



Challenges to detect genome-edited plants - consequences for surveillance authorities

Lutz Grohmann

BVL | Dept. Genetic Engineering | Unit 405

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General considerations (I)

What are the new analytical challenges ?

- Detection of small DNA-sequence modifications/variations
- Identification of the technique used to modify the plant genome
- Identification of the GE-plant (“event-specific” detection)

What is required for GE-GMO testing ?

- Analytical equipment and trained personnel (laboratory accreditation)
- Knowledge of target, reference material
- validated methods
- Reliability of the analysis result

General considerations (II)

Genome editing of plants by SDN-1, SDN-2, or ODM and

- no foreign DNA present
- no other permanent changes in the genome
- few small sequence variations (SNV, indels)
- no multiplex genome editing

not considered are

- insertion(s) of foreign DNA (SDN-3)
- targeted knock-out of several genes (metabolic pathway)

Detection

GTGAAGATACTCTTGTTAATGGCAACCAGCATCTTGGGAT
A



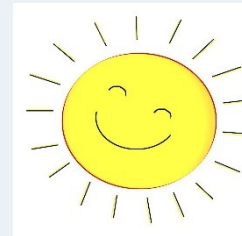
Identification of Genome Editing



untargeted
mutagenesis



genome-editing
(targeted mutagenesis)



random/natural
variation



Identification of GE plant

T
A G T

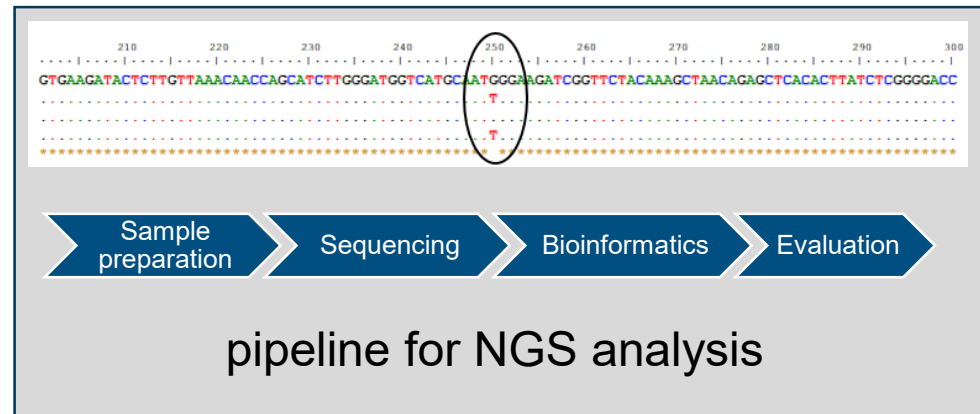
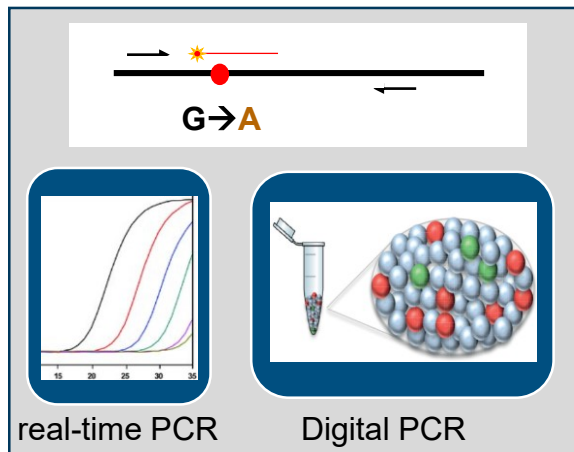
(several) unique modification(s) ?
genotyping, somaclonal variations?

A T G

same modification(s) present ?
Natural, other mutagenesis?

Detection of SNV require specific (new) methods of analysis

- Real-time PCR using specific reagents
 - (blocked or LNA/MGB probes, RNase H probe)
- Digital PCR
 - quantification of few SNV copies in background of wildtype DNA
- Next-Generation-Sequencing (NGS)
 - Whole-genome-sequencing or targeted sequencing



Requirements for GE-GMO detection

Surveillance laboratories will require additional new equipment

- New techniques and methods (targeted or untargeted detection)
 - Real-time PCR, digital PCR
 - Next-Generation-Sequencing (NGS)

Accreditation of laboratories

- new measurement instruments and IT (validation, verification)
- training of personnel
- Validation of methods

Detection, identification and quantification method for GE-GMOs

- for EU authorisation applicants must provide a method, which is validated (EURL and NRLs)
- certified reference material
- for unauthorised GE GMOs new methods must be developed by surveillance authorities

What else is challenging for the surveillance authorities?

Screening approaches not applicable

- no foreign DNAs (common genetic elements, P-35S or T-nos)

Without information and reference material → no method

- fail to detect GE plants (w/o foreign DNA) and products from third countries enter the market undetected
- court-proof evidence difficult or impossible to be achieved
- no reference material



Costs and turn-around-time for GE-GMO testing probably increase

- New instruments; IT and bioinformatic pipeline
- GE plants identification may require more than 1 method



Unclear method performance criteria

- sensitivity, specificity, robustness, precision etc.

Influence of sample material

- homogeneous or heterogeneous (e.g. seeds or composite feed)

What are the current activities ?

Report of the European Network of GMO Laboratories (ENGL) published:

Detection of food and feed plant products obtained by new mutagenesis techniques

Report endorsed by the ENGL Steering Committee

Publication date: 26 March 2019



<http://gmo-crl.jrc.ec.europa.eu/doc/JRC116289-GE-report-ENGL.pdf>

ENGL Working Group activated (Oct. 2019; request of EU COM)

Task:

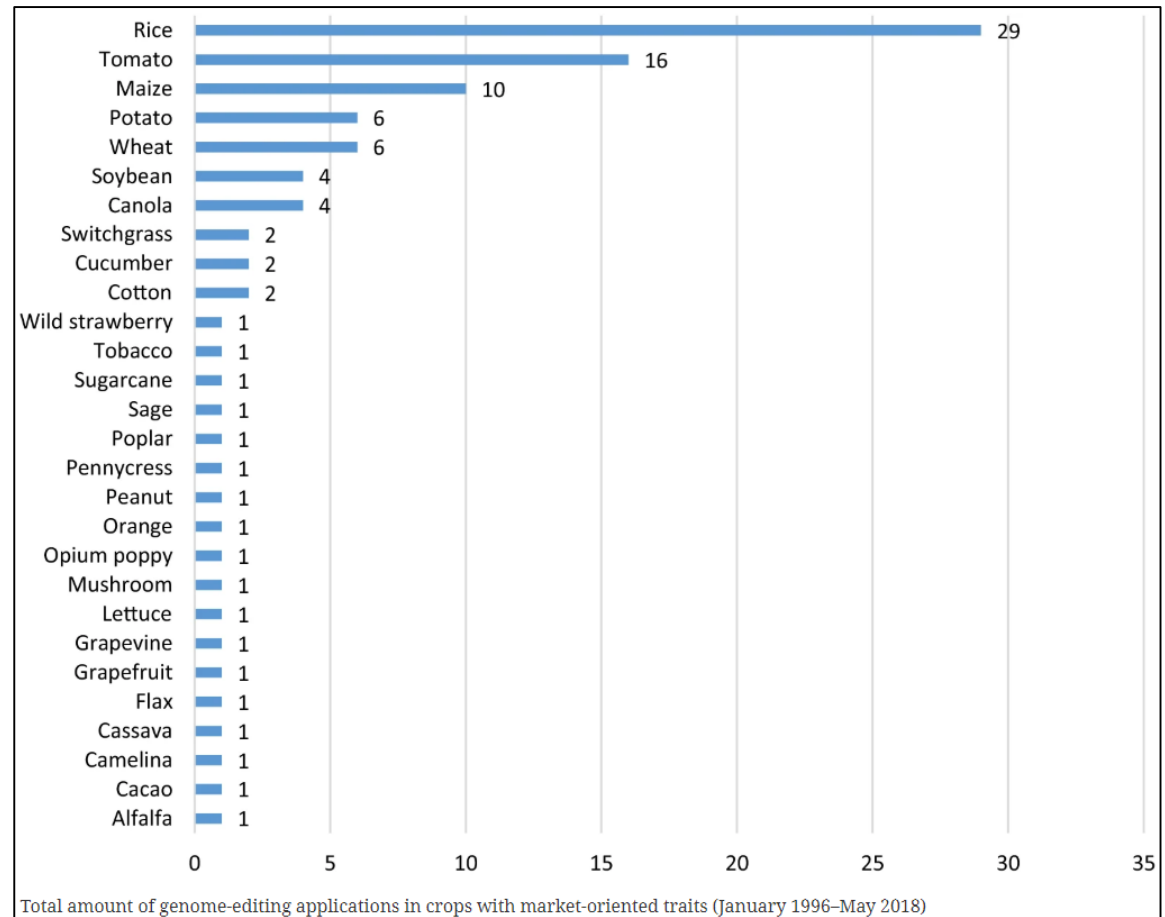
Expand existing Guidance on „Minimal Performance Requirements for Methods of GMO Testing“ and define acceptance criteria for (additional/new) methods for detection, identification and quantification of food/feed GE-plants

BVL Working Group (§28b GenTG)

- Case-study using a reference material to test the new analysis methods (rh-PCR, digital PCR, targeted NGS)
- non-commercial ODM or somaclonal modified rapeseed lines used for proof-of-principle and method evaluations
- **Challenging questions:**
 - Is it possible to achieve the limit of detection and for quantification ?
 - 0,9% labelling threshold (Reg. 1829/2003)
 - 0,1% threshold for feed with pending authorisation (Reg. 619/2011)
- What are the expected costs, turn-around-time ? Which equipment and expertise are required ?

Compilation of available knowledge on applications of genome-editing in plants

99 different market-oriented
applications
in 28 different crops



Modrzejewski et al. *Environ Evid* (2019) 8:27 <https://doi.org/10.1186/s13750-019-0171-5>

EUginius (EUropean GMO INitiative for a Unified database System)

- joint public website and GMO database hosted by BVL and WFSR (Wageningen Food Safety Research (previously RIKILT))
- 11 GE-GMOs (>20 in pipeline) with details on references (patent, publications etc.), authorization status (different countries), the genetic modification

GMO ¹	UID ¹	Species ¹	Traits	Companies	Developers	Tradenames	EU authorisation
GE-J2 Tomato		<i>Solanum lycopersicum (tomato)</i>	Improved fruit abscission		University of Paris-Saclay, University of Liège		
GE-PPO Potato		<i>Solanum tuberosum (potato)</i>	Alteration in growth, development or product quality	Calyxt	Calyxt		
GE-FAE1 Pennycress		<i>Thlaspi arvense L.</i>	Altered fatty acids and oils		Illinois State University		
GE-Vinv Potato		<i>Solanum tuberosum (potato)</i>	Alteration in growth, development or product quality	Calyxt	Collectis plant sciences		
GE-PPO Mushroom		<i>Agaricus bisporus</i>	Reduced browning		Pennsylvania State University		
Low PPO5 potato		<i>Solanum tuberosum (potato)</i>	Reduced black spot bruising	J.R. Simplot	J.R. Simplot		
FAD2KO Soybean		<i>Glycine max (soybean)</i>	Altered fatty acids and oils	Calyxt	Collectis plant sciences		
BHB Hi-Yield Maize		<i>Zea mays (maize, corn)</i>	Enhanced growth rate or yield	Benson Hill Biosystems	Benson Hill Biosystems		
FAD3KO Soybean		<i>Glycine max (soybean)</i>	Altered fatty acids and oils	Calyxt	Collectis plant sciences		
5715		<i>Brassica napus (canola, oilseed rape, rapeseed)</i>	Sulfonylurea tolerance, Imidazolinone tolerance	Cibus US LLC	Cibus US LLC	SU Canola	
CLB1		<i>Brassica napus (canola, oilseed rape, rapeseed)</i>	Imidazolinone tolerance		BASF		



www.euginius.eu (search for “GE”)



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PERSPECTIVE ARTICLE

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Lutz Grohmann^{1†}, Jens Keilwagen^{2†}, Nina Duensing¹, Emilie Dagand¹, Frank Hartung², Ralf Wilhelm², Joachim Bendiek¹ and Thorben Sprink²

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Contact:

lutz.grohmann@bvl.bund.de

