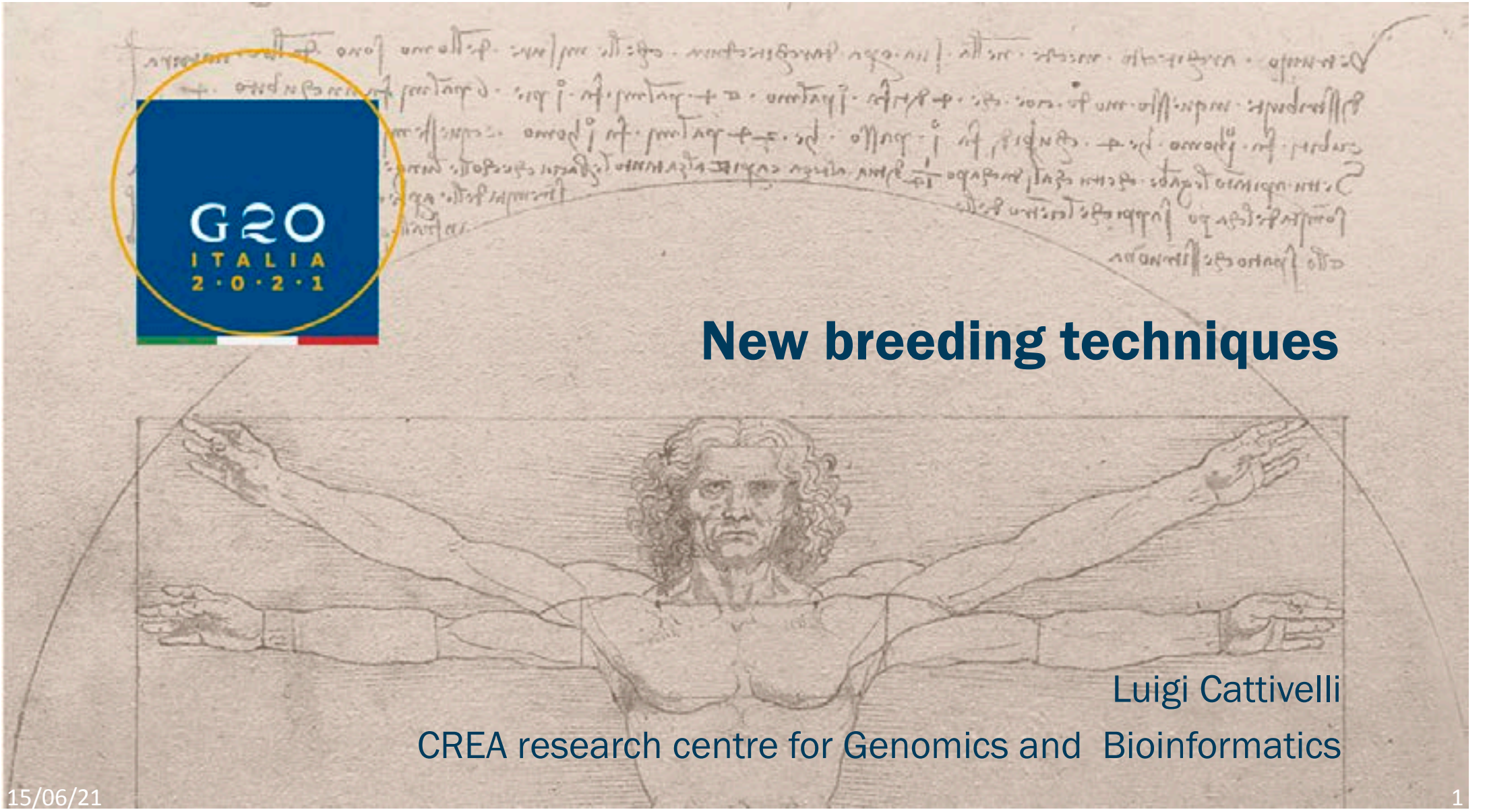
New breeding techniques



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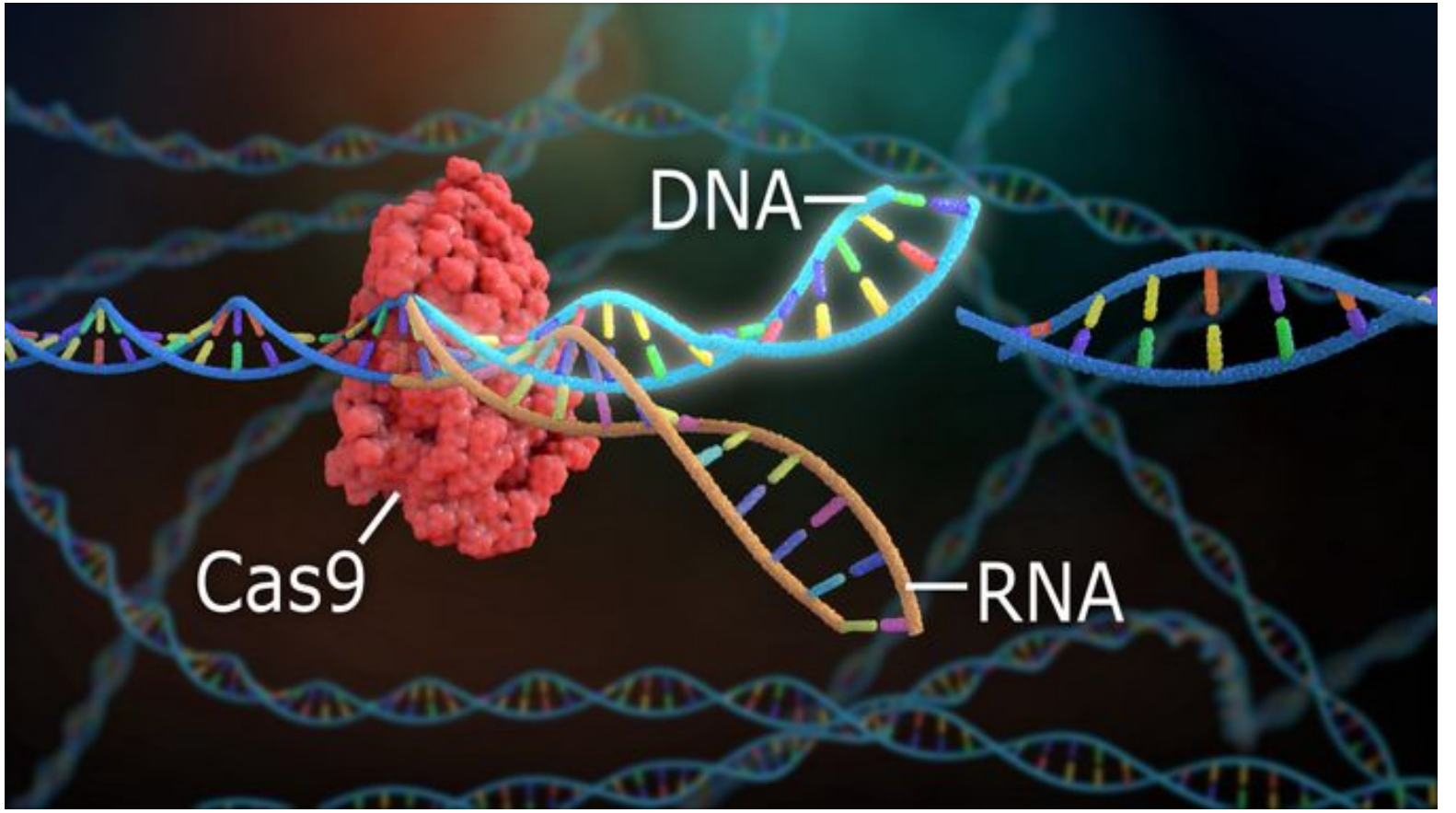
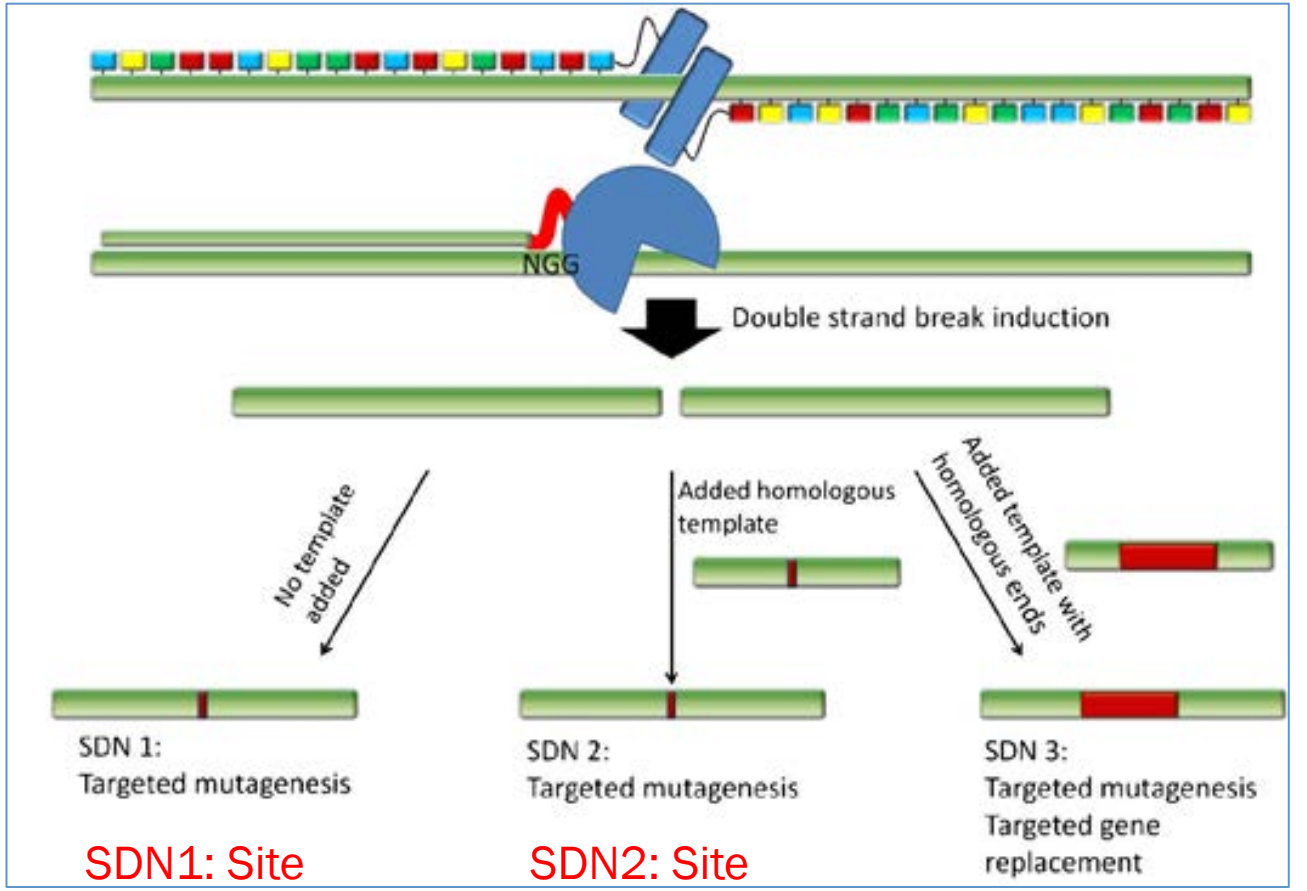
Genome editing based on CRISPR-Cas9



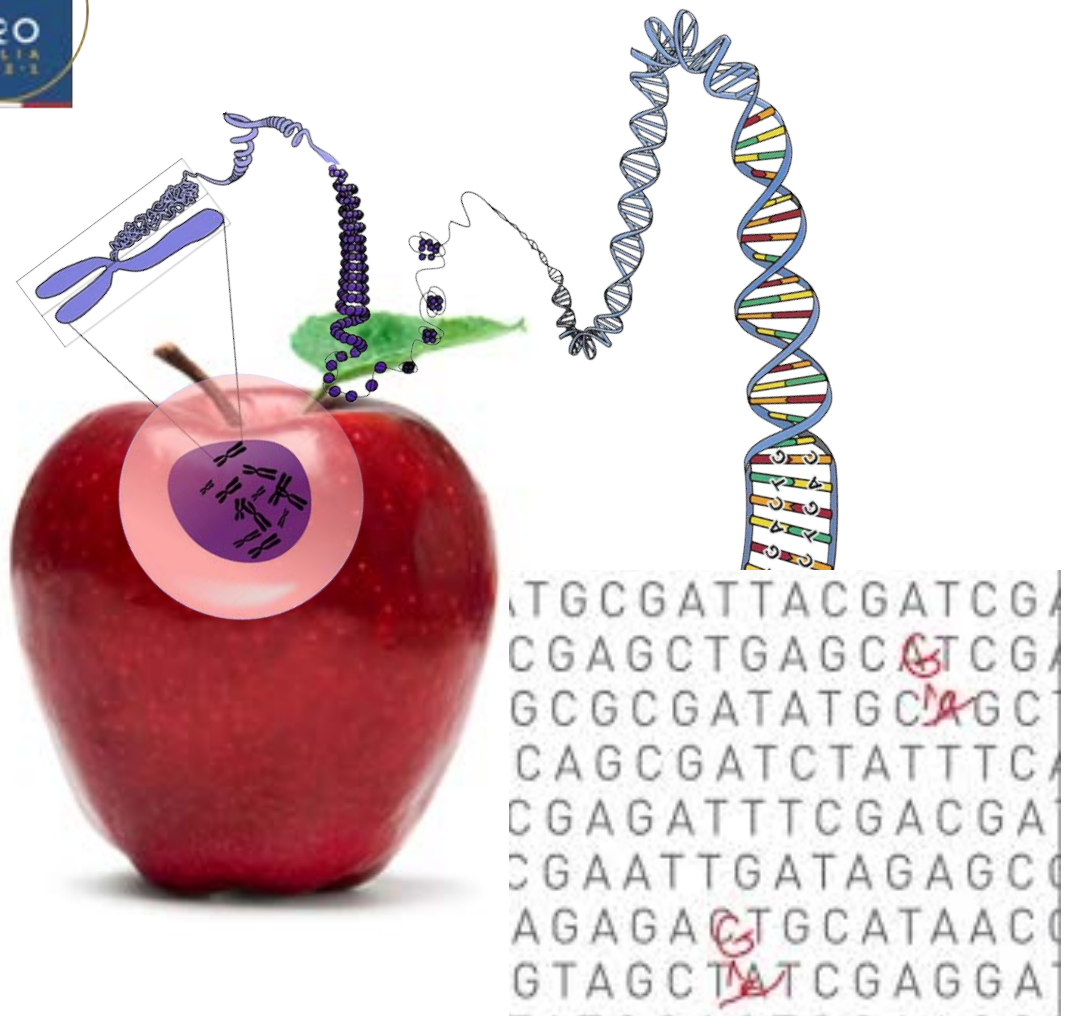
CRISPR-Cas9: a simple technology for Site Directed Nuclease



SDN1: Site directed unforeseen **mutations**

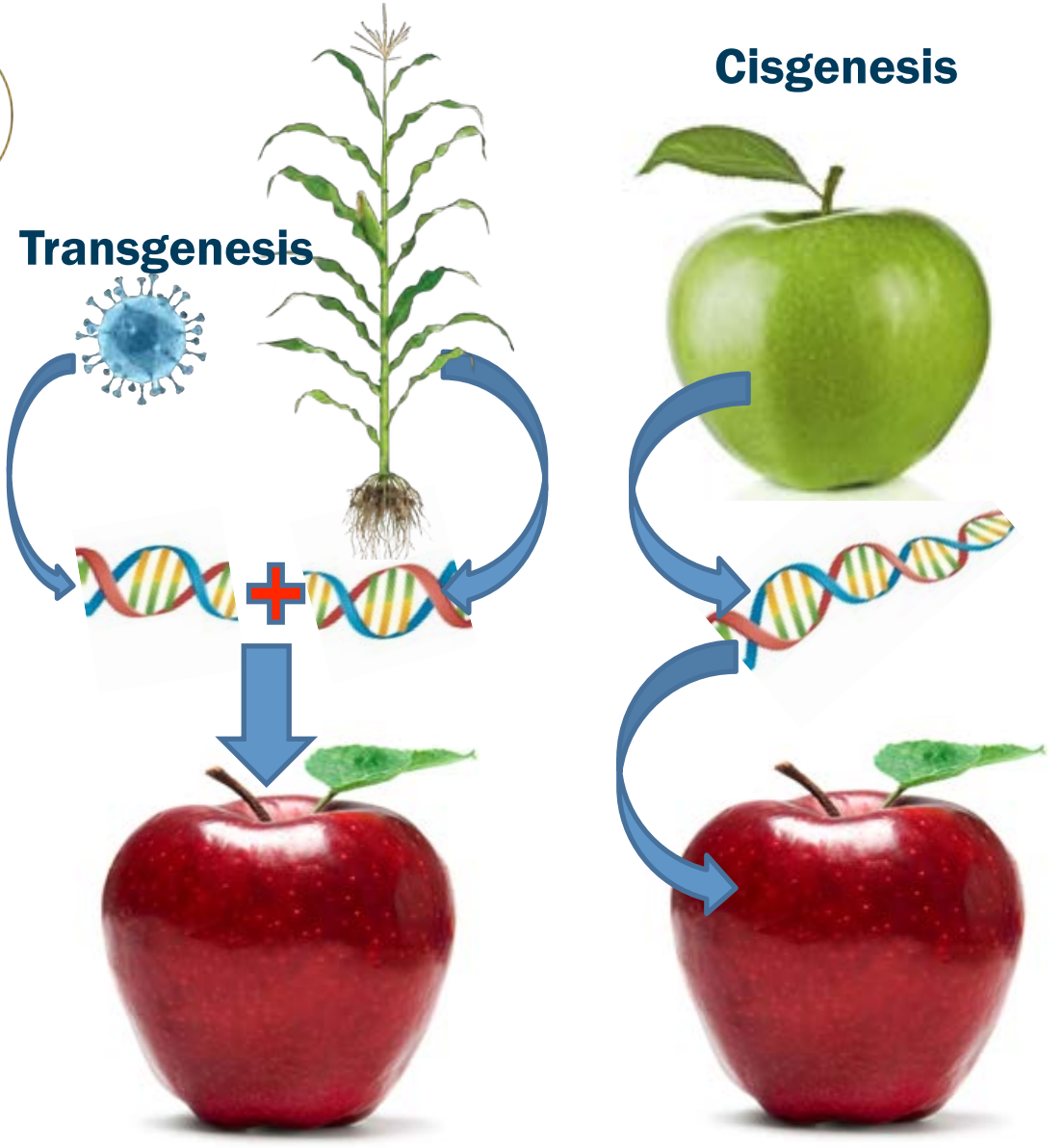
SDN2: Site directed pre-designed **mutations**

SDN3: Site directed insertion of DNA fragments



Genome Editing

- Genome editing (SDN1 and SDN2): modify a gene within the target species (without inserting any external gene).
- The result is equivalent to what is obtained with mutagenesis
- The mutation concerns a single gene defined a priori
- After the mutation the editing mechanism (Cas19) is removed



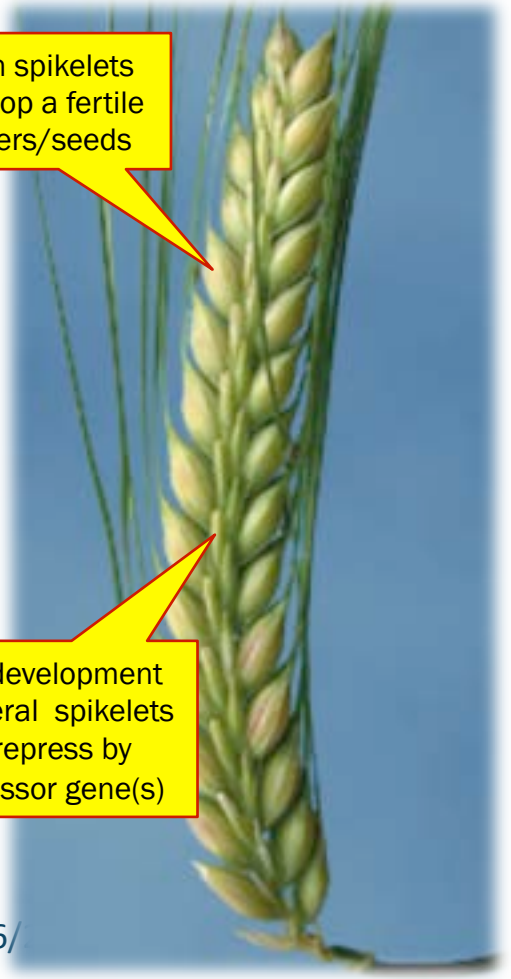
Cisgenesis

- Cisgenesis: when transformation is limited to the transfer of whole genes between plants of the same species (or interfertile).
- The inter-species barrier is fully respected and in principle, the gene transfer could be obtained with a backcross

Is it possible to demonstrate a genome edited events?

Although existing detection methods are able to detect even small mutations, this does not necessarily confirm the presence of a Genome Edited product; **the same alteration could have been obtained by conventional breeding (natural mutation, mutagenesis)**

Natural mutants that could have been generated by genome editing: barley



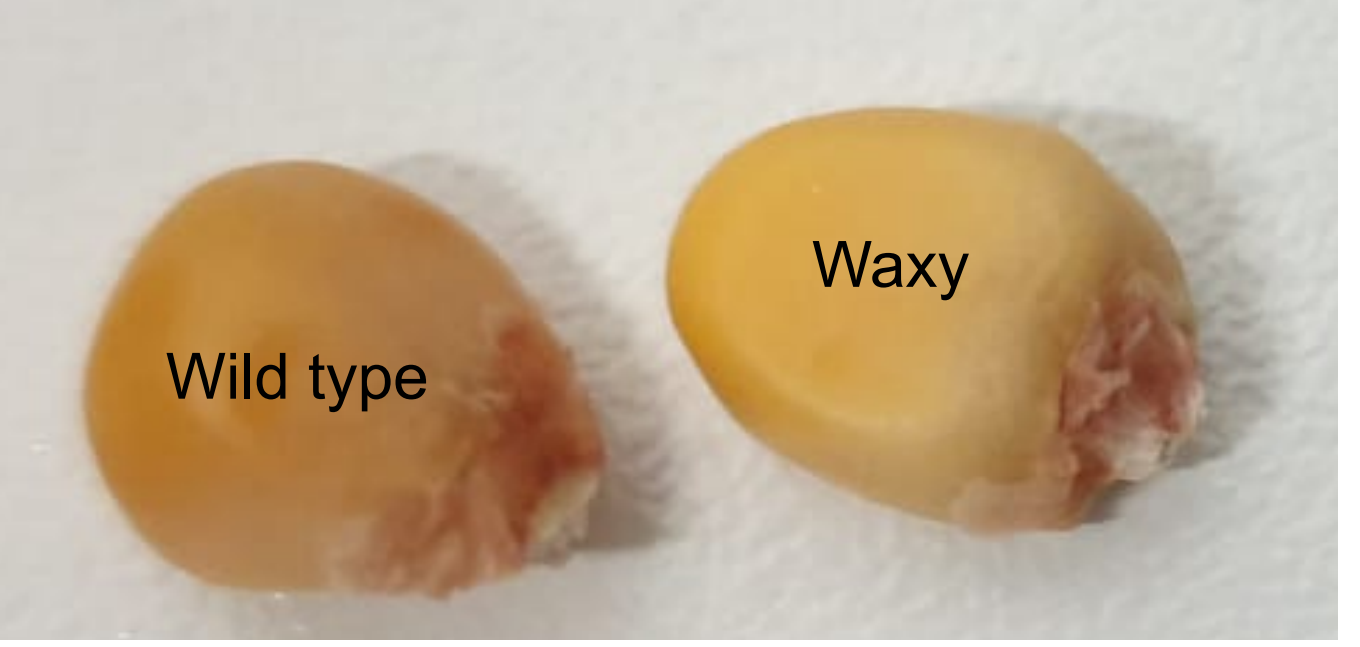
A single loss-of-function mutation transform two row barley (wild type allele) into six row barley (most of the barley grown today).

Natural mutants that could have been generated by genome editing: waxy corn

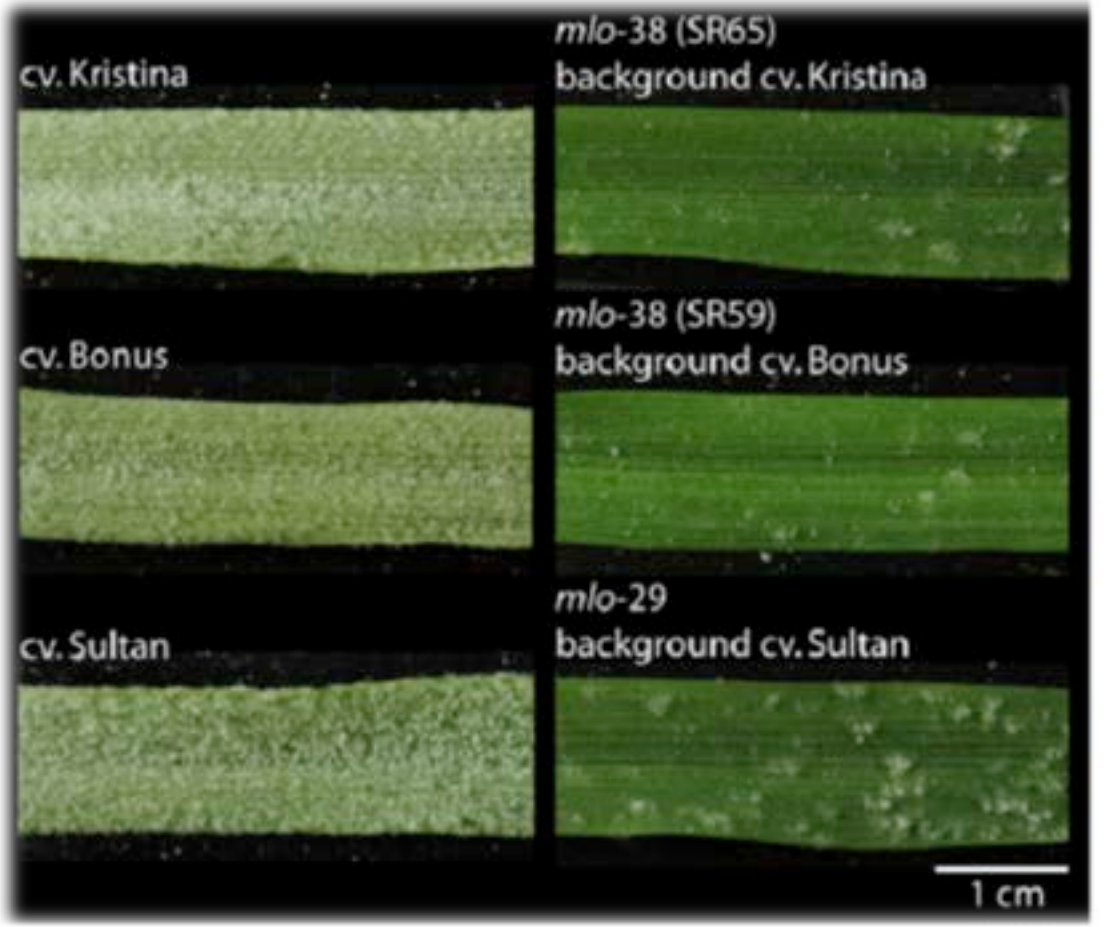
A knock out of the Granule Bound Starch Synthase gene leads to a maize waxy (with starch made of amylopectin only). Waxy mutants are known since 1908, many waxy-hybrids have been released.

Waxy starch is easy to gelatinize and it is used in adhesives industry.

Corteva has made a waxy corn with genome editing.



Natural mutants that could have been generated by genome editing: powdery mildew resistance in barley



Natural mutants that could have been generated by genome editing: powdery mildew resistance

- A knock-out mutation in the barley ***mlo*** gene confers resistance to powdery mildew
- Many ***mlo*** mutants have been selected after artificial mutagenesis, but are also known natural ***mlo*** mutants
- Powdery mildew exists in many plants and in all species there is an ***mlo*** gene homologs
- For example, a mutation induced by Genome Editing in the ***mlo*** gene of grapevine, bread wheat, strawberry, etc. leads to plants resistant to powdery mildew

Backcross or cisgenesis?
Resistance to downy mildew in grapevine

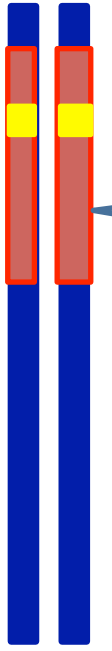
Backcrossing wild resistant genes on cultivated vines is possible, but a large genome region is transferred

Cisgenesis transfers the resistant gene only

Backcross or cisgenesis?

Resistant gene

Resistant cisgenic variety differs from the original variety for one gene out of 35-45,000 and can be obtained in one single generation

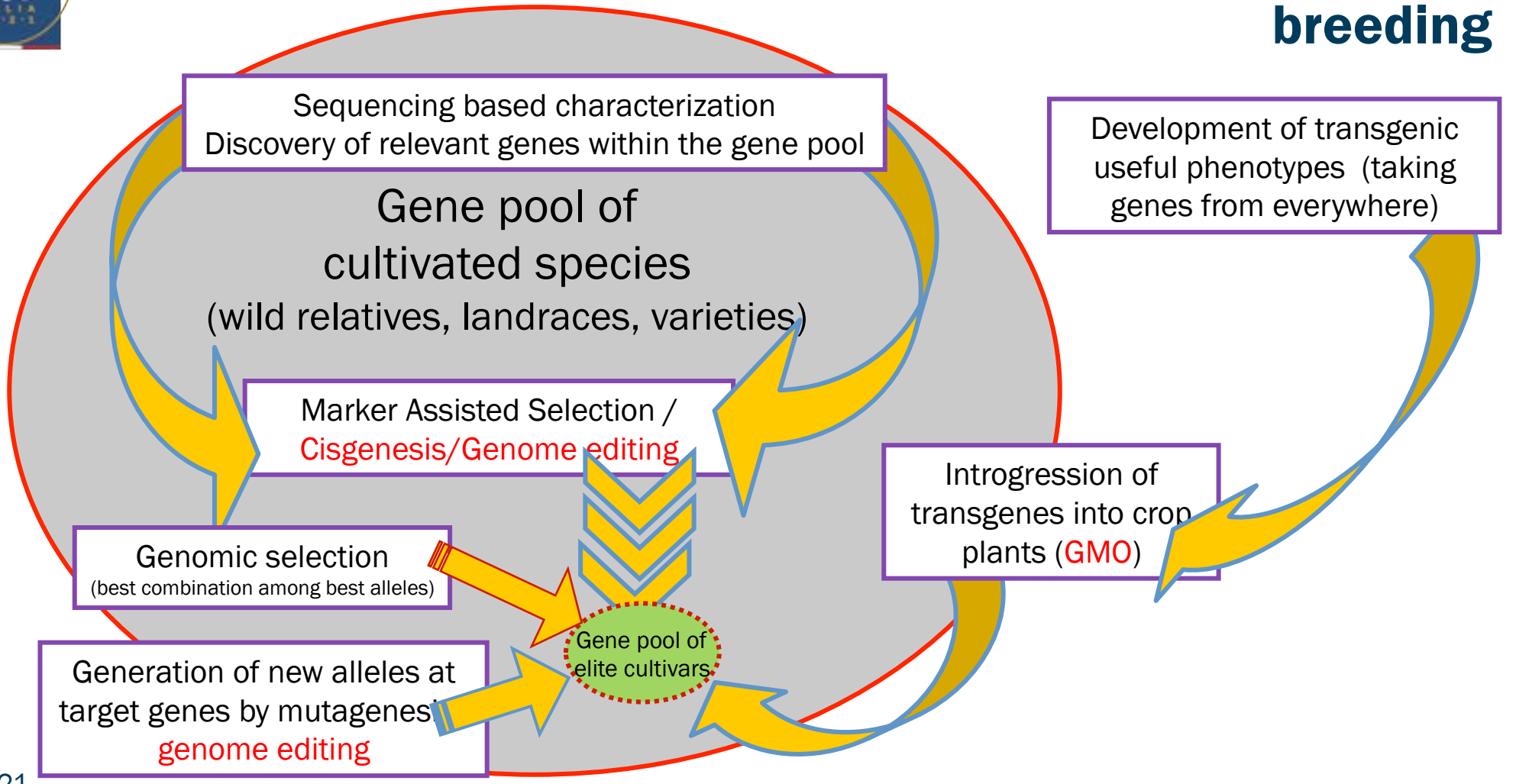


Adjacent DNA introgressed with the resistant gene (linkage drag)

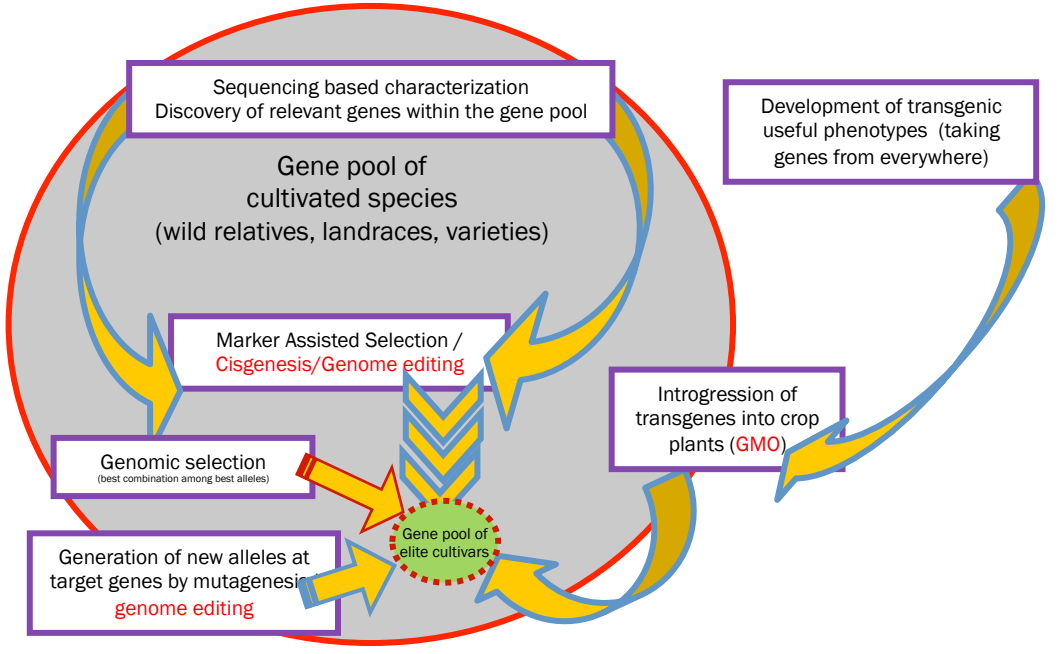
Resistant variety obtained by backcross differs from the original variety for 2-10% of its genome even after many backcross generations.

Cisgenesis varieties are identical to the original variety (except the introgressed gene)
e.g. cisgenic grapevine for a disease resistant gene have the same quality traits of the original one

The new breeding technique in the contest of plant breeding



The new breeding technique in the contest of plant breeding



- The impact of the New breeding techniques should be seen in the global contest of all breeding tools and strategies (diversity, sequencing, markers and gene mapping, mutants, NBT, crop modelling, …)
- A global coordination of the research aiming to enhance synergies could be useful.
- Wheat Initiative is an example.

WHEAT INITIATIVE

COORDINATING GLOBAL WHEAT RESEARCH

- New breeding techniques have been proven to be very useful for selecting the plants for the future
- Mutations in specific genes may confer very useful phenotypes and they are fully equivalent to natural or chemically induced mutations
- It is a fact that regulatory frameworks of genome editing differ from one country to another and a debate is ongoing in several countries on the role and regulation of genome edited plants

Concluding remarks and points for the discussion