Breeding Value

Pre-breeding strategies for obtaining new resilient and added value berries

From genomes to New Breeding Techniques to obtain new resilient and quality cultivars.

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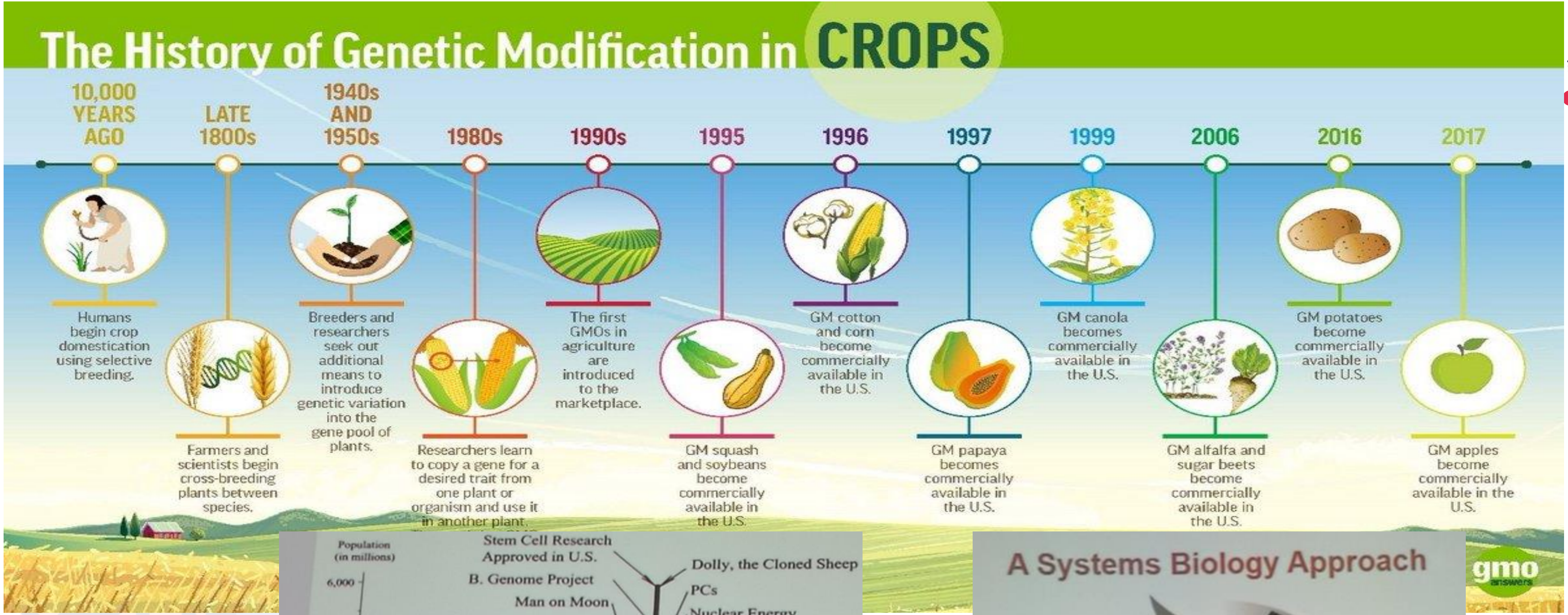
The BreedingValue project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000747.



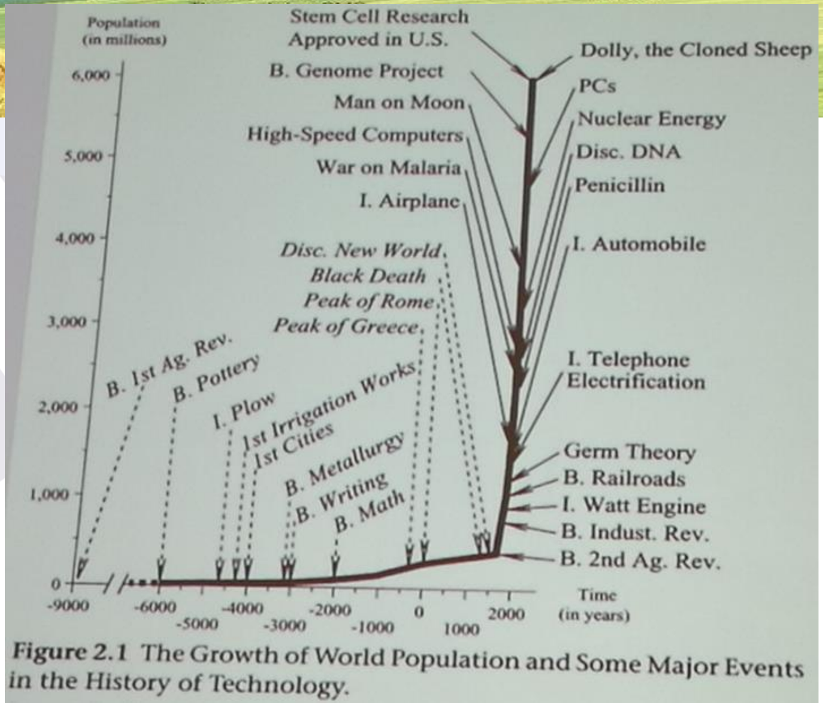
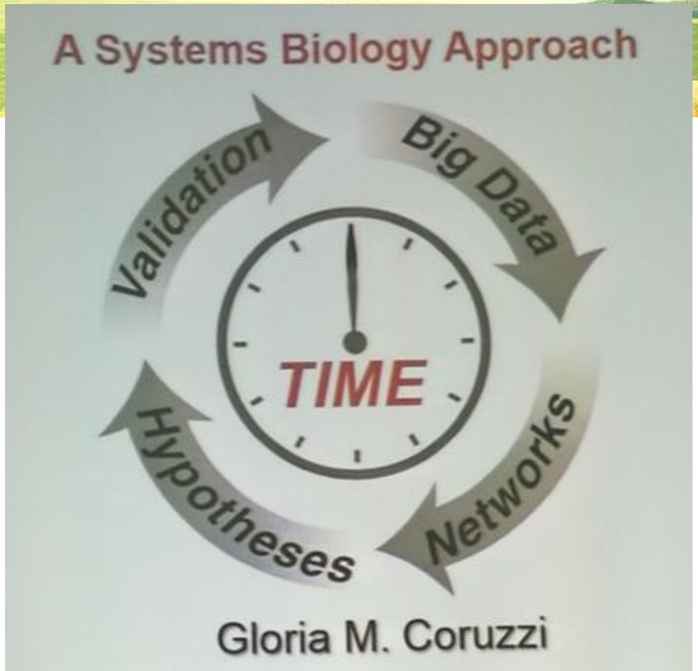
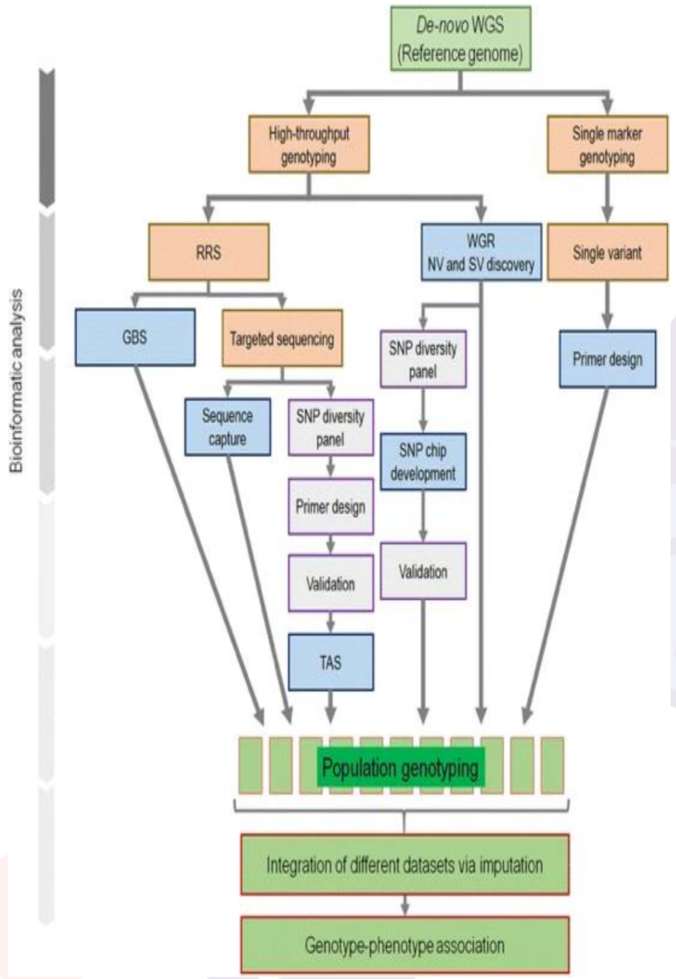


Figure 2.1 The Growth of World Population and Some Major Events in the History of Technology.

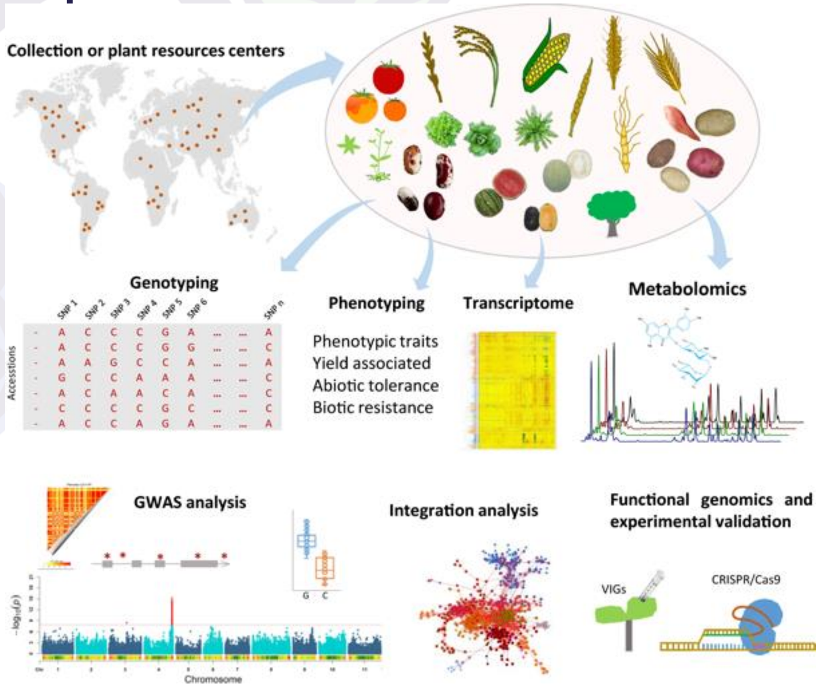


Bioinformatic tools and sequencing strategies



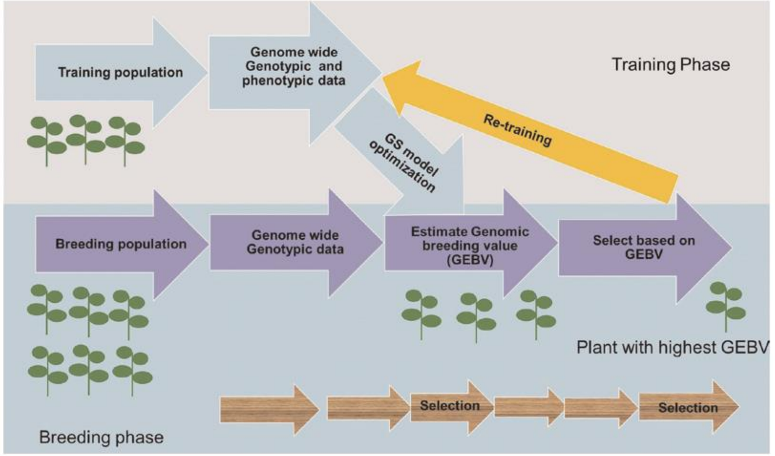
GENOME WIDE ASSOCIATION STUDIES - GWAS

Genome-wide association studies (GWAS) enable the detection and identification of quantitative trait loci (QTLs) and genes controlling phenotypic variations in a collection of cultivars and germplasm accessions.

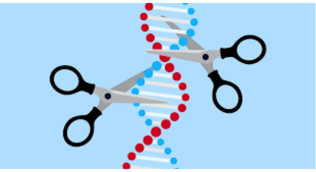


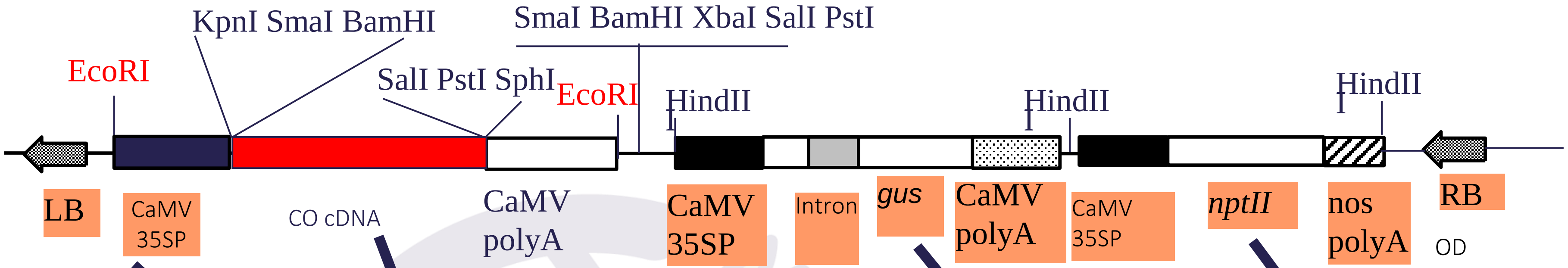
Genomic selection

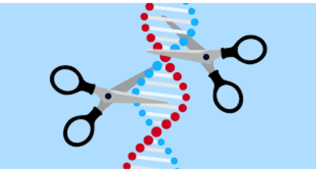
Genomic selection (GS) enables the selection of superior genotypes based on genomic estimated breeding values (GEBV) derived from the information of genome-wide DNA polymorphisms.



DNA AND GENES: Transgenes, Cisgenes, RNAi, gene editing







Promotor
Transgene/Cisgene

Regulator Gene

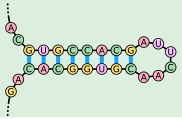
Constitutive
Site specific
Stress indoced

Gene

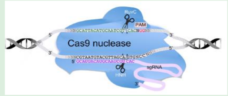
Transgene

Cis/Intragene

RNAi



CRISPR



Protein/enzime/RNAi/CAS9

TRAIT

GUS/GFP
Reporter

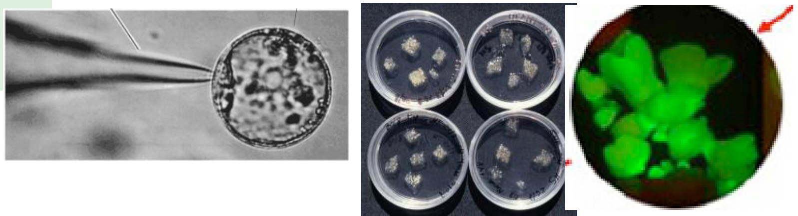
Transgene
TO AVOID

nptII
Antibiotic marker - to be avoided

Use new cisgenic markers such as the MYB gene

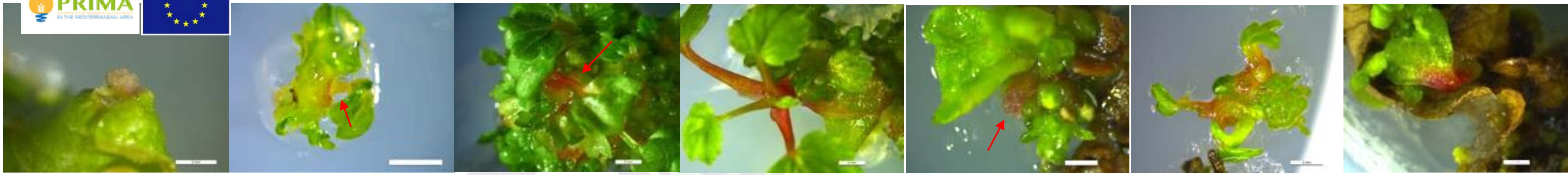
Problems in selection efficiency and chimerism

Clean Gene editing: transient expression in protoplast



Or plant segregation of Agro mediated transformed plant

Stable strawberry intragenic lines expressing *FaWRKY1*-RNAi *and FaNPR3.1*-RNAi driven by *FaAAT2* and *FaDOF2* promoters

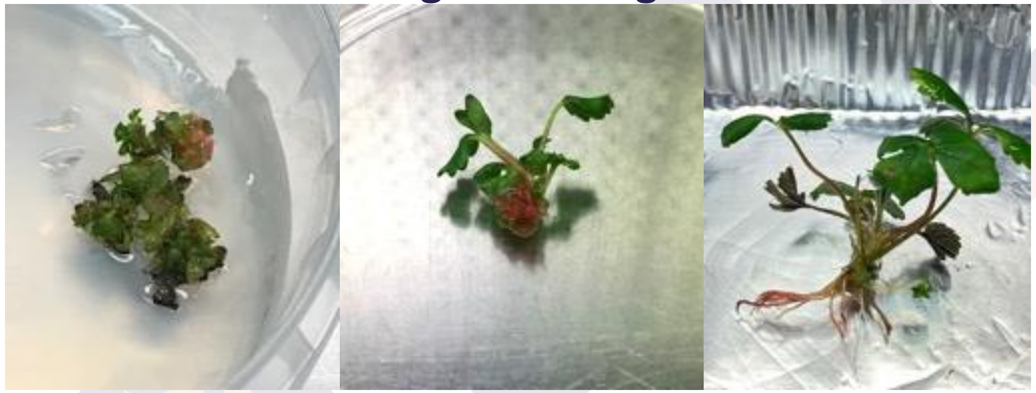


6 weeks after infection

15 weeks after infection

18 weeks after infection

Putative elongated intragenic lines



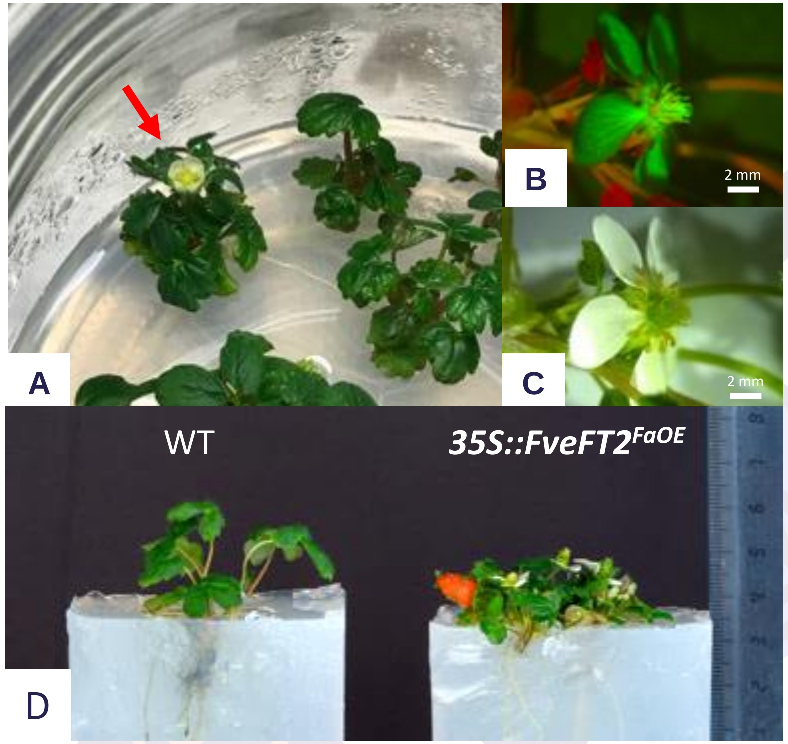
- Without using selectable marker genes, the possibility of obtaining a complete transformed shoot is very low
- High percentage of chimeric shoots

Alternative strategies are necessary

Transformation efficiency of four apple varieties without the use of selectable marker genes (through MYB expression only) (Transformation efficiency of apple with kanamycin is arounc 0.22 e 13.8%).

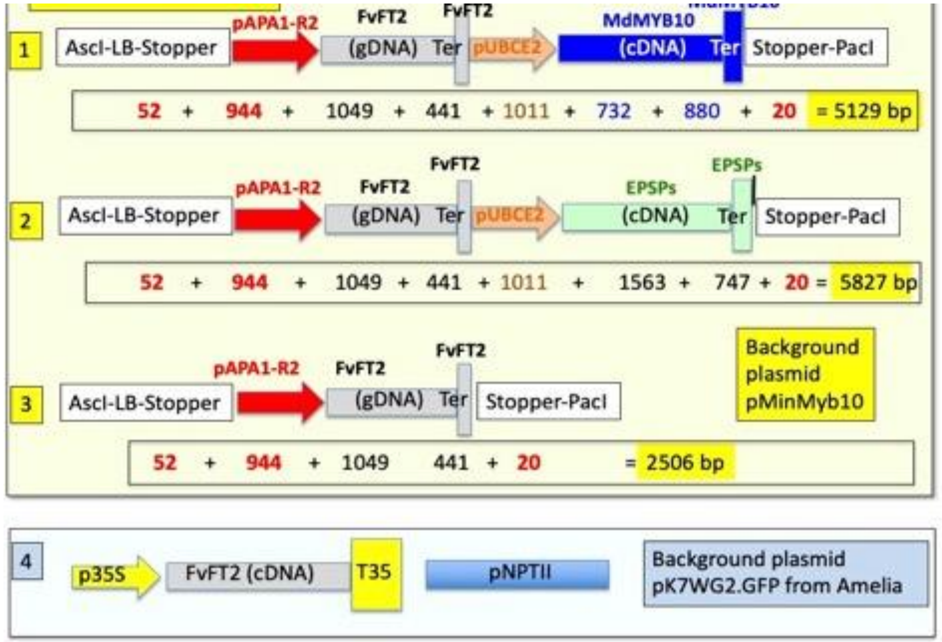
Krens et al., 2015

In vitro phenotype



In vivo phenotype

Gaston et al., 2021

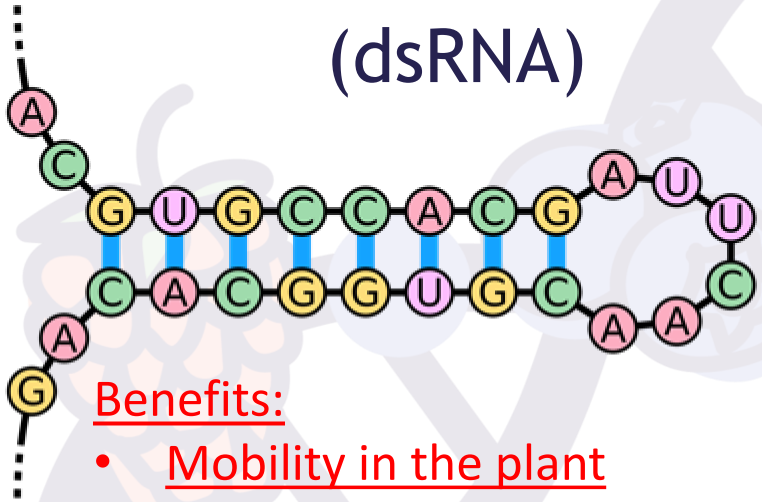


Intragenic constructs with Myb or EPSP genes from strawberry

RNAi versus CRISPR/Cas9

GENE EDITING/CRISPR

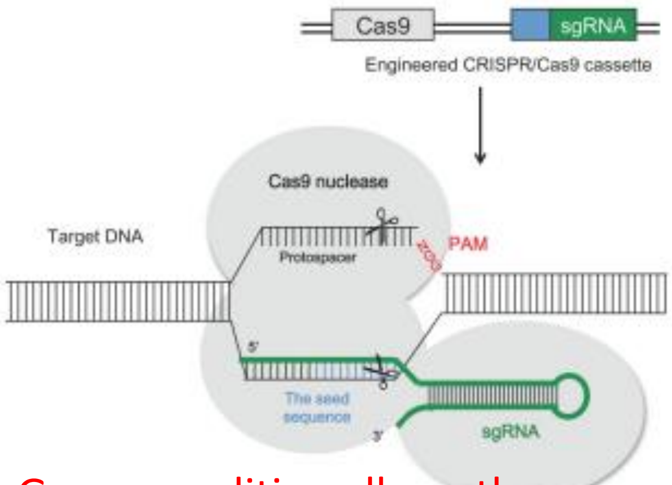
RNAi inhibits gene expression in a sequence-specific manner induced by double-stranded RNA (dsRNA)



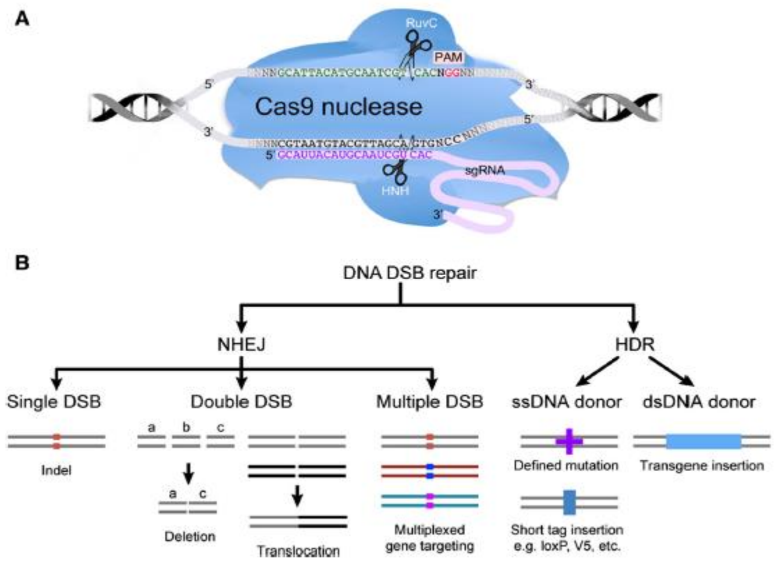
Benefits:

- Mobility in the plant
- Mobility between plant and target organisms
- Stable expression in planta
- Direct application - spray

Fire e Mello - Nobel prize for Medicine in 2006



Genome editing allows the integration, deletion, and/or mutation of genes of interest
In theory, replacing RNAi but only for cutting endogenous plant genes



CAS9 protein is the tool for induce gene editing and it must be inserted in the DNA or in the cell and cuts the DNA. For this reason, according to the legislation, it is still a GMO.

Charpentier e Doudna Nobel prize for Chemistry in 2020


frontiers in Plant Science

New Biotechnological Tools for the Genetic Improvement of Major Woody Fruit Species

Cecilia Limera[1], Silvia Sabbadini[1], Jeremy B. Sweet[2] and Bruno Mezzetti[1]*

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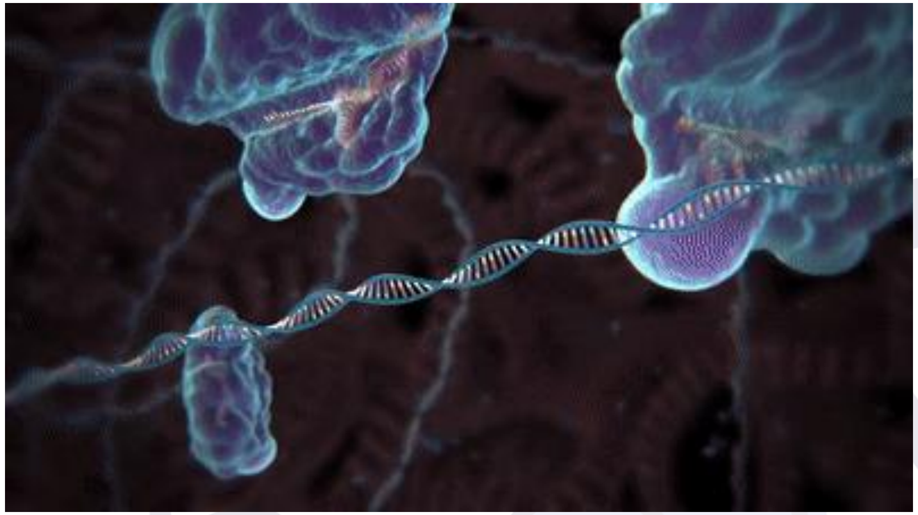
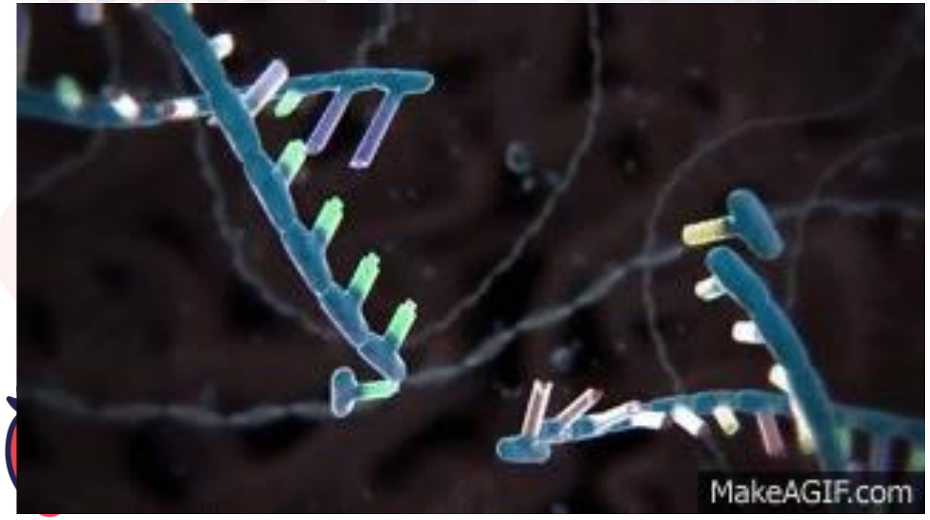
iPlanta

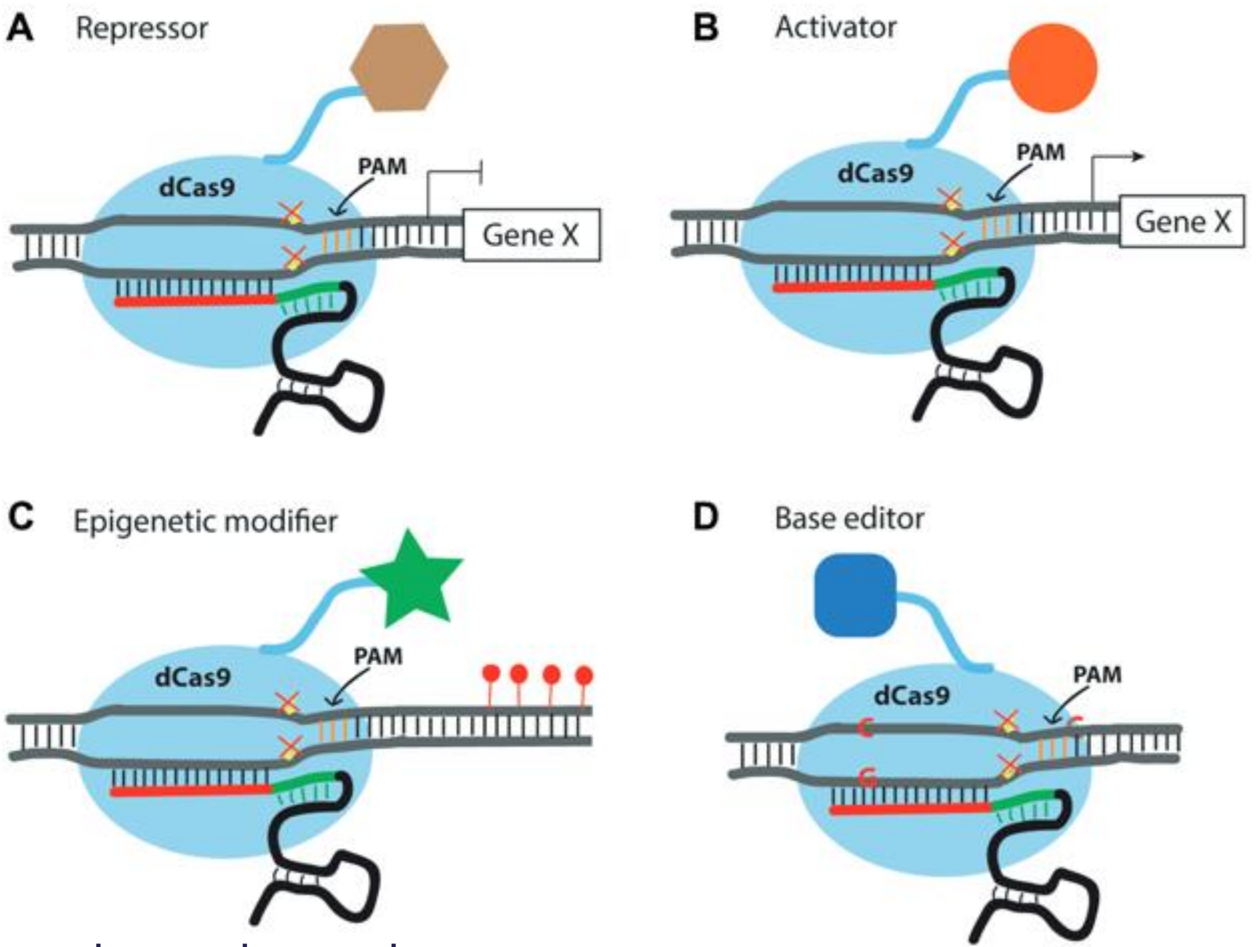
CRISPR/Cas9 mechanism

sgRNA that activates and guides Cas9 nuclease



DSBs mutation





Cas9 can be engineered:

- By linking it to a transcription activator or repressor
- To convert one base pair to another without performing a DSBs (base editing)
- Epigenetic modificator

CRISPR/Cas9 applied to fruit tree species

Reviewed by Fizikova et al., 2021

a) Proof of concept

PDS mutation = Chlorophyll-deficient variegated plant

grapevine

(b)

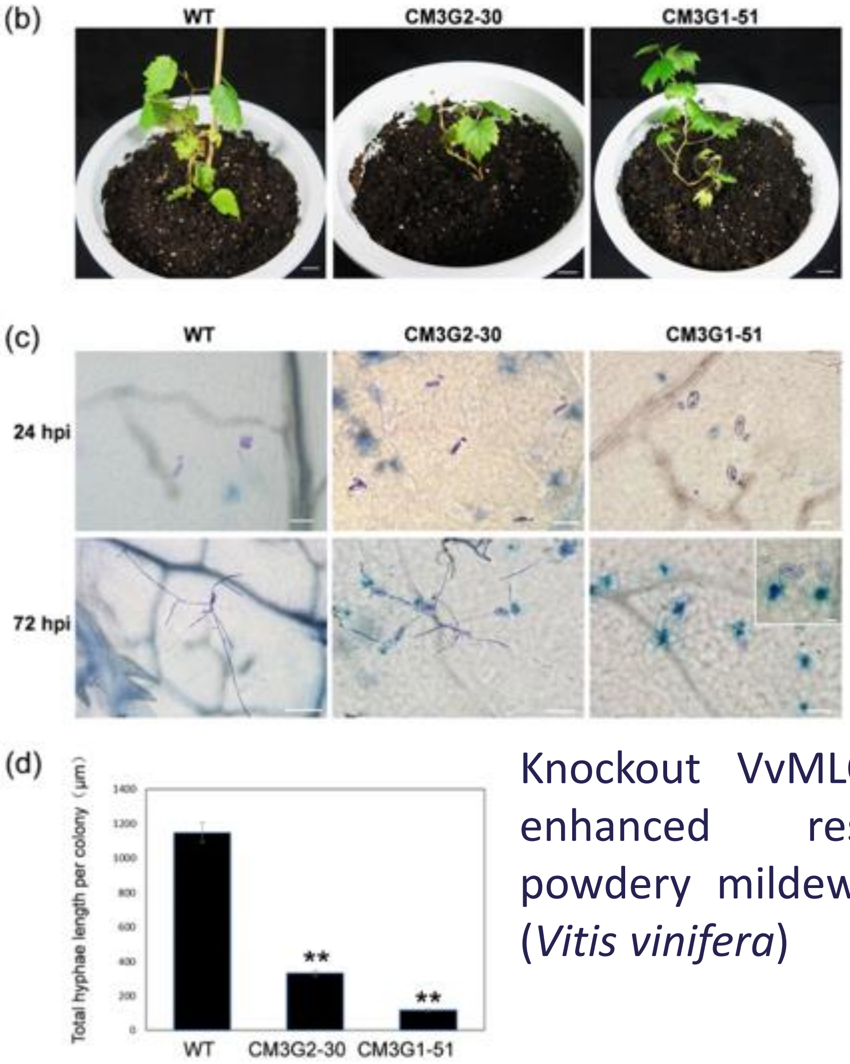
```
PDS-t2  GGCTGGTTTGTCTACTGCAAAATATTTGGCAGATGCAGGTCACAA WT

#1      GGCTGGTTTGTCTACTGCAAA--ATTTGGCAGATGCAGGTCACAA (-2)   1/14
        GGCTGGTTTGTCTACTGCAA---ATTTGGCAGATGCAGGTCACAA (-3)   1/14
        GGCTGGTTTGTCTACTGCA----ATTTGGCAGATGCAGGTCACAA (-4)   2/14

#2      GGCTGGTTTGTCTACTGCAAA--ATTTGGCAGATGCAGGTCACAA (-2)   2/14
        GGCTGGTTTGTCTACTGC-----ATTTGGCAGATGCAGGTCACAA (-5)   1/14
        GGCTGGTTTGTCTACTG-------TTTGGCAGATGCAGGTCACAA (-7)   1/14
        GGCTGGTTTGTCTACT-----------GGCAGATGCAGGTCACAA (-11)  1/14
        GGCTGGTTTGTCTACT------------GCAGATGCAGGTCACAA (-12)  1/14
        ---------------------------------------------  (-135) 1/14
```

apple

b) Pathogen resistance

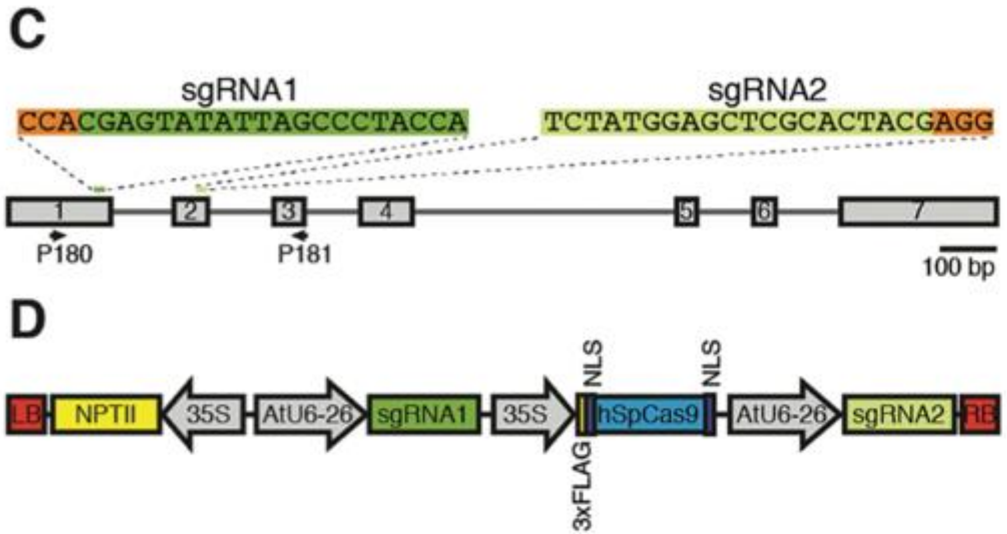


Knockout VvMLO3 results in enhanced resistance to powdery mildew in grapevine (*Vitis vinifera*)

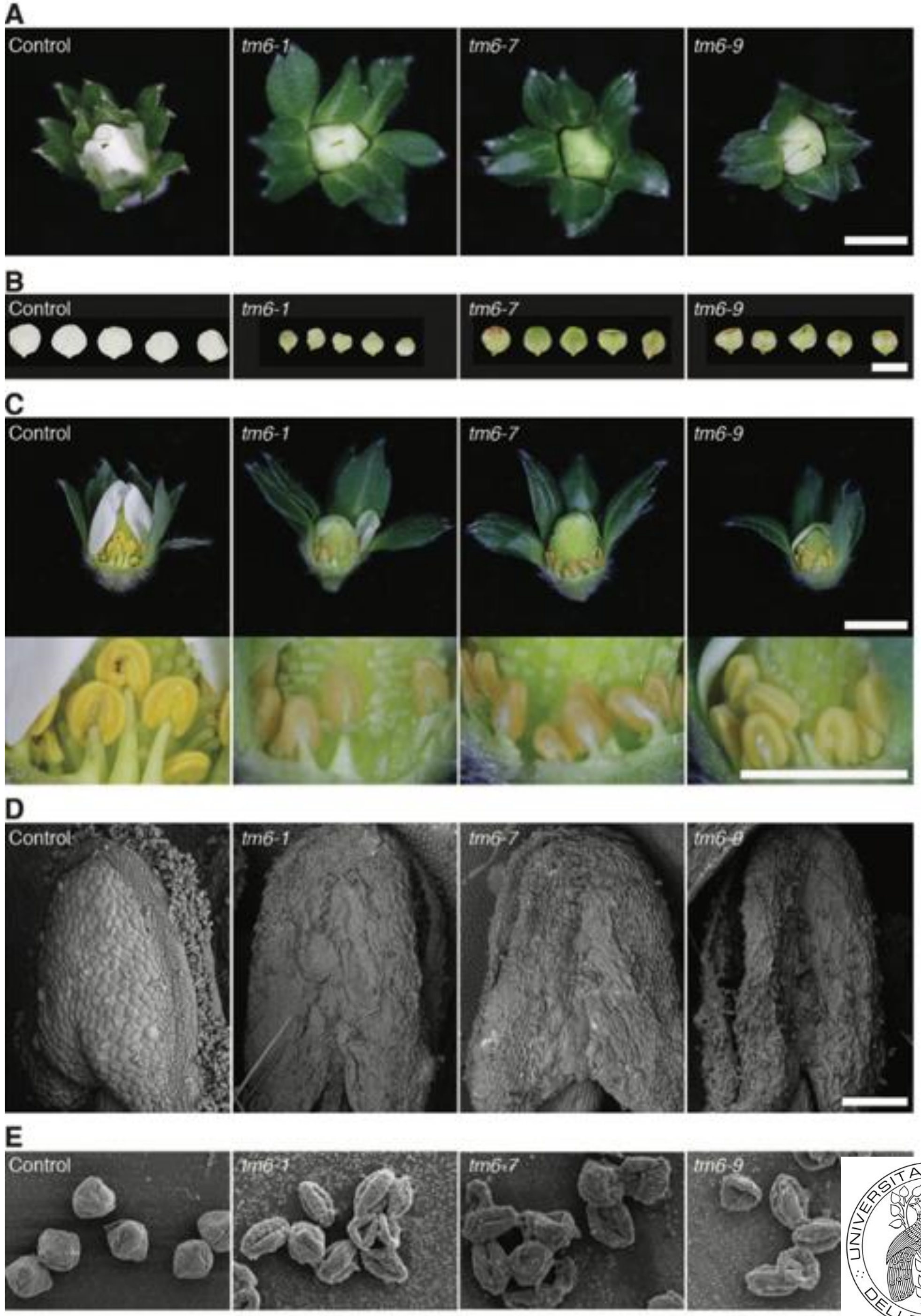
c) Functional genomic studies

APETALA3 (AP3)

Mutation in *Fragaria* x *ananassa*



Phenotypic characterization of the mutant lines indicated that FaTM6 plays a key role in anther development in strawberry.



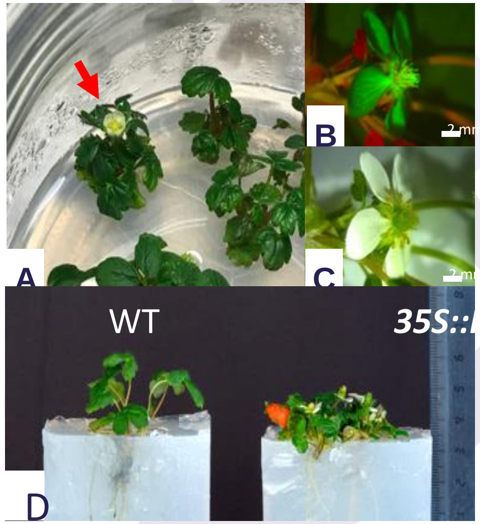
Plant flowering habitus

1. Isolation of *FT* genes from *F. vesca*, design and preparation of gene constructs (Gaston et al., 2021)

FveFT2 and *FveFT3* genes

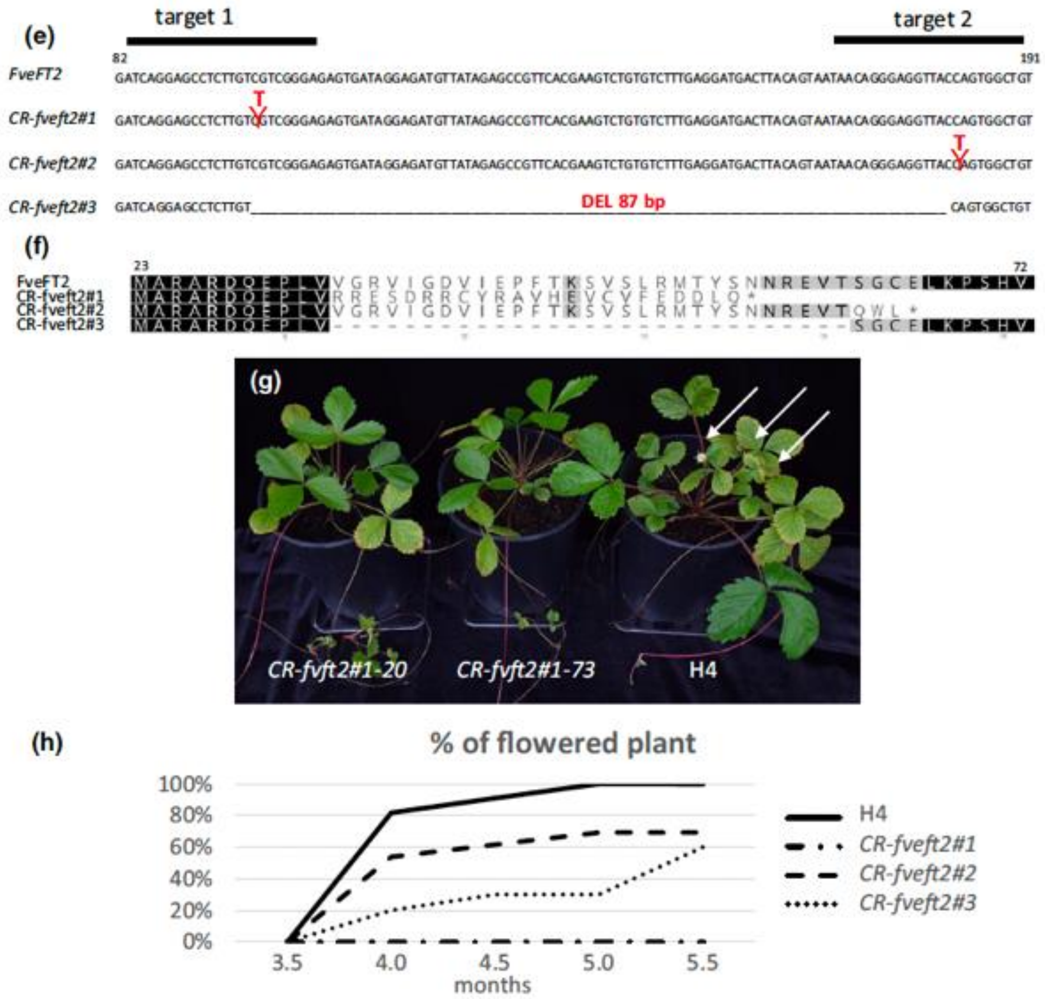
2. Overexpression of *FveFT2* and *FveFT3* genes in Sveva and *FveFT2* in Romina and phenotypical characterization of transgenic lines in vitro an greenhouse environment

In vitro phenotype



35S::FveFT2FaOE

3. Genome editing FT2 (Gaston et al. 2021)



RNAi for the control of Botritys cinerea: more advanced phase for product development

GREENHOUSE/FIELD EXPERIMENTATION OF dsRNA FORMULATES

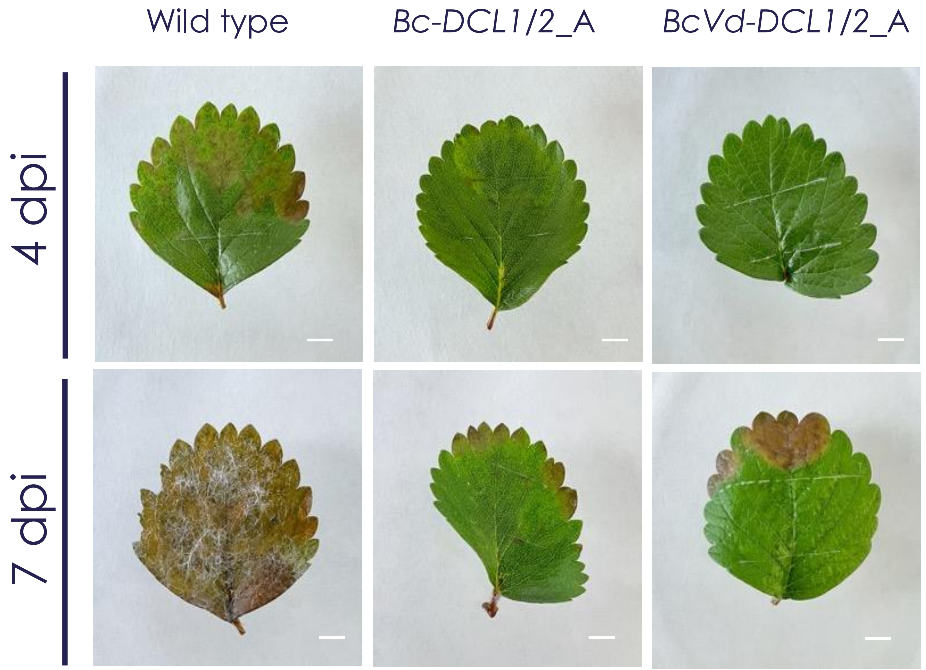
Test dsRNA formulations to identify the most effective ones

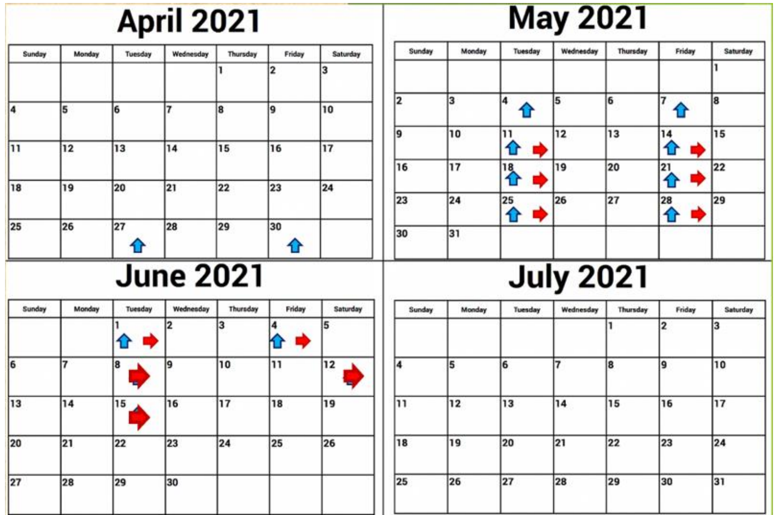
Compare dsRNA formulations with chemical and biological pesticides

Identify the level of protection of the dsRNA formulations in pre and post-harvest

Check for unexpected effects of dsRNA formulations

CONFIRMED THE EFFECTIVENESS OF EXPRESSION IN PLANTA: GMOs

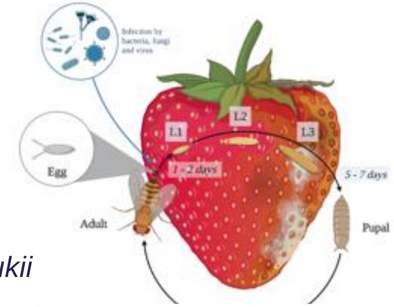




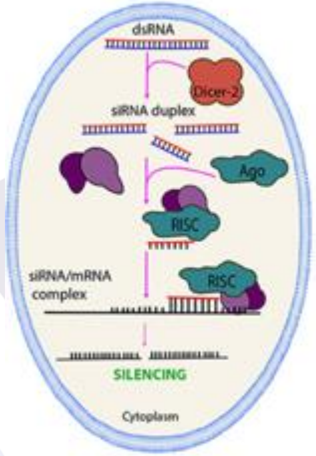
RNAi strategy in octoploid strawberry to induce resistance to Drosophila suzukii

New emergency: *Drosophila suzukii*

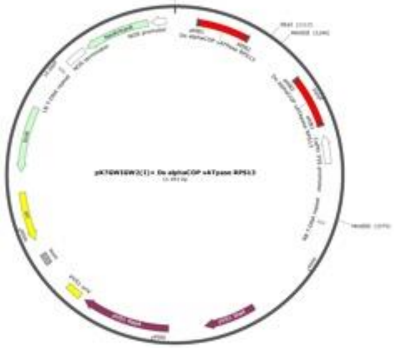
D. suzukii



RNAi mechanism

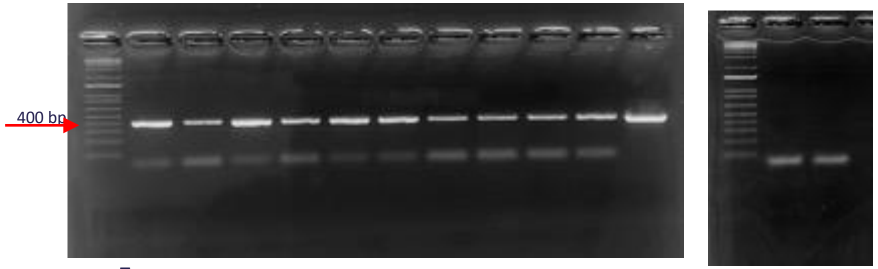


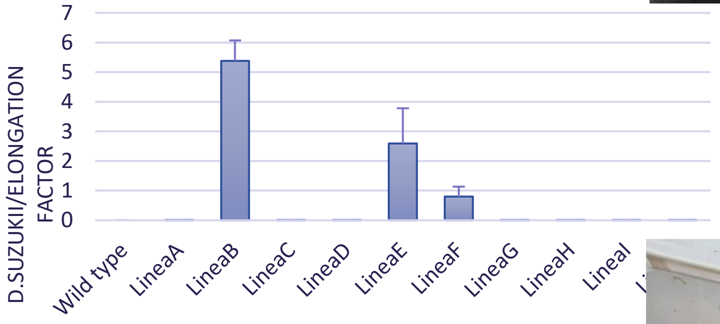
Harpin construct for D. suzukii gene target



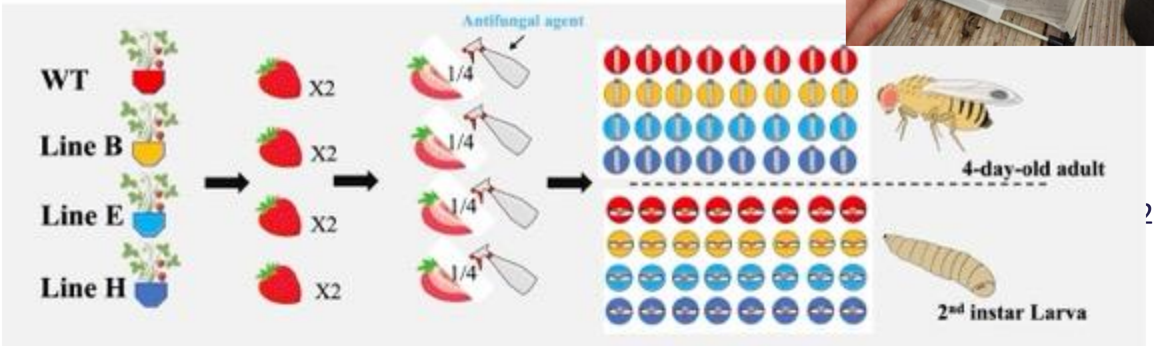
Agrobacterium transformation

Genomic characterization and gene expression





Infections to test the level of tolerance: Preliminari data to be confirmed in the coming season



TO BE OR NOT TO BE ….. GMO

Transformation with exogenous sequences	GMO
Cisgenesis / intragenesis E	GMO EU Court (25/07/2018)
Plant with a GMO intermediate grafting	no GMO
Non GMO cultivar grafted on GMO rootstock	no GMO
Genome editing	GMO EU Court (25/07/2018)
Stable expression of GMO dsRNA in plant	no GMO
DsRNA-based products for direct application	no GMOs

TO RESOLVE THE OPPOSITION AGAINST BIOTECHNOLOGIES IT IS IMPORTANT TO GIVE CORRECT INFORMATION AND SHOW THE PLANTS

Recent decision of the EU parliament

Note that it still needs to get approved by 2/3rds of the European Commission and then implementing regulations need to be written and implemented, so nothing will happen any time soon.

What did pass Parliament divides edited crops into two groups - those with the repair of a single double-strand cut (NGT1) -which are indistinguishable from mutagenesis, and those with more extensive edits (NGT2), such as a fragment deletion.

- NGT1 plants would still need to be labeled and listed (despite the lack of verification ability) and cannot be patented.
- NGT2 plants remain as GMOs, though an expedited review is planned.

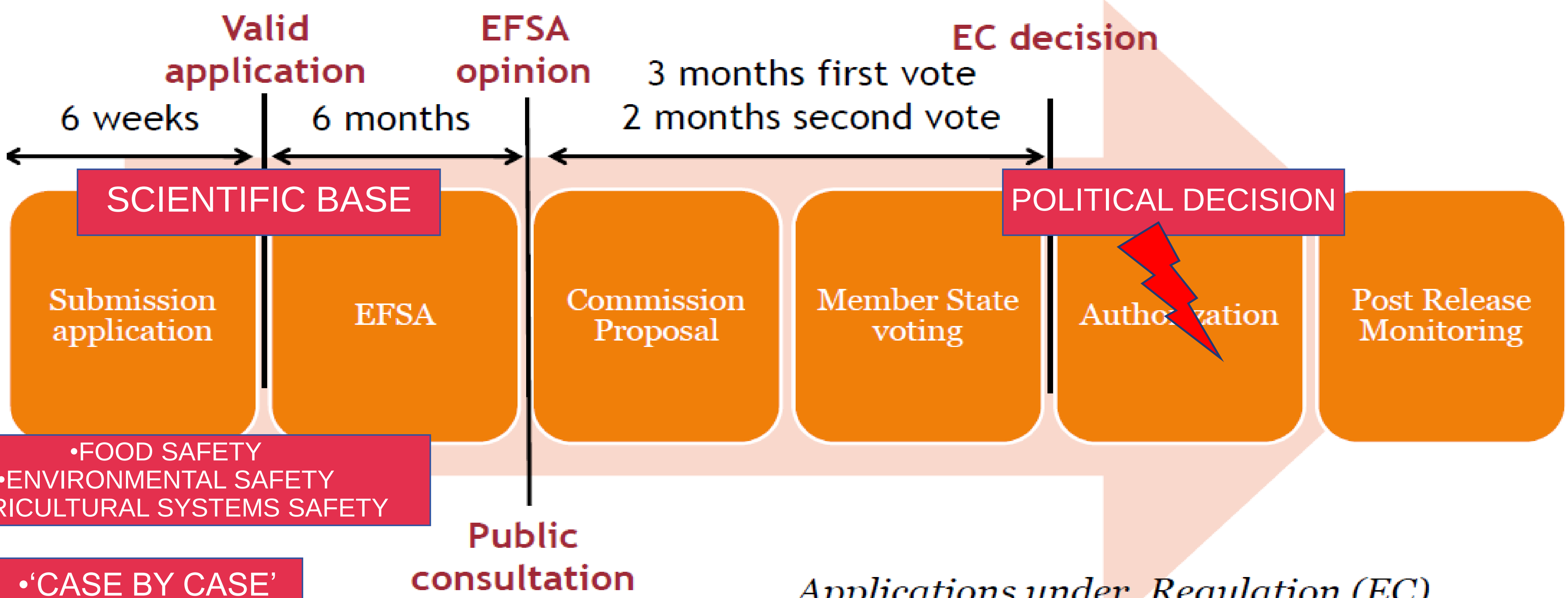
Overall, I see continued dysfunction, not enablement, when it comes to getting edited products on the market.

More at https://www.europarl.europa.eu/news/en/press-room/20240202IPR17320/new genomic-techniques-meps-back-rules-to-support-green-transition-of-farmers.

EU regulatory system

1829/2003 - IMPORT More than 100 EVENTS -------------------------2001/18 - CULTIVATION = MON810 ONLY

Approval process in EU for food, feed, import and processing



- FOOD SAFETY
- ENVIRONMENTAL SAFETY
- AGRICULTURAL SYSTEMS SAFETY

- ‘CASE BY CASE’ (Gene-Specie-Plant)

Applications under Regulation (EC) 1829/2003

Proposal for actions:

- Union between **SCIENCE, PRODUCTION and POLICY** to deal appropriately and efficiently with climatic and health emergencies. Don't be afraid to say that they are GMOs and that they are ALL GMOs.
- Communication on **UNATTACKABLE SCIENTIFIC BASIS** to gain consensus. It is impossible to reach unanimity - the majority is enough - working on biotech is a life that I am in the minority.
- It is true that biotechnologies are in the hands of multinationals but for conveniences (corn, soy, ...), **there is still room for public research and for small businesses interested in applying biotechnology** to solve emergencies in fruit and vegetable plants. The blockade from the 'organic' world favors multinationals and blocks research at the local level.
- Only by overcoming these barriers can we think of using biotechnologies to find useful solutions to reduce the impact of the emergencies that destroy our production.
- RNAi technology offers the double possibility of obtaining new products / formulations for defense and resistant plants.
- To extend the discussion to different institutional levels, on the procedures to be followed to ensure the **immediate experimentation in a controlled environment and field of new resistant RNAi/Gene Editing/cis/intragenic plants.**
- The application of **2001/18 can only be of help in demonstrating the benefits, even for organic crops, and the absence of risk.**
- IT IS FUNDAMENTAL THAT AGRICULTURAL ENTREPRENEURS AND ALL CITIZENS BE ABLE TO SEE **AND "TOUCH WITH HAND" THE RESULTS OF THE RESEARCH**

INTERNATIONAL AND NATIONAL PROJECTS

RESO: REsilience and SUSTAINABILITY of the fruit and vegetable and cereal supply chains to enhance the territories

BREEDINGVALUE: Pre-breeding strategies for obtaining new resilient and added value berries

Med-Berry PRIMA Project: Developing new strategies to protect strawberry crop in Mediterranean countries.

GOODBERRY: Improving the stability of high-quality traits of berry in different environments and cultivation systems for the benefit of European farmers and consumers

iPLANTA: Modifying plants to produce interfering RNA. 0C-2015-2-20281

MIUR-PRIN2017: Small RNAs and peptides for controlling diseases and development in horticultural plants 20173LBZM2,

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GENOLUTION

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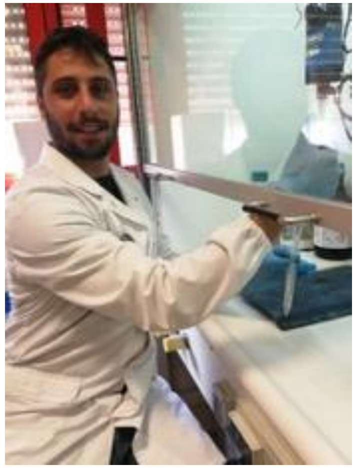

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