UPOV

International Union for the Protection of New Varieties of Plants

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Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular

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ADDENDUM TO DO NEW BREEDING TECHNIQUES LEAD TO ESSENTIALLY DERIVED VARIETIES?

Document prepared by an expert from the International Community of Breeders of Asexually Reproduced Ornamental and Fruit-Tree Varieties (CIOPORA)

Disclaimer: this document does not represent UPOV policies or guidance

The Annex to this document contains a copy of a presentation on “Do New Breeding Techniques lead to Essentially Derived Varieties?”, prepared by an expert from the International Community of Breeders of Asexually Reproduced Ornamental and Fruit-Tree Varieties (CIOPORA), which was made at the seventeenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular (BMT).

[Annex follows]

ANNEX

DO NEW BREEDING TECHNIQUES LEAD TO ESSENTIALLY DERIVED VARIETIES?

Presentation prepared by an expert from the International Community of Breeders of Asexually Reproduced Ornamental and Fruit-Tree Varieties (CIOPORA)

CIOPORA

Do New Breeding Techniques (NBT) lead to essentially derived varieties (EDV)?

Edgar Krieger & Jan De Riek

CIOPORA UNITING BREEDERS, PROTECTING INNOVATION

Contents

- Conventional mutation and mutation breeding
- New Breeding Techniques: short technical introduction
- Do new breeding techniques lead to essentially derived varieties?
- Regulatory status of genome edited plants (GMO status – CJEU decision – if time allows)

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Mutations in nature

- Mutations = great source of novelties in plant breeding!
- Spontaneous mutants

'(Red) Elstar'

'H. Vogel' bud sports

- Spontaneous mutation frequency: 1/100 Mbp

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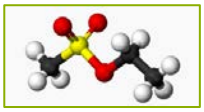
Mutation breeding

- Much higher frequency: up to 1/1000 bp
- EMS (ethyl-methanesulfonate)



- UV-C
- Gamma gardens
  E.g. Studiecentrum voor Kernenergie Mol (SCK-CEN)

- Space breeding
  Lotus 'Outer Space Sun' (China)

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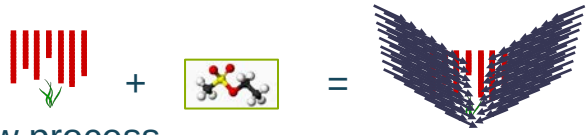
Mutation breeding

- Random and unpredictable



- Slow process
- More than 3000 novelties

https://mvd.iaea.org

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Mutations do not necessarily lead to plagiaristic varieties!

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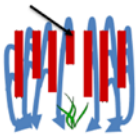
Mutation breeding 2.0



- Technology is moving forwards
- Mutations can be induced with chirurgical precision (molecular scissors)
- Without leaving behind foreign DNA in the plant genome
- Final product is often not discernable from 'classic' mutants

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New Breeding Techniques
(EU nomenclature)

1. Oligonucleotide Directed Mutagenesis (ODM)
2. Cis- en intragenesis
3. Grafting
4. Agro-infiltration
5. RNA-dependent DNA methylation
6. Reverse breeding
7. Synthetic biology
8. **Site-directed nucleases (SDN)**

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Schurft-resistente cisgene appels

Vier cisgene en één intragene genetisch gemodificeerde appellijnen van de cultivar 'Gala' worden in dit project in een boomgaard gedurende enkele jaren gevolgd.

Apple:

- reduction of long juvenile phase
- Scab resistance
- Red fruit flesh
- Browning

Oligonucleotide Directed Mutagenesis (ODM)

Broad application range – used intensively for herbicide tolerance

E.g. herbicide tolerant flax and canola

(mutation in *ALS*-gen –imidazolinone tolerance)

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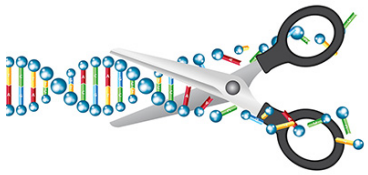
Site-directed nucleases



- Nuclease = protein (enzyme) that cuts the DNA

- Oriented to a recognition site
  - At least 4bp long
  - longer = more specific

EcoR I

5′. . . G|AATTC . . . 3′

3′. . . CTTAA|G . . . 5′

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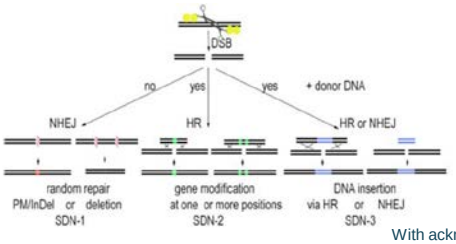
Site-directed nucleases

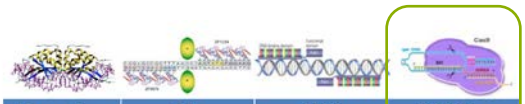
- SDN-1 (random repair): non-homologous end joining of the 2 parts; having a relatively high rate of mistakes leading to point mutations, short insertions/deletions or longer deletions.
- SDN-2 (gene modification): homology guided DNA repair by using a donor DNA and homologous recombination to introduce a targeted mutation.
- SDN-3 (DNA insertion): a new DNA sequence is inserted on a targeted site in the genome, DNA repair is based on homology (cfr. SDN-2) of by end joining (cfr. SDN-1)



With acknowledgement: Cécile Collonnier

Site-directed nucleases

ZFN: Zinc Finger Nucleases



	Meganuclease	ZFN	TALEN	CRISPR/Cas
Nb of proteins required	1	2	2	1 + gRNA
Off-target	Very low (target = 18-24 bp)	Medium high (target = 2 x 12-18 bp)	Low (target = 2 x 10-30 bp)	Low (target = 19-23 bp)
Diversity of target sequences	One cutting site every 250 bp	One cutting site every 500 bp	One cutting site every 35 bp	One cutting site every 8 pb
Difficulty of production	Heavy (modification of the peptid chain and selection)	Uneasy (interaction effects on specificity)	Relatively easy (modular assembly)	Very easy (synthesis of one oligonucleotid)
Approximate price	50 000 €	5000 €	1000 €	100 €

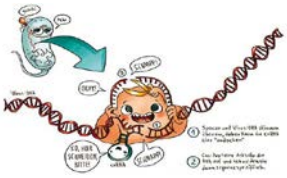
With acknowledgement: Cécile Collonnier

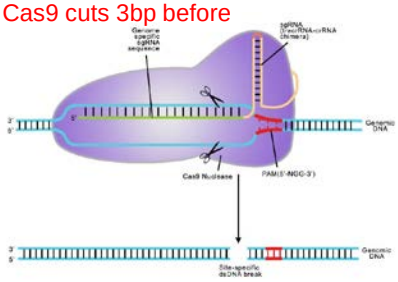
CRISPR/Cas

E. Charpentier

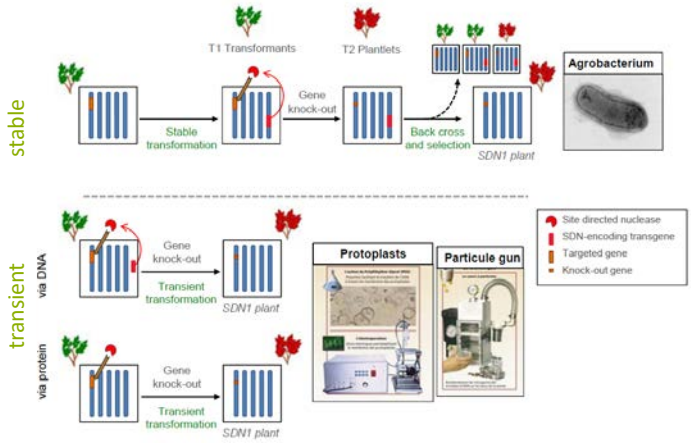
Clustered
Regularly
Interspaced
Short
Palindromic
Repeats

- Cas9 enzyme (scissors)
- Guide RNA (20bp known gene + tail) (sgRNA)
- NGG: PAM site (recognition site for restriction)
  => Cas9 cuts 3bp before





CRISPR/Cas



With acknowledgement: Cécile Collonnier

Implications of NBT technology on EDV status

- Common feature of all first generation varieties resulting from NBT is that they retain virtually the whole genome of the mother variety.
- Some NBT approaches are more suited for pre-breeding
  - (Recurrent) backcrossing is needed to get rid of foreign DNA of vectors and marker genes.
  - Not every variety is suited for transformation, protoplast regeneration etc.; therefore, a trait will be introduced once and then used in conventional breeding.
- How should these varieties be classified in terms of the EDV concept?

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EDV in the UPOV Convention

Article 14(5)(b) and (c) of the 1991 Act of the UPOV Convention

(b) For the purposes of subparagraph *(a)*(i), a variety shall be deemed to be essentially derived from another variety ("the initial variety") when

(i) it is predominantly derived from the initial variety, or from a variety that is itself predominantly derived from the initial variety, **while retaining the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety,**

(ii) it is clearly distinguishable from the initial variety and

(iii) except for the differences which result from the act of derivation, it conforms to the initial variety in the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety.

(c) Essentially derived varieties may be obtained for example by the selection of a natural or induced mutant, or of a somaclonal variant, the selection of a variant individual from plants of the initial variety, backcrossing, or transformation by genetic engineering.

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UPOVs´ way to its EXN on EDV

- UPOV has been discussing EDV since 2007
- In 2009, the first Explanatory Note has been approved by the UPOV Council, with an immediate opening for revision (on request of CIOPORA).
- In 2017, the second Explanatory Note (UPOV/EXN/EDV/2) has been approved, again with an opening for further discussion (on request of Russia and CIOPORA).

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Excerpts of EXN/EDV/2

….. a variety should only be essentially derived from another variety when it retains virtually the whole genotype of the other variety.

The phrase "while retaining the expression of the essential characteristics" requires that the expression of the essential characteristics conforms to and be derived from the initial variety.

6. The following might be considered in relation to the notion of "essential characteristics":

...

(ii) characteristics that are important from the perspective of the producer, seller, supplier, buyer, recipient, or user;

....

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Excerpts of EXN/EDV/2

The differences must not be such that the variety fails "to retain the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety".

The derived variety must retain almost the totality of the genotype of the initial variety and be different from that variety by a very limited number of characteristics (one or very few).

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Breeders ask for clarification on the interpretation of the EDV concept

- Varieties, although predominantly (or even solely) derived from one initial variety (like mutants or GMO), but not retaining the essential characteristics of the initial variety, will **not** be considered EDV?
- An extensive list of essential characteristics: characteristics that are important from the perspective of producer, seller, supplier, buyer, recipient, or user.
- Practical consequences: What will be the future status of
  - **Color** mutations of protected varieties?
  - **Disease resistant** or **tolerant** NBT or GMO varieties?
  - Mutations with **earlier ripening** time?
  - **Non-browning** apple NBT or GMO varieties?

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Breeders ask for clarification on the interpretation of the EDV concept

- CIOPORA has concerns that only plagiaristic mutants, plagiaristic GMO and plagiaristic results from repeated backcrossing are considered EDV.
- An EDV concept, limited to prevent plagiarism only, would not particularly strengthen the right of the breeder of the original variety, because UPOV 1991 has extended the scope of the protected variety to *varieties which* are *not clearly distinguishable* from the protected variety. This provision, dealing with **Minimum Distance**, was and is meant to prevent plagiarism, not the EDV concept.

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Do new breeding techniques lead to Essentially Derived Varieties (EDV)?

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Subsumption

(= squeezing facts under the law)

(b) For the purposes of subparagraph *(a)*(i), a variety shall be deemed to be essentially derived from another variety ("the initial variety") when

(i) it is predominantly derived from the initial variety, or from a variety that is itself predominantly derived from the initial variety, **while retaining the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety,**

(ii) it is clearly distinguishable from the initial variety and

(iii) except for the differences which result from the act of derivation, it conforms to the initial variety in the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety.

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Do new breeding techniques lead to Essentially Derived Varieties (EDV)?

Sometimes YES, sometimes NO ??? – Issue seems not to be set yet

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CIOPORA current views and concerns

- The basic purpose of the EDV concept is to strengthen the right of the breeder (IOM/IV/2, page 2, no. B. 5. (i), of October 1989). The very objective of dependence is to give a breeder of an original genotype an additional source of remuneration (IOM/IV/2, page 12, no. 6. (iv)).
  - Wording of the EDV provision in the UPOV 1991 Act gives room for interpretation.
  - EDV provisions in PBR laws of UPOV members differ from UPOV text and are not harmonized, which might lead to different results as regards the classification of a variety as EDV or not.
  - Too narrow EDV approach does not take into consideration new developments in breeding. It does not support traditional breeding of initial varieties (crossing and selection), because the results are not effectively protected.

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CIOPORA current views and concerns

- Too narrow EDV approach disadvantages small and medium sized breeders in particular, because they solely rely on clear and effective laws.
- Too narrow EDV approach might quickly lead to an overall decrease of initial varieties, so that NBT lose their basis. Breeders might be reluctant to introduce innovative initial varieties into countries which follow a too narrow EDV approach.
- UPOV members should apply a fair EDV approach, which takes into consideration the interests of both the traditional breeders and the developers of NBT
- UPOV should consider to revise the EDV provision in the UPOV 1991 Act.

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Regulatory status of genome edited plants

- Regulatory dilemma: same phenotype can be obtained by
  - Loss of gene function by natural mutation
  - Idem, but induced by chemical mutagenesis or irradiation
  - Gene editing by SDN1 or SDN2 action e.g. from CRISPR-Cas technology
  - Loss of gene function by insertion of a transgene
- Balance between process-based and trait-based regulation is lost
  - Identical genotypes obtained by different processes will have a different regulation
  - Compared to conventional mutagenesis, CRISPR-Cas is generally considered to be
    - More versatile
    - More rapid
    - More precise
    - Unwanted modifications in the genome are strongly reduced
  - Potentially better technology is treated more strictly because of being newer

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Regulatory status of genome edited plants

- CJEU interpretation of 25/07/2018 choses a juridical sound but technically absurd solution
  - If obtained by natural mutation, no regulation
  - Organisms obtained by mutagenesis are GMO within the meaning of the GMO Directive, in so far as the techniques and methods of mutagenesis alter the genetic material of an organism in a way that does not occur naturally
  - Those organisms come, in principle, within the scope of the GMO Directive and are subject to the obligations laid down by that directive
  - However, organisms obtained by certain mutagenesis techniques which have conventionally been used and have a long safety record are exempt from those obligations
  - In vitro mutagenesis techniques (NBT) might prove to have risks that are similar to those that result from the production and release of GMO through transgenesis

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[End of Annex and of document]