requires other techniques or equipments than real-time PCR the laboratory is able to respond to changes.

2. Seed samples:
Samples for GMO testing (annual sampling plan for GMO testing) are selected by NFCSO-Seed Inspection Department (SID). The screening tests of plant propagating materials are performed in the Central Seed Testing Laboratory (ISTA accredited).
In case of plant propagating material seed samples risk-based selection of samples is performed. 10% of the import items are marked out for GMO testing and 5% of the Hungarian production. If the seed lot is coming from a country where GMO cultivation is allowed, the sampling is more intensive than if it is coming from a country where GMO cultivation is prohibited.
PCR technique is used to screen and identify GMOs. Contaminated seed lots are officially destroyed. NGTs have not appeared in our system yet.

Annual report is sent to the Ministry of Agriculture about the test results of seed, food and feed samples.

* 3 bis. Have you encountered challenges or limitations, including administrative burden or costs?

- ○ Yes
- ◉ No

* Please explain why not

We have not yet encountered any challenges or limitations, including administrative burden or costs, because in the inspection system these situations have not yet appeared due to the unavailability of validated methods and reference materials.

* **4. Do you have experience or information on traceability strategies, which could be used for tracing NGT-products?**

- ◉ Yes
- ○ No

* Please describe the traceability strategy, including details on the required financial, human resources and technical expertise required

Currently we do not have concrete information on possible traceability strategies, however targeting the unique, genome edited sequence in the product obtained by NGT or protospacer adjacent motifs in products obtained by CRISPR/Cas9 allows a possibility for analysis. Alternatively, fingerprint genes (if any) possibly introduced by variety developers (but not related to the genetic modification by NGT) could be used for tracing.

Those farms that are GMO-free (including NGT-free) – actually all farms in Hungary, as there is no cultivation of any kind of GM plants (including plants derived from NGTs) in the country– need transparency with regard to the techniques used in the production of new varieties. In order to ensure this, the respective

techniques that had been used in seeds and seedlings should be declared. Traceability should be guaranteed in case of all NGT-related food and feed products through the whole supply chain.

* What best practices can you share?

See the details in the previous sub-reply.

* 4 bis. Have you encountered any challenges or limitations, including administrative burden or costs?

- (●) Yes
- ( ) No

* Please describe

Yes, there are certainly limitations, i.e. fingerprint genes may not exist in certain cases, and we may also face with the problem which relates to proprietary, confidential information.

* How could these challenges or limitations be overcome?

See the details in the reply to the main question 4.

* **5. What other experience can you share on the application of the GMO legislation, including experimental releases (such as field trials and clinical trials), concerning NGT-products in the:**

- [x] **Agri-food sector?**
- [x] **Industrial sector?**
- [ ] **Medicinal sector?**

Agri-food sector

In Hungary there is neither ongoing field trial nor clinical trial regarding NGTs in relation to the agri-food sector.
However, when talking about experimental releases, field or clinical trials, it is important to take into account, that NGTs are expected to show the same hazards as transgenic organisms used in open technologies: cross-fertilization or cross-pollination, in case if the genetic modification is inherited.

Industrial sector

In Hungary there is neither ongoing field trial nor clinical trial regarding NGTs in relation to the industrial sector.

* **6. Have plant varieties obtained by NGTs been registered in national catalogues?**

- ( ) Yes
- (●) No

* **7. Do you require specific information in national catalogue when registering plant varieties obtained by NGTs?**

- ( ) Yes
- (●) No

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

The maximum file size is 1 MB

B - Information on research and innovation

* **8. Have you supported with national funding programmes NGT-related research projects/programs (ongoing or finalised in the last 5 years), including on identification or traceability?**

- (●) Yes
- ( ) No

* Please provide an overview of the project/program including title of project, a brief summary with scope and objectives, the amount of national funding received and possibly specify if the receiving entity is public or private

Yes, there are twenty NGT-related research projects supported with national funding programmes. Please find the titles, brief summaries and the amount of the national funding received of the main state funded NGT-related research programmes' (= OTKA, Hungarian Scientific Research Fund) (ongoing or finalised in the last 5 years) in the Attachment 1.
Within the framework of the Thematic Excellence Programme started in 2019, 55 research themes of 26 universities and research institutions received around HUF 14.6 billion funding altogether. The programme aims to align research conditions to societal and economic challenges, to strengthen their R&D and innovation focus and increase the productivity of science. Some NGT-related programmes also gained support within the above mentioned framework in 2019.
As it was already mentioned in question 2, in February 2020 the EAI-CA requested the users, who are currently carrying out researches in contained uses to report about all the gene technology activities relating to new genomic techniques (NGTs) according to the definition and examples determined in the introduction of this questionnaire. Please find the results of the data gathering in the Attachment 2, including three figures. All the NGT-related researches are being carried out only in contained uses in Hungary. Currently there are no ongoing deliberate releases to the environment other than placing on the market (experimental field trials and clinical trials) of products obtained by NGTs. According to the results of the data gathering, altogether in Hungary there are about 100 authorised premises carrying out more than 130 NGT-related gene technological contained use activities (only in class 1 and class 2).
Figure 1. shows the proportion of NGT-related researches using the different techniques in contained uses in Hungary in 2020. The overwhelming majority of researches are carried out by CRISPR techniques; however, there are also examples for other techniques.
In Hungary, in 2020 the NGT-related researches are used mainly for basic research purposes (Figure 2.). However, industrial applied researches are also being carried out, but only by private companies. Some universities and national academic institutions are doing the researches for experimental development and for innovation purposes.
According to Figure 3., the proportion of the different funding types for NGT-related researches can be found. 33 % of the total number of researches is fully nationally funded. This funding type is used only by universities and national academic institutions. Researches which are not funded either partially or fully by the state are conducted mainly by companies, but by some universities and national academic institutions as well.

* 8 bis. Please highlight the potential challenges encountered when supporting/funding NGT-related research and any consequences from these challenges.

No challenges were encountered in course of supporting NGT-related research.

*** 9. How do you see NGT-related research evolving?**

The various NGT technologies developed during the last decade (e.g.: ZFN, TALEN, CRISPR/Cas9, etc) represent the most important, fastest evolving and spreading, most widely used methods, both in the academic laboratories, and in the companies dealing with practical applications.
In the meantime, hazard/risk assessment, unfortunately, is not developing at the outstanding pace of NGT development, which raises the likelihood of unknown hazard factors. Although NGTs are more accurate than earlier GM technologies, but not sufficiently. The accuracy of CRISPR/Cas9, although indeed being far more targeted than transgenesis achieved e.g., via Agrobacterium mediated techniques or gene gun, has been shown not to be as accurate as claimed before (in CRISPR/Cas9, both the cut and the insertion results in several mismatches). On the other hand, precision cannot be considered an indication of safety, especially in relation to the novel traits created by such modifications.
In case of the medical sector, the development of human GMO medicinal products is extremely fast and promising especially regarding rare and debilitating genetic diseases when other treatments are not available or not effective.

*** 10. Have you identified any NGT-related research needs from private or public entities?**

- (●) Yes
- ( ) No

* Please specify which needs and how they could be addressed

The applications which relates to NGTs are fairly new, therefore only a few plants obtained by NGTs have gone through risk assessment for cultivation purposes so far. These applications contain novel traits that are not present in agricultural plants currently in cultivation and have never been assessed. Because of this insufficient knowledge and experience for a variety of applications, and based on the findings of Eckerstorfer et al. (2019) a case-specific risk assessment is needed. These applications (e.g. crop protection by resistant crops) are prone to the same limitations as conventional ones (emergence of pest resistance or secondary pests), which raises the question, that should be answered on a case by case basis, whether it is worth releasing certain new varieties produced by NGTs. Risk assessment is not yet at the level to answer/address these questions, therefore hazard researches should be carried out.
A crucial issue relating to the products obtained by NGTs is traceability. Detection and the possibility to distinguishing products produced by these techniques is of utmost importance for implementation and also for socio-economic considerations. Therefore, dedicated research and funding is needed to address this problem and to develop an appropriate traceability methodology and strategy.

*** 11. Could NGT-related research bring opportunities/benefits to science, to society and to the agri-food, medicinal or industrial sector?**

- (●) Yes
- ( ) No

* Please provide concrete examples/data

1. NGT-related research on identification and detection:
A crucial issue relating to the products obtained by NGTs as already mentioned is traceability. Detection and the possibility to distinguishing products produced by these techniques is of utmost importance for implementation and also for socio-economic considerations, therefore the development of these methods relating to identification and detection of NGTs and NGT-related products could bring benefits not only to science, but to the society and also to the agri-food, medicinal or industrial sectors.
2. NGT-related environmental and health risk assessment research:
NGT-related environmental risk assessment researches studying the effects for example on non-target species could also bring considerable benefits mainly to science, but also to the society. Carrying out more NGT-related health impact assessments are of utmost importance to the society in order to prove the safety of the products obtained by NGTs.
3. NGT-related research in the medical sector:
NGT-related research could bring opportunities in particular for the medicinal sector.
Overall, the global research effort must remain focused on treating disease rather than engineering new traits or creating new organisms.

* **12. Could NGT-related research bring challenges/concerns to science, to society and to the agri-food, medicinal or industrial sector?**

- (●) Yes
- ( ) No

* Please provide concrete examples/data

1. NGT-related research on identification and detection:
The absence of validated methods and reference materials is a huge risk that could bring challenges to society and to the agri-food sector through the difficulties of monitoring those products obtained by NGTs which are already in the market in third countries.
2. NGT-related environmental and health risk assessment research:
The absence of NGT-related environmental and health risk assessment research is also a critical issue that could bring challenges and raise deep concerns to society and to the agri-food sector. The risks should be assessed in accordance with a coherent, efficient, predictable and scientifically sound framework and a complete risk assessment should be carried out for all products obtained by NGTs that are planned to be marketed and applied for authorisation. To our knowledge there are only some risk assessment studies done or currently ongoing in the territory of the EU, particularly field trials. For the agri-food sector cross-pollination remains a similar untenable problem with NGT products as with transgenic GMOs, just like management of organic farming and co-existence. The absence of these environmental and health impact studies could definitely raise concerns to the whole society.
3. NGT-related research in the medical sector:
For the medicinal sectors (and for society) new challenges abound. At present any heritable human genetic modification (i.e. germline gene therapy) is forbidden but there is increasing pressure, that with radical safety improvement, such modifications should eventually be allowed. This is a hotly debated issue. Even in the case of somatic gene therapy which is already possible, the danger of unintended effects (off-target mutations) still exists, and serious efforts are needed to diminish, or exclude these possibilities.
Overall, cross-sector collaboration, informational transparency, honest dialogue, and a commitment to scientific excellence and integrity are all essential elements to treating genetic disease through the modification of a patient's DNA (regardless the type of technology).
4. Concerns in relation to gene drives-related research
In certain cases, e.g., when NGTs are used to create gene drives, drastic environmental biological consequences can occur that can severely affect biodiversity. (In fact, such gene drives are targeted to cause extinction of species.)

For the society several questions induced from the experimental releases of gene drive organisms raised concerns and doubts, i.e. the morality of editing genes in wildlife to decrease or eliminate local populations of animals or plants that are causing environmental and/or health problems. An ecosystem can sometimes adapt to human alterations in ways that cannot be reversed without bringing about still more unwanted changes. According to the literature to date the potential adverse ecological outcomes following the deliberate or accidental release of gene drive organisms are the same as, or at least similar to, those identified for transgenic crops and animals, invasive species and biocontrol agents (Hayes et al. 2018).
Research on gene drives presents questions of justice. Questions relating to who would be affected by the benefits and harms, who will be able to conduct research into gene drive technologies and study the release of gene-drive modified organisms, and who will make the decisions about whether to pursue the benefits and risk the potential harms. Questions about the distribution of potential benefits and harms, about liberty, about the nature of legitimate decision making for matters affecting the public. They are about how communities and nations are affected by gene drive technologies, the ability of scientists and funders to undertake the research, and the relationship of citizens to nations and of nations to each other (Gene Drives on the Horizon 2016).
Selection of sites for field trials or open environmental releases, it is important that researchers consider the values of the publics affected by the release and their understanding of the balance of benefits and harms. The expectation that people should have a voice in fundamental decisions that affect their health and their environment is particularly important and may generate additional guidelines for the release of gene-drive modified organisms. Approaches to ensure that communities participate meaningfully in decision making about the use of gene-drive modified organisms will be essential, particularly in low- and middle-income countries where power differentials may affect such participation.

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

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C - Information on public dialogues and national surveys

* **13. Have you or other institutions/bodies/entities organised national dialogues concerning NGTs?**

- (●) Yes
- ( ) No

* Please describe briefly the content, methodology and conclusions

After the Court Ruling, certain institutions organised national dialogues concerning NGTs.

The Ministry of Agriculture (EAI-CA) organised a GMO thematic event in May 2019 focusing on the following four topics:
- actualities of the national and European Union legal environment relating to NGTs;
- questions on the possible human and environmental risks of products obtained by NGTs – the relevance of the current risk assessment to products obtained by NGTs;
- the possible socio-economic aspects of products obtained by NGTs; and
- the concerns and difficulties of detection and identification of products obtained by NGTs. The event was organised in a closed formation by dedicated invitation. On the thematic day, more than 50 experts represented Hungarian research institutes, enterprises, universities, plant breeders, as well as licensing and inspection authorities engaged in genetic engineering activities. NGOs, beekeepers and experts in the food

and feed industry, among others, also took part in the discussion.

The Parliamentary Committee on Sustainable Development, the Advocate of Future Generations, the Hungarian Friends of the Earth partner, the Hungarian Bioculture Association and the Central Hungarian Green Circle organised an open conference in September 2018 titled as New breeding techniques and genetic modification.

The Hungarian Academy of Sciences in cooperation with the OECD organised a symposium, CRISPRing – A New Beginning for the Genetic Improvement of Plants and Microbes, in September 2018 in order to review achievements, new developments, trends and challenges in the field of gene editing, and to foster a broad exchange of information within the scientific community (https://crispring.agrar.mta.hu/).

* **14. Have you or other institutions/bodies/entities organised national surveys, which assessed public opinion on NGTs?**

- ○ Yes
- ◉ No

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

The maximum file size is 1 MB

D Information on ethical aspects

* **15. Have any national bodies or expert groups discussed or issued opinion on the ethical aspects of NGTs?**

- ○ Yes
- ◉ No

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

The maximum file size is 1 MB

E - Information on opportunities and benefits from the use of NGTs and NGT-products

* **16. Could the use of NGTs and NGT-products bring opportunities/benefits to the agri-food, medicinal or industrial sector?**

- ◉ Yes
- ○ No

* Please provide concrete examples/data

NGTs and NGT-related products may constitute advancements in the field of the agri-food, medicinal and industrial sector.
However, since there have not yet been NGT products in Hungary, and there are no ongoing field trials with plants produced by NGTs, there is practically no experience on the possible real benefits to the agri-food or industrial sector.
According to the present experience, mainly the medicinal sector could benefit from using these technologies and products. In all cases, and in all sectors, approval however, should be a subject of strict and precautious risk assessment.

* **17. Could the use of NGTs and NGT-products bring opportunities/benefits to society in general, such as for the environment, human, animal and plant health, consumers, animal welfare, as well as social and economic benefits, in the short, medium and long term?**

- (●) Yes
- ( ) No

* Please provide concrete examples/data

When new technologies are invented, and this is valid also for NGTs, our task is not only to consider whether they are safe to use, safe for the environment or human, animal and plant health, but also, we should ask where, how, and for what purpose we want to use these technologies. Therefore, not only scientific knowledge is relevant, but also practical aspects of the application of the technology and societal goals that may be realized or threatened by the technology.

* Under which conditions do you consider this would be the case?

Not appropriate.

* **18. Do you see particular opportunities for SMEs on the market access to NGTs?**

- (●) Yes
- ( ) No

* Please explain under which conditions

Regarding medicinal products containing GMOs SMEs may find opportunities during the clinical trial processes. Nonetheless up to now, HH-CA has not received any scientific advice or regulatory requests from Hungarian SMEs that has reached clinical trials at the level of development.

* **19. Do you see benefits/opportunities in patenting or accessing patented NGTs or NGT-products?**

- (●) Yes
- ( ) No

* Please describe and provide concrete examples/data

NGTs or NGT-products can be patented, which is an important factor in progress in biotechnological industry. In addition, some of these new technologies, such as gene editing by the CRISPR/Cas9 system, are accessible to thousands of individuals, therefore patenting is one of the most important issues in case of these technologies.

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

The maximum file size is 1 MB

F - Information on potential challenges and concerns of NGT products

* **20. Could the use of NGTs and NGT-products raise challenges/concerns for the agri-food, medicinal or industrial sector?**

- (●) Yes
- ( ) No

* Please provide concrete examples/data

Also, see question 12.
Yes, NGTs and NGT-products definitely raise concerns for each of the sectors. Please find below the main areas of challenges:

1. Agricultural structure

In the EU, and also in Hungary family farming is an important tradition (Eurostat 2018). Many farms have been owned and run by the same family for generations. Rural areas (farm land and forests) cover over 90% of EU territory and are home to around half of its population (farming community and other residents). These small farms can play an important role in reducing the risk of rural poverty, providing additional income and food.

Since in the EU there is no NGT-related product approved for cultivation, the only socio-economic considerations can be drawn from experiences outside the EU. Therefore, we should be very cautious when comparing the EU's agricultural schemes with third countries. Thus, conclusions of the production of GM /NGT-related crops cannot be drawn from the current experiences in the USA based on the fundamental structural differences between the USA and Europe, as such (USDA, NASS 2019):

- There are 10.5 million farms in the EU, with an average mean size of 16.6 ha while the two-thirds of all the farms are less than 5 ha in size. In comparison, the number of farms in the USA is estimated at 2,029,200. The average farm size is 179 hectares.2. The overwhelming majority (96.0 %) of the EU's farms are classed as being family farm as already mentioned above. More than nine in every ten farms (93.0 %) in the EU only had family workers (the farmer and his/her family members) in 2016.
- Corporate farming – where the farm (i.e. the land, buildings, machinery and livestock) is owned by a commercial company that employs a manager to run it – is rather unusual in Europe.
- In the EU in case of small family farms the food is local and travels short distances to get to market. In the USA in case of giant factory farms the food travels hundreds if not thousands of miles to get to market. Thus, food products in the USA requires a great deal of processing and preservatives in order to remain edible after traveling over vast distances.

The above mentioned differences have significant cultural, social and also economic implications for the respective societies.

2. Transparency on the information of seeds and seedlings

Those farms that are GMO-free (including NGT-free) – actually all farms in Hungary, as there is no cultivation of any kind of GM plants (including plants derived from NGTs) in the country– need transparency with regard to the techniques used in the production of new varieties. In order to ensure this, the respective techniques that had been used in seeds and seedlings should be declared. Traceability should be

guaranteed in case of all NGT-related food and feed products through the whole supply chain. (see also response to question 4.)

* **21. Could the use of NGTs and NGT-products raise challenges/concerns society in general, such as for the environment, human, animal and plant health, consumers, animal welfare, as well as social and economic challenges, in the short, medium and long term?**

- (●) Yes
- ( ) No

* Please provide concrete examples/data

Since Hungary is amongst the five largest sowing seed exporters in the world (Seed exports 2016) proving and protecting the genetic purity and GMO-free status of seeds is an issue of primary importance for the producers and foreign trading partners as well. Therefore, it is crucial to guarantee that crops as well as seeds and propagating materials produced in Hungary are free from any GMOs (including organisms obtained by NGTs), since no cultivation of any kind of genetically modified plant variety/hybrid is authorised in Hungary according to the legislation in force.
NGTs can target areas of the genome that are normally highly resistant to mutation. Research has found that gene editing can result in numerous unexpected, unpredictable and undesirable outcomes, even at the intended gene editing site. This includes large deletions and complex rearrangements of DNA and the creation of new proteins. It is important to note that these unpredictable and undesirable genetic mutations result after the gene editing tool has completed its task (e.g. of creating a break in the DNA) and will occur regardless of the precision of the initial edit. There is no guarantee that the use of these techniques will result in predictable outcomes – or that any resulting products will be safe.
From an ecological point of view, the biggest concern is the time. NGTs can lead to modifications to genes as different or even more pronounced than introducing genes from other species. This is due to the ability to apply these techniques rapidly and repeatedly to the same genes or to simultaneously or serially alter many genes at once – either in a laboratory or in the open environment – with unknown ecological consequences. With these techniques, modified organisms can be engineered and produced in a very short time, while those would either never appear naturally or would take hundreds of thousands or even millions of years to develop. No one is prepared to these extremely rapid changes, especially the biosphere. So, the main question is not the precision of a technique, but the fate of the modified genome. In contained use (e.g.: vaccine production), both the modification of the genome and the selection thereof remain in our hands (provided that we can guarantee closed circumstances). In case of release into nature however, these two processes go separately and we lose control. The released modified genome can interact with the genomes of the natural ecosystems (through gene transfer, cross-breeding) and we do not know how these genomes or genes would integrate in these natural systems, what would be there impact on other organisms, what kind of population genetics or even evolutionary changes they generate. The important question with regard to these organisms is whether we can keep them under our control, or lose our control over them.
Furthermore, long term effects on the environment cannot be foreseen at this stage. NGTs and the related products potentially trigger similar environmental, social and economic concerns as transgenic GMOs.
It should also be taken into account, that intensification of technologies that are open to the environment can be achieved only at environmental costs (pollution, biodiversity loss, emergence of resistance, etc.). NGTs are no exceptions from this. Just such environmental costs are less apparent at the current level of use.
Organic farming and seed production are particularly counter-interested in the using of such technologies and their products. Therefore, appropriate legal measures are needed to be in place measures to protect breeding and seed production for organic farming against GMO contamination. Difficulties in traceability of new technologies also pose a risk, as the lack of such methods/strategies make impossible to apply the coexistence rules on conventional and NGT-related crops.
In the light of rapid technological development of NGTs, time pressure constitutes a serious problem for

taking regulatory decisions, including labelling requirements, regarding the agricultural use of NGTs (Bechtold S. (2018)). Freedom of choice should be given to all consumers, both who have negative attitude to such techniques and its products but also for those who endorse the use of NGTs. Food and feed products obtained by NGTs should be labelled and provide the necessary information and should not be ambiguous.
With establishing the legal framework of GMO-free labelling and the GMO-free trademark system our goal is to clearly differentiate the products produced by GMO-free production in the range of goods, to establish a GMO-free product chain, thereby developing GMO-free agriculture and strengthening its reputation. With treating NGT-related products differently from conventional GMOs would constitute the risk that we will no longer be able to guarantee that our food products are GM free. Also, our organic products may lose their organic status.

* Under which conditions do you consider this would be the case?

The following responses strictly relate to the ones to the previous question.
1. Economic concerns:
For Hungary, being a major sowing seed producer, it is important to keep its agriculture free from genetically modified organisms. GMO-free seeds, propagating materials as well as other GMO-free products have a specific added value on international markets and may help to improve the country's export positions. If seeds produced by NGTs were to be placed on the market without labelling, Hungary would not be able to secure its GMO-free status, and Hungary would lose the economic benefit of providing GMO-free status.
2. Concerns in the food and feed chain in general
For food and feed produced from or containing GMOs, EU legislation lays down rules for authorization, labelling and monitoring, which Member States must comply with. If food and feed products obtained by NGTs were marketed without labelling, consumers would not be able to distinguish between products on store shelves, even though products created by such processes are also GMOs.
3. Concerns in the food chain in relation to GMO-free labelling
If NGT-related food and feed products were to be placed on the market without labelling, they could also be included in foods that were otherwise labelled as GMO-free, clearly misleading the consumer. In summary, GMO-free products have great advantages on the sowing seed, and food markets. Consequently, the GMO-free strategy is highly important and advantageous for Hungary not only in terms of biodiversity protection, but also as an economic incentive, because that can boost the competitiveness of our products on the global market and help us accessing new markets.
4. Socio-economic concerns in the medicinal sector:
As regards the medicinal sector, decisions about pricing and reimbursement of human medicinal products containing GMOs (possibly including also NGTs) may raise challenges in the context of the national health system.

*** 22. Do you see particular challenges for SMEs on market access to NGTs?**

- (●) Yes
- ( ) No

* Please explain under which conditions

A common argument in favor of new technologies is that these processes will no longer be available only to large multinational companies, but also to SMEs that will have the opportunity to apply the technology and patent varieties. However, the facts show just the opposite. All of these current patents are in the hands of some multinational giants, as has been the case with GMOs in the past. Again, small and medium-sized enterprises do not seem to benefit from these technologies.

*** 23. Do you see challenges/concerns in patenting or accessing patented NGTs or NGT-products?**

- (●) Yes
- ( ) No

* Please describe and provide concrete examples/data

In certain medical areas, the abuse of the monopoly of the patent owners can be responsible for unethically high prices of certain new drugs or treatments. Furthermore, producers and/or patent owners of these NGTs and the NGT-related products should be held responsible and liable for any adverse impacts they may cause including the payment of any costs.

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

The maximum file size is 1 MB

G - Final question

*** 24. Do you have other comments you would like to make?**

- (●) Yes
- ( ) No

Please provide your comments here

The precautionary principle must be the guiding principle during the production of NGT-related products and also during the activities in contained use and in the environment (such as field and clinical trials).
A wide array of different products can be produced with NGTs. Therefore, a case-by case approach is needed for the safety assessment and no general safety statements addressing all NGT-applications are thus justified. When classified into a group of breeding techniques, this classification does not automatically indicate the safety or the lack of safety of a particular product. The currently used comparative approach using a conventional variety as the baseline for the safety assessment has been acknowledged. Certain risk mitigation measures can already be built in when designing a certain approach (e.g. in the case of gene drives). These should, however, not replace the safety assessment.
Without carrying out a comprehensive and independent risk assessment both to the environment and to human health no products should be approved, even in those cases where the end product no longer contains traces of foreign DNA.

Please find the literature in the Attachment 3.

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

The maximum file size is 1 MB

Contact

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Figure 1. Percentages of NGT-related researches using the different techniques in contained uses in Hungary (2020)

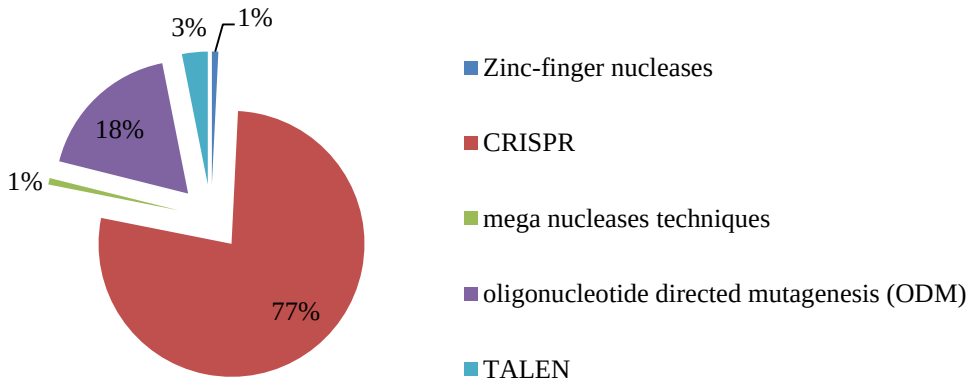


Figure 2. Percentages of NGT-related researches in contained uses in Hungary by research type (2020)

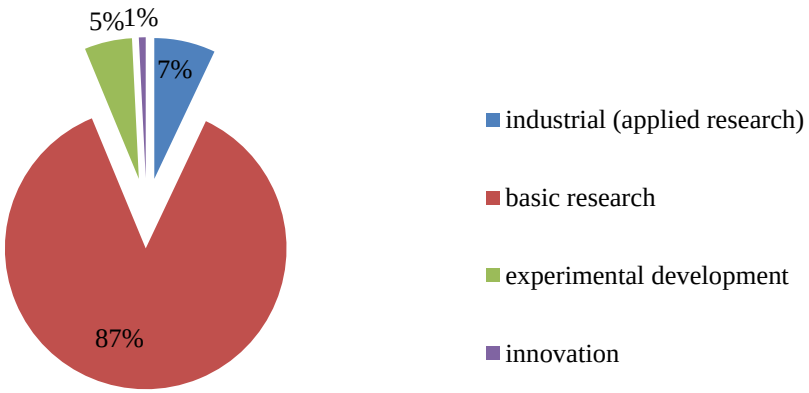
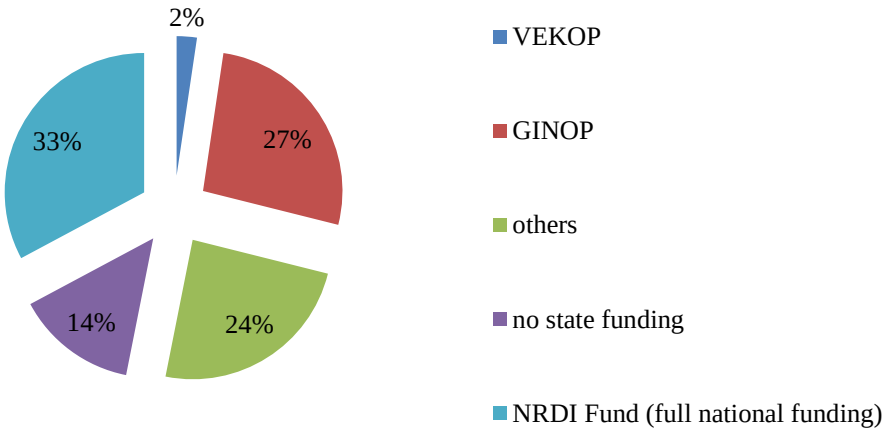


Figure 3. Percentages of NGT-related researches in contained uses in Hungary by funding type (2020)



No.	Research Institution (all of them public)	Support (million HUF)	Project title	Project summary
1.	Biological Research Centre Szeged (Institute of Biochemistry)	15.36	Improving bacterial metabolic efficiency and genomic stability by directed genome shuffling.	In summary, using our unique tools and expertise in genetic engineering of bacteria, we plan to construct genetic hybrids of two strains of the bacterium E. coli. This bacterium is one of the most important model organisms for basic research and host cell for biotechnological applications. There are many variants of the bacterium, each displaying different characteristics. We plan to construct genetic hybrids of two strains by a rational, targeted approach, with the aim of combining the beneficial features of both parents in a single cell. The proposed research would (i) explore novel ways of constructing "a la carte", semi-synthetic genomes for various (e.g., medical or industrial) purposes, (ii) would advance our knowledge regarding the genetic basis of genetic stability, tolerance and enhanced metabolic efficiency, and (iii) could produce host cells with superior performance for biotechnological applications.
2.	National Agricultural Research and Innovation Centre (Agricultural Biotechnology Institute (ABC))	81.66	Silencing related RNAs, effectors and their mechanims in virus-host interplay	Silencing related small RNAs are key players in silencing based antiviral mechanism, which operates in plant and insects, however gene silencing is also a very important gene inactivation mechanism, which controls gene expression in many organisms including human, animals, plants and insects. The aims of the proposed research is to get better insight into the interaction between the invading virus and the plant and how the plant tries to defend itself by activating the RNA silencing based defence system. This defence system is able to degrade the attacking virus and prevent the development of viral diseases. However, viruses are very efficient pathogens and they are able to overcome the RNA silencing based defence producing silencing suppressor proteins, which inhibit the plant defence and the plant becomes infected and shows diseases. Therefore we also want to extend our knowledge about this arm race between viruses and their hosts at the molecular level. We expect that the proposed research will significantly extend our knowledge about this small RNA molecules based antiviral mechanism and not only will help us to develop antiviral strategies to protect agricultural crops but can also provide important information to fight against dangerous viral diseases in other organisms. In addition, this work will extend our understanding about small RNA mediated regulatory mechanisms, which operate in almost all higher organisms.

No.	Research Institution (all of them public)	Support (million HUF)	Project title	Project summary
3.	University of Debrecen (Department of Clinical Physiology)	26.4	Posttranslational titin isoform modifications in perinatal diastolic dysfunction	In newborns the background of the frequently occurring cardiac diastolic dysfunction is unknown. In this condition cardiac relaxations following systoles are incomplete, despite preserved systolic performance (similarly to adult heart failure with preserved ejection fraction, HFPEF). Earlier, we have pointed out the significance of increased cardiomyocyte stiffness (Fpassive) in the pathogenesis of adult HFPEF. One of the most important myocardial determinants of increased Fpassive is the giant cardiomyocyte protein, titin. We hypothesize, that physiological and pathological adaptation processes to the extrauterine life involves molecular modifications (phosphorylation and oxidation) of titin isoforms, and that these together coordinate Fpassive of newborns. Therefore, in model experiments we will follow the titin isoform dependence of Fpassive in isolated cardiomyocytes during the perinatal period in healthy mice, rats, and adult humans, and in genetically engineered mice expressing high amounts of the phosphodiesterase 5 isoform. In addition, we will test how signalling pathways alone or in combination with oxidative insults affect Fpassive at different stages of postnatal development. In a parallel clinical study, advanced echocardiographic examinations aiming at diastolic ventricular functions together with the determination of oxidative plasma/urine markers will reveal their hypothetical relationships during the postnatal period of term and preterm human newborns. Altogether, our investigations will reveal the pathomechanism of perinatal diastolic dysfunction and assist the development of new therapeutic approaches for its pharmacological management.
4.	Biological Research Centre Szeged (Institute of Biochemistry)	22.563	Development of genome engineering approaches to study the evolution of antibiotic resistance	Just like plants or animals, microbes can also be selectively bred. Through a process called directed evolution, bacteria with advantageous properties (such as an increased ability to produce pharmaceutically, energetically, or chemically important products) can be created. This process however has several limitations: it is time-consuming and it is uncertain whether the most suitable genetic combinations will form by just chance. A novel technique, termed multiplex automated genetic engineering presents a solution to these problems. Using this method, up to 50 different specific genes can be modified at once in a matter of hours and from the scores of combinations generated, the ones with the most advantageous properties can be selected. The method however has a major drawback: it is not precise enough. Besides the targeted modifications, lots of unwanted changes also occur in the genetic material. This is a problem we are looking to solve. By proper optimization, the precision of the method can be dramatically increased therefore the „breeding” process will be faster and more efficient. With our optimized genetic editing tool in hand, we will focus our studies on the resistance of bacteria to antibiotics. One of the most severe public health problems of our day is the resistance of infectious bacteria to antibiotics. When treated with a drug, bacteria have the ability to adapt by generating mutations that result in resistance. With our optimized genome engineering tool, we will be able to generate and combine these mutations with great efficiency, therefore gaining clinically valuable information about the exact mechanisms behind resistance to antibiotics.

No.	Research Institution (all of them public)	Support (million HUF)	Project title	Project summary
5.	National Agricultural Research and Innovation Centre (Agricultural Biotechnology Institute (ABC))	25.315	Functional analysis of a mutation in the cattle motilin gene	Left displaced abomasum (LDA) of cattle is a common disease of the dairy breeds. This disease mainly affects the high yield Holstein-Friesian cattle kept under intensive housing conditions mostly in the month after calving. Because of the displacement of abomasum the animals fluid balance is upset, digestion changes, which result in a significant loss of production and loss of weight. The disease requires a surgical intervention which increases the cost of dairy farm. In the Hungarian and also in the international dairy breeds the occurrence of LDA disease is growing, therefore it is necessity of the correct understanding of the disease. The aim of the project is to identify genetic changes that are linked to the LDA disease. With the identification of this genetic background we will able to evolve selection programs which can significantly reduce the possibility of developing the disease.
6.	National Agricultural Research and Innovation Centre (Agricultural Biotechnology Institute (ABC))	43.968	Exploring molecular mechanisms responsible for plant recovery from virus infection	The present proposal aims to explore a very old still not fully understood phenomenon in plant virology: " the recovery" when the plant recovers from the initially strong virus induced symptoms and upper leaves become healthy and resistant against the virus used for primary inoculation. Our goal is to get better insight into the interaction between the invading virus and the plant and how the plant tries to defend itself by activating different defence systems. These defence systems can be very efficient and the plant finally recover from viral diseases. This recovery likely based on distinct molecular mechanisms, which in some cases interacts a synergistic ways and the plant is able to overcome the virus infection. Exploring this very interesting mechanism at molecular level will help us to develop successful strategies against pathogenic viral attack and protect/recover crop plants against devastating viral diseases. In addition, our extended knowledge about recovery may provide also important information to fight against dangerous viral diseases in other organisms.
7.	National Agricultural Research and Innovation Centre (Agricultural Biotechnology Institute (ABC))	30.7	Establishment of a guinea pig platform to study disease models in vivo	Model animals play crucial role in biomedical researches. We can understand the molecular background of diseases and potential drugs can be tested in animal models. The most frequently used animal models are mice and rats. Unfortunately some diseases like respiratory diseases and allergy are very difficult to study by these species. Guinea pigs have biological similarities to humans which makes them more useful in these fields of research than rodents. The best animal models harbor exactly the same genetic modification which can be found in the corresponding diseased humans. That's why scientist use genetically modified animals. There were several attempts to produce transgenic Guinea pigs but all of them failed. In the last few years, new transgenic technologies like transposon mediated transgenesis appeared. Transposons are mobile genetic elements. They can relocate themselves and integrate into a new genomic region. That is, what we use in biotechnology applications. We can produce safe and efficient vectors from transposons, and hopefully we would establish the FIRST TRANSGENIC GUINEA PIGS IN THE WORLD. Our plan is to express a reporter gene first. Our project can open a new era in modeling some special human diseases in Guinea pigs. Using better animal models we could also reduce the number of experimental animals in biomedical applications.

No.	Research Institution (all of them public)	Support (million HUF)	Project title	Project summary
8.	Research Center of Natural Sciences (Institute of Organic Chemistry)	30	Development of new fluorogenic dyes for super-resolution microscopy of site-specifically engineered proteins	Latest, Nobel-prize winning innovations in microscopy technology enabled routine nanoscale observation of biomolecular processes. These so-called super-resolution microscopy (SRM) applications utilize fluorescent dyes that stain the area of interest. Although recent hardware developments could offer even better resolution for the exploration of living matter, the unavailability of suitable fluorescent dyes limits further improvements. Major limitations for SRM in biology are the quality, size, light-emitting features, biocompatibility and non-invasive specific installation of such dyes. To reach the ultimate goal of further increasing resolution to match the typical size of biomolecules novel dyes must be developed. It is also crucial how these fluorescent labels are implemented into the biomolecule of interest. Bioorthogonal reactions offer biocompatible and selective means to manipulate proteins with light emitting dyes. In combination with genetic engineering even site selective modulation of proteins is possible. In this project we aim at exploring the possibility to create new light emitting (fluorescent) dyes that have complex features. They bear a bioorthogonal function, which enables selective reaction with target proteins and quench the fluorescence of the dye molecule. When reacting with the protein of interest, however, this quenching effect is ceased therefore light emission is reinstated. Such turn-on dyes are highly demanded to improve resolution of lens-based microscopy methods. Development of new dyes might have a large impact on SRM applications and on the understanding of biomolecular processes, consequently on medical diagnostics and drug development.
9.	Biological Research Centre Szeged (Institute of Biochemistry)	40.542	The evolutionary limits of genomic transposon expansion in Escherichia coli, and its biotechnological implications	One of the most surprising discoveries of the past century was the observation that certain genes are capable of transposition, that is jumping from one position to the other on the chromosome. Such mobile elements, also called transposable elements (TEs) are important for multiple reasons. From the aspect of evolution, TEs contribute to the mutations arising in the genome and thereby facilitate adaptation of the host to environmental changes. Like all mutational events, transposition can have detrimental effects as well; therefore an extremely high copy-number of TEs within a cell is unlikely. The first part of this project investigates the evolutionary limits of TE-expansion within a bacterial genome by artificially increasing their copy-number, and inspecting the resulting effect on cellular fitness. The second part aims at controlling and exploiting TEs for biotechnological purposes. Today, hundreds of fine chemicals such as pharmaceuticals, hormones or food additives are made by genetically reprogrammed bacterial cells. Spontaneous mutations of the engineered genetic changes are highly unwanted, for they lead to loss of the industrially useful cellular functions. Our aim is to develop a simple way to silence TE-mobility within numerous existing biotechnologically useful bacterial strains. Using the most up to date techniques, we also wish to use TEs to aid the integration of the genetic constructs into the bacterial genome during the process of cellular reprogramming. Finally we wish to use mobile elements as vehicles to increase gene copy-number, and thereby improve productivity of industrially relevant bacterial strains.

No.	Research Institution (all of them public)	Support (million HUF)	Project title	Project summary
10.	National Agricultural Research and Innovation Centre (Agricultural Biotechnology Institute (ABC))	20	Increasing the efficiency of precision breeding in potato	Genome-editing is the latest technique of molecular biology suitable for simple and precise genome modification of very different organisms. This technique can be used to get information on gene functions, for targeted correction of malfunctions and to turn disadvantageous traits to advantageous ones. Especially this last feature of the technique is important for plant breeders whose responsibility is getting enormous due to increase of human population and climate change. Thus, beside the classical breeding methods, precision breeding techniques are widely used. One of the precision breeding techniques is genome-editing. Nevertheless, to avoid introduction of foreign genes by genome-editing into crops there is a need for technical development. The aim of the proposed research is the development of a vector system suitable for foreign gene-free genome-editing in plants and testing its efficiency in potato. Furthermore, we are interested in knowing whether complex genomes, as potato has, can be modified by the current genome-editing tools. If yes, this would open the way for targeted breeding of potato, the World's fourth most important food crop.
11.	National Agricultural Research and Innovation Centre (Agricultural Biotechnology Institute (ABC))	33.1	Consortional main: The functional study of nodule-specific cystein-rich (NCR) peptide genes required for bacterial differentiation in Medicago truncatula.	Legumes compose the third largest family of flowering plants. Medicago truncatula and other legumes are able to establish nitrogen-fixing symbiotic associations with soil bacteria, termed rhizobia. The legume-rhizobial symbiosis is a major contributor to biological nitrogen fixation worldwide. Because of the ease how legumes can acquire nitrogen, their seeds and forage are highly proteinaceous and therefore legumes are an invaluable source of protein in both human and animal nutrition. In addition, legumes also play an important role in sustainable agriculture by enhancing soil fertility without the need for fertilizer application. The symbiotic interaction between rhizobia and legume plants induces the formation of legume root nodules, wherein intracellular bacteria are present in plant-derived membrane compartments. Bacteria in the nodules of M. truncatula and related legumes adapt to an endosymbiotic life-style and undergo irreversible elongation. It is presumed that this terminal differentiation of bacteria affects the effectiveness of the symbiosis and indeed, the legumes containing terminally differentiated rhizobia exhibit higher symbiotic performance. The terminal differentiation of the bacteroids is directed by hundreds of nodule specific cysteine-rich peptides (NCRs) produced by the host plant. In this proposal, we attempt to identify essential NCRs that have unique role in bacteroid differentiation and analyze their regulation and sequence composition that required for their activity. The completion of the proposal will give better insights into process of the bacteroid differentiation and may help to improve the effectiveness of symbiotic nitrogen fixation.
	Biological Research Centre Szeged (Institute of Plant Biology)	14.9	Consortional assoc.: A functional study on nodule-specific cystein-rich (NCR) peptide genes required for bacterial differentiation in Medicago truncatula	

No.	Research Institution (all of them public)	Support (million HUF)	Project title	Project summary
12.	Research Center of Natural Sciences (Institute of Enzymology)	15.216	Efficient and specific gene editing with Cpf1 (Cas12a) nuclease variants: intracellular localization of tagged prion protein family members	The discovery and the innovative implementation of the bacterial immune system called CRISPR as a tool for genome editing during the past few years has triggered an explosion of developments in molecular biology and related fields bringing the impossible within reach in many areas of research. The great potential of the system is that it allows for precise, targeted modification (such as correction, removal, addition) of almost any gene in the genome which opens up a wide area of applications. Being a modular system with few key but alterable elements it also provides possibility for its own retailoring and perfecting to suite versatile needs. At present developing the palette and versatility of the CRISPR-based genome modification tools is a competitive field with spectacularly intense developments aiming to expand the areas of applicability by perfecting them or searching for new solutions. One of the major limitations of the CRISPR-based method is the appearance of off-target hits. Our aim in this proposal is to increase further the productivity and broaden the applicability of CRISPR-based tools while minimizing its off-target effects. The tools and methods to be developed here may be utilized in various research applications, as such may have many and yet unforeseeable benefits for the society in many areas like: gene therapy applications, such as the reprograming of the immune system to kill cancerous cells, the study of diseases by using model systems not available by now, development of humanized animal model systems for testing drugs, food industry applications, just to name a few.
13.	University of Debrecen (Department of Anatomy, Histology and Embryology)	37.976	Elucidation the role of p-S10H3 in the development of heat hyperalgesia with utilizing cell-type-specific in vivo gene editing	Neurons in the superficial spinal dorsal horn constitute one of the most important groups of cells engaged in pain sensation (nociceptive processing) because they are components of a neural network that integrates painful (nociceptive) and non-painful experiences from the periphery, and descending inhibitory and excitatory signals from different brain areas. Hence, superficial spinal dorsal horn neurons significantly contribute to controlling the nociceptive information that is forwarded to the brain and ultimately to the cortex where the pain experience develops. Burn injury is one of the most frequently occurring traumatic tissue injury which is associated with one of the most severe pain experiences which becomes chronic and present even after the healing process is terminated. However, satisfactory control of pain in burn injured patients is an unmet medical need. Currently available treatments to control burn-injury associated pain are very often inadequate, which imposes a huge financial and emotional burden on the sufferers. Therefore, in this proposal, we are about to gain better understanding of the molecular mechamisms to lead to the development of inflammation-related pain. We believe that this set of experiments can provide us with the key to develop novel effective control of tissue-damage induced inflammatory pain.

No.	Research Institution (all of them public)	Support (million HUF)	Project title	Project summary
14.	National Agricultural Research and Innovation Centre (Agricultural Biotechnology Institute (ABC))	47.946	Establishment of genome editing in barley and other crop species for research and crop improvement.	The CRISPR/Cas9 system is an efficient, and highly specific genome editing technology which has been introduced into basic and applied sciences with stunning velocity. CRISPR/Cas9 system provides precise genome modifications directly in valuable local cultivars, even the introduction of multiple traits, saving the time-consuming backcrossing procedure of conventional breeding approaches. In addition, the potential of CRISPR/Cas9 provides alternative approaches circumventing the drawbacks associated with traditional genetically modified organisms. It is inevitable to establish this ground breaking technology in Hungary and exploits its potential in basic and applied researches. The economically important barley (Hordeum vulgare) will be used to generate CRISPR/Cas9 edited plants. The genome editing targets will be selected to analyse basic biological processes and assess the efficiency of the technology in trait improvement, such as virus resistance and enhanced seed development. In long term, the generated basic knowledge can be translated into development genetic approaches leading to the improvement of economically important traits of currently used important local cultivars.
15.	National Agricultural Research and Innovation Centre (Agricultural Biotechnology Institute (ABC))	31.852	Molecular studies on early tuber bulking in potato	Potato is the world's fourth most important food crop. Potato production exceeds 350 million tonnes worldwide and is around 500-600 thousand tonnes in Hungary, however, with an average yield of only 22-27 tonnes/hectare. The major environmental factors adversely affecting potato production are heat and drought. Early bulking potato varieties are candidates for stress escapers, as they can complete their life cycle before stress becomes a serious constraint. Furthermore, production profitability of early harvest potatoes is higher compared with late harvest potatoes. Thus there is a high interest in breeding early harvest potato cultivars. The Dutch partner of the current proposal did a pioneer work in identification of a transcription factor that regulates tuberisation and plant life cycle length, by acting as a mediator between the circadian clock and a mobile tuberisation signal similar to a peptide hormone influencing flowering. As an external partner of the Dutch consortium that aims to study the relationship between tuberisation and flowering, the objectives of our research will be the metabolic signalling influencing the bulking time of potato tubers. The experimental approach will be based on comparison and grafting early and late bulking potato varieties and analysing the metabolite composition of plants at different stages of development. Influence of the tuberisation factor identified by the Dutch partner on potato leaf metabolism will be studied by utilising a novel genome-editing technique. We attempt to increase the earliness of tuberisation by increasing leaf expansion rate in potato based on previous model plant experiments.

No.	Research Institution (all of them public)	Support (million HUF)	Project title	Project summary
16.	University of Szeged (Department of Medical Biology)	27	Using OMICS approach for the analysis of viral gene expression	Our aim is to analyze the gene expression of different human and non-human pathogenic viruses during lytic infection. We will characterize the different RNAs and proteins which are expressed during different time points after infection. We will use the so-called long-read sequencing method, which is able to identify the different length and splice variants of RNAs. According to our previous results, this method is able to multiply the number of known RNAs of an organism. It is capable to detect for example long non-coding RNAs, splice isoforms, transcriptional overlaps, which might have important regulatory role in viral infection We will test the results of our previous experiments which showed the viral transcriptome is much more complex than it was earlier believed We will examine the effect of viral infection on gene expression of the host cells Our aim is to analyze the presence and role of other regulatory RNAs (mi-, circRNAs). It has been shown that miRNAs have key roles in virus-host interactions in several viruses. It has also been demonstrated that circRNAs play a role in the regulation of miRNAs We will analyze the DNA and RNA methylation patterns of viral genomes by using various methods. It is well known that methylation has an important role on the regulation of gene expression in higher organisms. We will develop an algorithm for the methylation analysis. Although, the project plan is basic scientific research, results can serve as potential clinical relevance: eg. understanding of viral miRNAs can led to new therapeutic strategies, our data will be useful in the field of gene regulation as well in developing potential antiviral therapies.
17.	National Agricultural Research and Innovation Centre (Agricultural Biotechnology Institute (ABC))	47.986	Engineering tomato fruit ripening variation by genome editing	Providing enough food to the entire human population is an emerging problem of the 21st century. Currently, the human population is rapidly growing and it is projected to reach 9 billion by 2050. According to recent estimation, food supplies would need to increase by 60% (estimated at 2005 food production levels) in order to meet the food demand in 2050. As the available land for food production has reached its maximum the only way to assure food security if we can increase crop yields, improve distribution and in the same time reduce yield losses. FAO predicts that about 1.3 billion tons of food are globally wasted or lost per year. Thus, reduction of post-harvest food losses is a critical component of ensuring future global food security. Furthermore, food losses also have negative effects to society through costs of waste management, greenhouse gas production, and loss of resources used in their production. Tomato is one of the most popular vegetables on the globe. Beside its economic and nutritional importance, tomato is the principal model to study fleshy fruit development and ripening. The long shelf life of tomato is an important trait for the quality of fleshy fruit, and it is one of the main objectives in breeding programs as it influences fruit marketability and reduces post-harvest losses. This project will use state-of-the-art genome editing method to provide new genetic material to breeders to help to improve the shelf life of tomato. The increased shelf life has an important economic and environmental protection consequence, since it is a major contributor to the reduction of post-harvest losses.

No.	Research Institution (all of them public)	Support (million HUF)	Project title	Project summary
18.	National Agricultural Research and Innovation Centre (Agricultural Biotechnology Institute (ABC))	19.98	Investigation of heat stress linked RNAi and crop quality determining genes by genome editing technology in barley.	Genome editing is a powerful molecular biology technology introducing site specific mutations into the genome of plants, animals and microbes. The successful routine utilization of genome editing in the laboratory allows the precise investigation of target genes providing an efficient tool to understand complex biological processes, not only model plants but also in important crop species. Our aim is to investigate the major genes involved in small RNA mediated regulation in heat stress adaption of barley by introducing precise mutations by genome editing into target genes. Moreover we would like to use this technology to mutate barley gene controlling seed number potentially enhancing the production rate of important barley cultivars.
19.	University of Szeged (Department of Microbiology)	46.89	Background and consequences of the azole-resistance in mucormycosis-causing fungi	Mucormycosis is a severe, life-threatening, opportunistic infection caused by fungi belonging to the phylum Mucoromycota (primarily in the order Mucorales). These infections occur mainly in immunocompromised patients but diabetes (especially diabetic ketoacidosis), trauma, injuries or injection drug usage also can be regarded as risk factors of them. Although mucormycoses are relatively rare, these infections are characterized with a continuously increasing incidence, difficulties in their diagnosis, extremely high mortality rates and the fact that these fungi are inherently resistant to most antifungal drugs. All these factors urge the understanding of the pathogenicity mechanisms of Mucoral fungi and the development of new, effective antifungal strategies against them. As azoles are the widest group of the currently used antifungal agents and majority of them are inefficient against these fungi, the aim of the proposed project is to clarify the genetic background and the mechanism of azole-resistance in mucormycosis-causing moulds and to use this information to find new potential antifungal targets and propose new strategies against them.

No.	Research Institution (all of them public)	Support (million HUF)	Project title	Project summary
20.	Centre for Agricultural Research (Applied Genomics Department)	32.041	Consortional main: Exploring disease susceptibility genes to produce bacterium wilt-resistant potato (RESPOTA)	Our research is focused on potato brown rot disease in Hungary. This bacterial infection of tropical origin entered the country in the late 1990s and became so widespread that it has contributed considerably to the disappearance of domestic seed potato production. However, it also causes serious damage to food potato production due to the lack of effective chemical control. The solution would therefore be the breeding of improved varieties that are genetically resistant to the pathogen. Based on our preliminary results, in our view the resistance can be provided by destroying or modifying potato genes that are exploited by the pathogen and are necessary for its entrance and subsequent proliferation within the plants. Artificial infection of plants carrying point mutations in these so-called susceptibility genes should determine whether the desired resistance or tolerance can be achieved this way. To this end, numerous potential susceptibility genes will be tested. We also attempt to discover novel genes exploited by the bacterium by applying the latest genome editing and precision mutagenesis techniques. Obviously, emphasis will also be placed on generating minimal and punctual mutations resulting in increased resistance without causing disadvantages like yield penalty.
	National Agricultural Research and Innovation Centre (Agricultural Biotechnology Institute (ABC))	15.509	Consortional assoc.: Exploring disease susceptibility genes to produce bacterium wilt-resistant potato	

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