

10 June 2021



# *In vivo and in vitro* random mutagenesis techniques in plants

GMO Unit

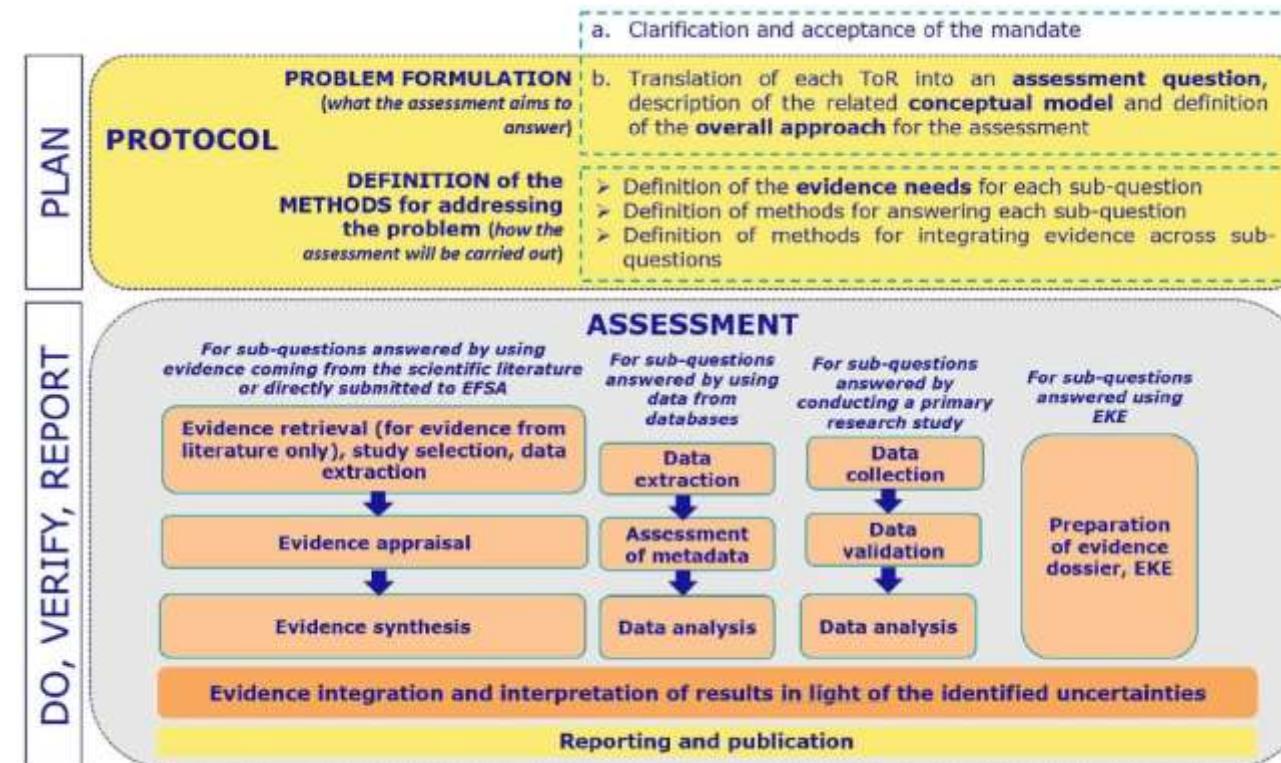
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- In the judgment in Case C-528/16 the Court of Justice of the European Union (CJEU) interpreted the Article 3 of Directive 2001/18 on the deliberate release of Genetically Modified Organisms (OGM) referring to the “application of conventional methods of random mutagenesis” without distinguishing further between *in vivo* and *in vitro* random mutagenesis
- The Conseil d’Etat of France issued on 7 February 2020 a judgment on organisms obtained by mutagenesis, distinguishing between *in vivo* and *in vitro* random mutagenesis techniques
- The EC asked EFSA to clarify whether the distinction between *in vitro* and *in vivo* is scientifically justified

- Protocol development
- Draft Opinion
- Timeline

- **Random mutagenesis mandate** selected as a pilot
- 'Draft framework for protocol development for EFSA's scientific assessments' (EFSA, 2020)
  - Protocol applied to EFSA's non-application scientific assessments

- Step 1. Problem formulation
  - Translation of ToRs into assessment questions
- Step 2. Definition of methods for addressing the problem
  - Literature search: review papers and book chapters



- Assistance from the AMU Unit
- Literature search outcome:
  - **517** documents
- Screening of the literature:
  - 2 independent reviewers screened the documents by title and abstract
  - **294** documents retained
  - **75** publications cited in the DO

- ✓ ToR1: To provide a more detailed description of random mutagenesis techniques as applied *in vivo* and *in vitro*.
- ✓ ToR2: To assess whether the types of genetic modification induced by random mutagenesis techniques are different depending on whether the technique is applied *in vivo* or *in vitro*.
- ✓ ToR3: To assess whether the molecular mechanism underlying random mutagenesis techniques is different if the techniques are applied *in vivo* or *in vitro*.
- ✓ ToR4: To assess whether *in vitro* random mutagenesis techniques are to be considered as different techniques compared to *in vivo* random mutagenesis techniques or on the contrary, if they are to be considered as a continuum.

- Introduction
  - Spontaneous and induced mutations in the context of plant breeding
  - Historical view of random mutagenesis in mutation breeding
- Addressing ToR1
  - Q1: What random mutagenesis techniques are used to obtain mutant plants?
    - Mutation breeding: summary of the main steps
    - General considerations of *in vivo* and *in vitro* random mutagenesis techniques in plants
    - Physical mutagenesis techniques applied in mutation breeding
    - Chemical mutagenesis techniques applied in mutation breeding
    - Example of *in vivo* and *in vitro* mutagenesis applications in plants
  - Q2: Are all these techniques applied both *in vivo* and *in vitro*?
    - Sub-conclusion



## ■ Addressing ToR3

- Q1: What are the underlying molecular mechanisms which generate the mutations?
  - Mechanisms leading to DNA damage
  - Mechanisms leading to repair
- Q2: Is there any difference between these molecular mechanisms whether they happen *in vivo* or *in vitro*?
  - Sub-conclusion

## ■ Addressing ToR2

- Q1: What type of alterations at the DNA level are induced by random mutagenesis?
  - Types of mutations
- Q2: Is there any difference between the mutations whether they are obtained *in vivo* or *in vitro*?
  - Sub-conclusion

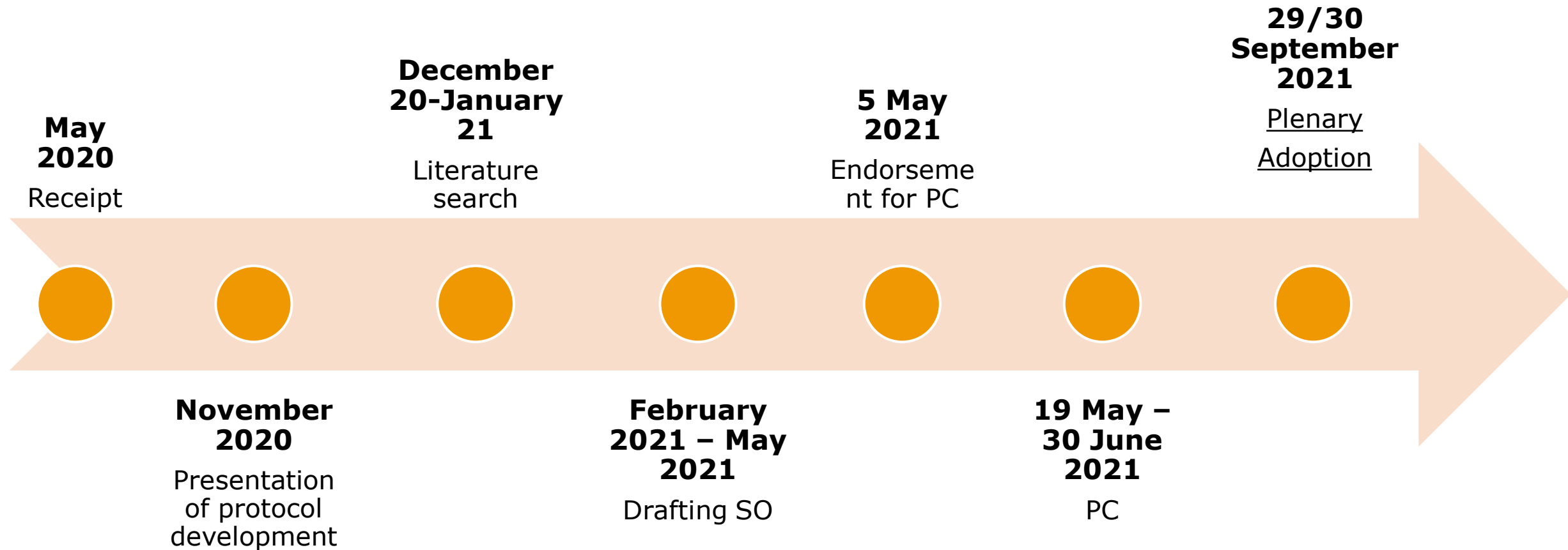
## ■ Addressing ToR4

- Q: Are *in vitro* and *in vivo* random mutagenesis techniques considered to be different or not?
  - General conclusion based on the assessment

## ■ Conclusions


- different physical and chemical mutagenesis techniques have been applied to both *in vivo* and *in vitro*
- the process and the repair mechanisms that are triggered by the mutagen act at the cellular level and thus there is no difference in the way the mutagen will affect the DNA whether the mutagen is applied *in vivo* or *in vitro*;
- the type of mutations induced by a specific mutagen are expected to be the same, regardless of whether such mutagen is applied *in vivo* or *in vitro*.
- The distinction between plants obtained by *in vitro* or *in vivo* approaches is, therefore, not justified. Indeed, the same mutation can be potentially obtained using both *in vivo* and *in vitro* random mutagenesis and the resulting mutants would be indistinguishable.

# Timeline



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## Public Consultations

 Public Consultation  
Draft Scientific Opinion on in vitro random mutagenesis techniques

Status	Food Domain	Start Date	End Date
Open	GMO	19/05/2021	30/06/2021

Title  
Draft Scientific Opinion on in vitro random mutagenesis techniques

Full Name  
Draft Scientific Opinion on in vitro random mutagenesis techniques

▼ Public Consultation Details

Food Domain GMO	Start Date 19/05/2021
	End Date 30/06/2021

**Please, submit your comments!**

**Deadline 30-06-2021**

<https://connect.efsa.europa.eu/RM/s/publicconsultation/a0c1v00000DOI9dAAD/pc0011>

# Thanks!

- MC subWG
  - Nils Rostoks (Chair)
  - Josep Casacuberta
  - Philippe Guerche
  - Tamas Dalmay
- MC WG
- MC Team
- GMO panel members
- Irene Pilar Munoz Guajardo, Laura Martino (AMU Unit)



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