Current and future market applications of new genomic techniques (NGTMs)

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National Research Institute in Radzikow, Poland
"As the science and knowledge service of the Commission our mission is to support EU policies with independent evidence throughout the whole policy cycle"

3000 staff Almost 75% are scientists and researchers. Headquarters in Brussels and research facilities located in 5 Member States:

• Belgium (Geel)
• Germany (Karlsruhe)
• Italy (Ispra)
• The Netherlands (Petten)
• Spain (Seville)
New Genomic Techniques

NGTs = techniques which are capable to alter the genetic material of an organism, developed after the publication of Directive 2001/18/EC
Background

• **July 2018**, the Court of Justice of the European Union (CJEU) clarified that organisms from new mutagenesis techniques fall within the scope of the EU GMO legislation.

• **November 2019**, The EU Council requested the Commission to submit, by 30 April 2021, a study on the status of NGTs.

• The [JRC](https://ec.europa.eu/jrc) was requested to provide, as part of the study, “An overview of current and future scientific and technological developments in New Genomic Techniques as well as of new products that are, or are expected to be marketed”.

• **April 2021** European Commission study published including JRC Reports

• **September 2021** launching of EC initiative that will propose a legal framework for plants obtained by targeted mutagenesis and cisgenesis
Classification of NGTs (Broothaerts et al. 2021, JRC121847)

- **Group 1:** Genome editing involving a DNA double-strand break
- **Group 2:** Genome editing without DNA double-strand break
- **Group 3:** Editing of the epigenome
- **Group 4:** Site-directed RNA editing
Double strand break

No donor DNA

NHEJ

Gene disruption SDN-1

Donor DNA

HR

New alleles SDN-2

Delivered to plants cells:
- DNA
- mRNA
- proteins

HR

Gene insertion/stacking SDN-3
Current and future market applications of new genomic techniques
Scope

**Sectors:**
- Agriculture
- Bio-based industry
- Medical

**Organisms:**
- Plants (& mushrooms)
- Animals
- Microorganisms
- Human cells

**Uses of NGTs:**

**Within the scope:**
- Product/variety development (traits)
- Use as breeding tool (e.g. reproductive characteristics)

**Outside the scope:**
- Technology development (e.g. new/improved genome editing tools)
- Gene discovery research
• Screening of public authorities’ databases of different countries
• Search in scientific literature and datasets, including clinical trials databases worldwide
• Identification of companies/institutions developing NGT products and screening of their websites and press releases
• Expert consultation: More than 20 videoconferences with regulators and public/private technology providers from several countries worldwide
• Survey of public and private technology developers: 47 organisations participated (37 private companies and 10 public/academic organisations)
• Integration and cleaning of the data from different sources in the database
The NGT applications identified were classified, using the information available, as being at the following development stages:

1. **Commercial stage**
   - NGT applications currently marketed in at least one country worldwide

2. **Pre-commercial stage**
   - NGT applications ready to be commercialised in at least one country worldwide but not yet on the market

3. **Advanced R&D stage**
   - NGT applications at a late stage of development and likely to reach the market in the medium term

4. **Early R&D stage**
   - NGT applications at proof of concept stage (i.e., testing gene targets for trait enhancement of commercial interest).
Results: web dashboard

Web dashboard at this link: https://datam.jrc.ec.europa.eu/datam/embed/NEW_GENOMIC_TECHNIQUES/
Plants in the NGT database

Number of applications

426

Development stage

2. Pre-commercial stage
3. Advanced...
4. Early R&D...

Technique share

CRISPR: 70.8%
ODM: 10.2%
TALEN: 7.4%
Genome editing: 8.4%

European Commission
NGTs - Plants – breakdown by crop groups
NGT - plants – breakdown by traits

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Modified composition</td>
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<td>38</td>
<td>71</td>
<td>115</td>
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<tr>
<td>Biotic stress tolerance</td>
<td>15</td>
<td>23</td>
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<td>113</td>
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<td>Plant yield and architecture</td>
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<td>24</td>
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<tr>
<td>Abiotic stress tolerance</td>
<td>11</td>
<td>12</td>
<td>25</td>
<td>34</td>
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<tr>
<td>Breeding tools</td>
<td>10</td>
<td>11</td>
<td>21</td>
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<td>Herbicide tolerance</td>
<td>10</td>
<td>11</td>
<td>21</td>
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<tr>
<td>Storage performance</td>
<td>9</td>
<td>11</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Modified colour/flavour</td>
<td>9</td>
<td>11</td>
<td>20</td>
<td></td>
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<tr>
<td>Other traits</td>
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<tr>
<td>NA</td>
<td>1</td>
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</table>
Modified composition

- Consumers health & consumers convenience
SOYBEAN - High Oleic Calyno

**Stage:** COMMERCIAL  
**Technique:** TALEN  
**Trait:** High Oleic (Calyno oil)  
**Developer:** Calyxt (US)
TOMATO- Enhanced GABA ("Sicilian rouge")

Stage: COMMERCIAL
Technique: CRISPR
Trait: high GABA content (low blood pressure association)
Developer: Sanatech (Japan)
B. Juncea greens - low pungency/low bitterness

Stage: field trials

Technique: CRISPR-SDN1

Trait: eliminate bitterness from nutrient rich species in “orphan crop” (new salads)

Developer: Pairwise (USA)
WHEAT - safe gluten for coeliacs

Stage: greenhouse trials
Technique: CRISPR-SDN1
Trait: alfa-gliadin family, reduced alfa-gliadin production
Developer: CSIC (Spain), WUR (NL)
<table>
<thead>
<tr>
<th></th>
<th>Celiac Disease (CD)</th>
<th>Wheat allergies (WA)</th>
<th>NCWS</th>
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<tbody>
<tr>
<td>Prevalence</td>
<td>0.2 – 2.4%</td>
<td>0.2 – 2.1%</td>
<td>0.6 – 13%</td>
</tr>
</tbody>
</table>
Pest and disease resistance
Resistance to pests and diseases

Number of applications: 113

Traits and development stage

- 2. Pre-commercial stage: 2
- Biotic stress tolerance
- 3. Advanced R&D stage: 37
- 4. Early R&D stage: 74

Technique share

- CRISPR: 67.2%
- Genome editing: 13.8%
- TALEN: 6.9%
- ODM: 8.6%
- ZFN: 2.6%
- Meganuclease: 3.4%
WHEAT - broad and wide fungal R (PILTON)

Stage: close to field trials

Technique: CRISPR SDN1

Traits: KO repressors of natural defense reaction in wheat (rust, Septoria, Fusarium)

Developer: BDP Consortium of DE breeding companies
Disruption of susceptibility (S) genes in crops is an attractive breeding strategy for conferring disease resistance. However, S genes are implicated in many essential biological functions and deletion of these genes typically results in undesirable pleiotropic effects. Loss-of-function mutations in one such S gene, *Mildew resistance locus O* (*MLO*), confers durable and broad-spectrum resistance to powdery mildew in various plant species. However, *mlo*-associated resistance is also accompanied by growth penalties and yield losses, thereby limiting its widespread use in agriculture.
## CGIAR gene editing plants/animals – developing countries

<table>
<thead>
<tr>
<th>Research centre</th>
<th>Species</th>
<th>Trait category</th>
<th>SDN type (1, 2, 3)</th>
<th>Development stage</th>
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<tbody>
<tr>
<td>IITA</td>
<td>Banana</td>
<td>Biotic stress tolerance</td>
<td>SDN1</td>
<td>4. Early R &amp; D stage</td>
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<tr>
<td>IITA</td>
<td>Banana</td>
<td>Biotic stress tolerance</td>
<td>SDN1</td>
<td>4. Early R &amp; D stage</td>
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<tr>
<td>IITA</td>
<td>Banana</td>
<td>Biotic stress tolerance</td>
<td>SDN1</td>
<td>4. Early R &amp; D stage</td>
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<tr>
<td>CIAT</td>
<td>Beans</td>
<td>Modified composition</td>
<td>SDN1, SDN2</td>
<td>4. Early R &amp; D stage</td>
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<td>CIAT</td>
<td>Cacao</td>
<td>Modified composition</td>
<td>SDN1, SDN2</td>
<td>4. Early R &amp; D stage</td>
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<tr>
<td>CIAT</td>
<td>Cassava</td>
<td>Haploid techniques</td>
<td>SDN1, SDN2</td>
<td>4. Early R &amp; D stage</td>
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<tr>
<td>ICARDA</td>
<td>Chickpea</td>
<td>Abiotic stress tolerance</td>
<td>SDN1</td>
<td>4. Early R &amp; D stage</td>
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<tr>
<td>ICRISAT</td>
<td>Chickpea</td>
<td>Modified composition; plant yield and architecture</td>
<td>SDN1, SDN2</td>
<td>4. Early R &amp; D stage</td>
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<tr>
<td>CIMMYT</td>
<td>Maize</td>
<td>Biotic stress tolerance</td>
<td>SDN1, SDN2</td>
<td>3. Advanced R &amp; D stage</td>
</tr>
<tr>
<td>CIMMYT</td>
<td>Maize</td>
<td>Biotic stress tolerance</td>
<td>SDN1</td>
<td>3. Advanced R &amp; D stage</td>
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<tr>
<td>ICRISAT</td>
<td>Millet</td>
<td>Storage performance</td>
<td>SDN1, SDN2</td>
<td>4. Early R &amp; D stage</td>
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<tr>
<td>ICRISAT</td>
<td>Pigeon pea</td>
<td>Reproductive/flowering characteristics</td>
<td>SDN1, SDN2</td>
<td>4. Early R &amp; D stage</td>
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<tr>
<td>CIP</td>
<td>Potato</td>
<td>Biotic stress tolerance</td>
<td>SDN1, SDN2</td>
<td>4. Early R &amp; D stage</td>
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<tr>
<td>CIAT</td>
<td>Rice</td>
<td>Biotic stress tolerance</td>
<td>SDN1, SDN2</td>
<td>3. Advanced R &amp; D stage</td>
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<tr>
<td>CIAT</td>
<td>Rice</td>
<td>Plant yield and architecture</td>
<td>SDN1, SDN2</td>
<td>4. Early R &amp; D stage</td>
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<tr>
<td>IRRI</td>
<td>Rice</td>
<td>Plant yield and architecture</td>
<td>SDN1</td>
<td>4. Early R &amp; D stage</td>
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<tr>
<td>IRRI</td>
<td>Rice</td>
<td>Modified composition</td>
<td>SDN1, SDN2, SDN3</td>
<td>4. Early R &amp; D stage</td>
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<tr>
<td>IRRI</td>
<td>Rice</td>
<td>Reproductive/flowering characteristics</td>
<td>SDN2</td>
<td>4. Early R &amp; D stage</td>
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<tr>
<td>IRRI</td>
<td>Rice</td>
<td>Biotic stress tolerance</td>
<td>SDN1</td>
<td>4. Early R &amp; D stage</td>
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<td>ICRISAT</td>
<td>Sorghum</td>
<td>Biotic stress tolerance</td>
<td>SDN1, SDN2</td>
<td>4. Early R &amp; D stage</td>
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<td>CIMMYT</td>
<td>Wheat</td>
<td>Biotic stress tolerance</td>
<td>SDN1</td>
<td>4. Early R &amp; D stage</td>
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<tr>
<td>CIMMYT</td>
<td>Wheat</td>
<td>Biotic stress tolerance</td>
<td>SDN1</td>
<td>4. Early R &amp; D stage</td>
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<td>ILRI</td>
<td>Cattle</td>
<td>Biotic stress tolerance</td>
<td>SDN3</td>
<td>4. Early R &amp; D stage</td>
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</tbody>
</table>
MAIZE – Lethal Necrosis viruses- Africa

Stage: field trials

 Technique: SDN1

 Traits: editing African-adapted parental lines to mimic R genes identified in tolerant germplasm

Developer: CYMMIT
Plant yield
SUGAR CANE – Flex I Flex II

Stage: field trials
Technique: CRISPR
Traits easier digestibility of cell walls (ethanol) and higher yield in sugars
Developer: EMBRAPA Brazil

• Photo Hugo Molinari (EMBRAPA)
Improved storage
LETTUCE - non-browning

- Photo Green Venus

**Stage:** field trials

**Technique:** SDN1

**Traits:** longer storage

**Developer:** GREEN VENUS (USA)
NGT- Animals- Traits under development

<table>
<thead>
<tr>
<th>Trait</th>
<th>Number of applications</th>
</tr>
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<tbody>
<tr>
<td>Gene drive</td>
<td>10 (Pre-commercial) 7</td>
</tr>
<tr>
<td>Improved meat yield/quality</td>
<td>1 (Pre-commercial) 3</td>
</tr>
<tr>
<td>Biotic stress tolerance</td>
<td>6 (Advanced R&amp;D) 7</td>
</tr>
<tr>
<td>Other traits</td>
<td>3 (Advanced R&amp;D) 3</td>
</tr>
<tr>
<td>Reproductive characteristics</td>
<td>4 (Advanced R&amp;D) 1</td>
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<tr>
<td>Hypoallergenic properties</td>
<td>2 (Pre-commercial) 2</td>
</tr>
<tr>
<td>Abiotic stress tolerance</td>
<td>1 (Pre-commercial) 2</td>
</tr>
</tbody>
</table>

Development stage:
- 2. Pre-commercial stage
- 3. Advanced R&D stage
- 4. Early R&D stage
Red Sea Bream (Madai)

**Stage:** COMMERCIAL

**Technique:** CRISPR

**Trait:** higher flesh/muscle (up to 20%), more feed efficiency (up 14%)

**Developer:** Regional Fish Institute (start-up) (Kyoto) + Univ. of Kyoto + Kindai University
Tiger pufferfish (Torafugu)

**Stage:** COMMERCIAL

**Technique:** CRISPR K.O leptin receptors

**Trait:** higher flesh/muscle (up to 1.9 times)

**Developer:** Regional Fish (Kyoto) + Univ. of Kyoto + Kindai University
NGT- microorganisms for contained use

- NGTs are already applied commercially in microorganisms for the bio-production of industrial molecules and the R&D pipeline is active.
- Technology developers continuously use genetic techniques (including both established and new) to improve microbial strains.
- NGTs (CRISPR) are becoming standard tools in some cases.
- NGTs are mainly used to knock out unfavourable genes (e.g. toxins, intrinsic antibiotic resistance or by-products).
- It is difficult to estimate the current share of microorganism strains used by bio-industry worldwide that have been improved with NGTs.
- Sectors: biofuels, bio-based enzyme production...
NGT microorganisms for deliberate release
Klebsiella-N fixing-corn specific

Stage: COMMERCIAL

Technique: Gene editing, synthetic biology

Trait: sup: Nitrogen-fixing-bacteria associated to maize/sorghum seeds

Developer: Pivot Bio (US)
Results in human health applications - conditions

- Cancer: 29 applications, 19 in early R&D, 48 total
- Viral diseases: 12 applications, 11 in early R&D, 23 total
- Haematological diseases: 11 applications, 5 in early R&D, 16 total
- Other hereditary diseases: 9 applications, 6 in early R&D, 16 total
- Cancer/viral diseases: 8 applications, 3 in early R&D, 16 total
- Neurodegenerative diseases: 6 applications, 6 in early R&D, 16 total
- Hereditary eye diseases: 5 applications, 4 in early R&D, 9 total
- Cardiovascular/metabolic diseases: 4 applications, 4 in early R&D, 8 total
Conclusions I

- NGTs Group I (mainly CRISPR) are actively and increasingly used in agri-food, industrial and medicinal applications (over 600 items identified)
- NGTs Group I used in >90% of cases
- Few applications are marketed worldwide (two crop plants, two fish, one microorganism for release and microorganisms for contained production of industrial molecules)
- Plants the largest pipeline (>400), composition and resistance to pests and diseases the largest pipelines
Conclusions II

- NGT now incorporated in toolkit for improving strains of microorganisms for contained use commercial (biofuels/enzymes).
- NGT microorganisms for release on agricultural soil commercial (N fixation) Pipeline active
- NGTs actively used to tackle several human diseases, and many applications have already reached patients (phase I and phase I/II clinical trials).
- United States and China are the most frequent countries of origin, particularly in the stages closest to market. The EU, particularly Germany and France, is also active in the use of NGTs. Several developing countries very active, mostly in the agricultural sector.
- Private and public sector both source of applications
Thank you

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