Nuove frontiere della genetica agraria per la bioeconomia

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XX Convegno
AISSA
“Le scienze agrarie nella bioeconomia”
Bologna, 16-17 febbraio 2023

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

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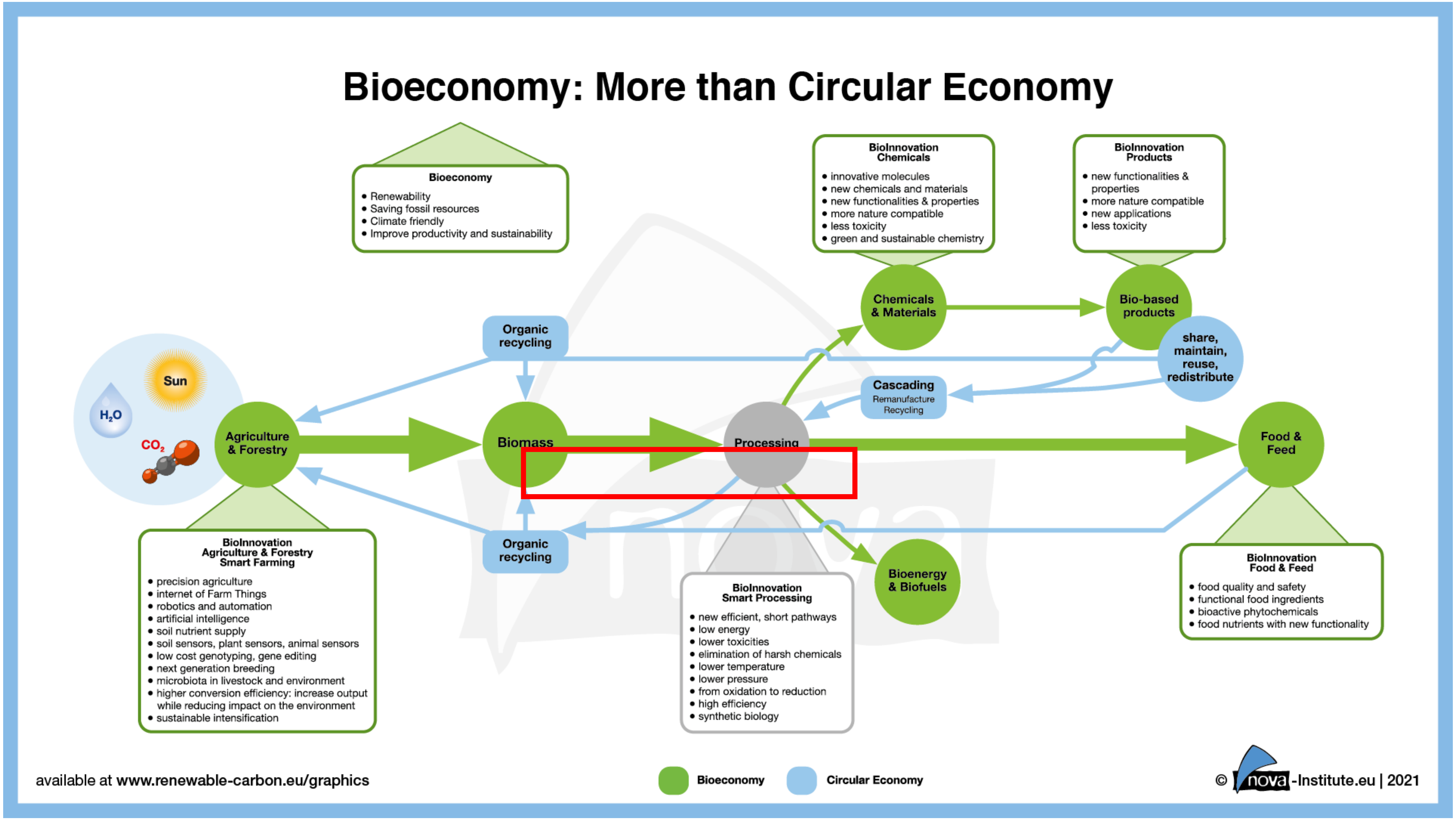
Bioeconomy in the circular economy cycle



Bioeconomy: More than Circular Economy

Bioeconomy
- Renewability
- Saving fossil resources
- Climate friendly
- Improve productivity and sustainability

BioInnovation Chemicals
- innovative molecules
- new chemicals and materials
- new functionalities & properties
- more nature compatible
- less toxicity
- green and sustainable chemistry

BioInnovation Products
- new functionalities & properties
- more nature compatible
- new applications
- less toxicity

Chemicals & Materials

Bio-based products

share, maintain, reuse, redistribute

Organic recycling

Cascading
Remanufacture
Recycling

Sun

H_2O

CO_2

Agriculture & Forestry

Biomass

Processing

Food & Feed

Organic recycling

Bioenergy & Biofuels

BioInnovation Agriculture & Forestry Smart Farming
- precision agriculture
- internet of Farm Things
- robotics and automation
- artificial intelligence
- soil nutrient supply
- soil sensors, plant sensors, animal sensors
- low cost genotyping, gene editing
- next generation breeding
- microbiota in livestock and environment
- higher conversion efficiency: increase output while reducing impact on the environment
- sustainable intensification

BioInnovation Smart Processing
- new efficient, short pathways
- low energy
- lower toxicities
- elimination of harsh chemicals
- lower temperature
- lower pressure
- from oxidation to reduction
- high efficiency
- synthetic biology

BioInnovation Food & Feed
- food quality and safety
- functional food ingredients
- bioactive phytochemicals
- food nutrients with new functionality

available at www.renewable-carbon.eu/graphics

🟩 Bioeconomy 🟦 Circular Economy

Next generation breeding

Genetic resources : genetic material of actual or potential value for exploitation.

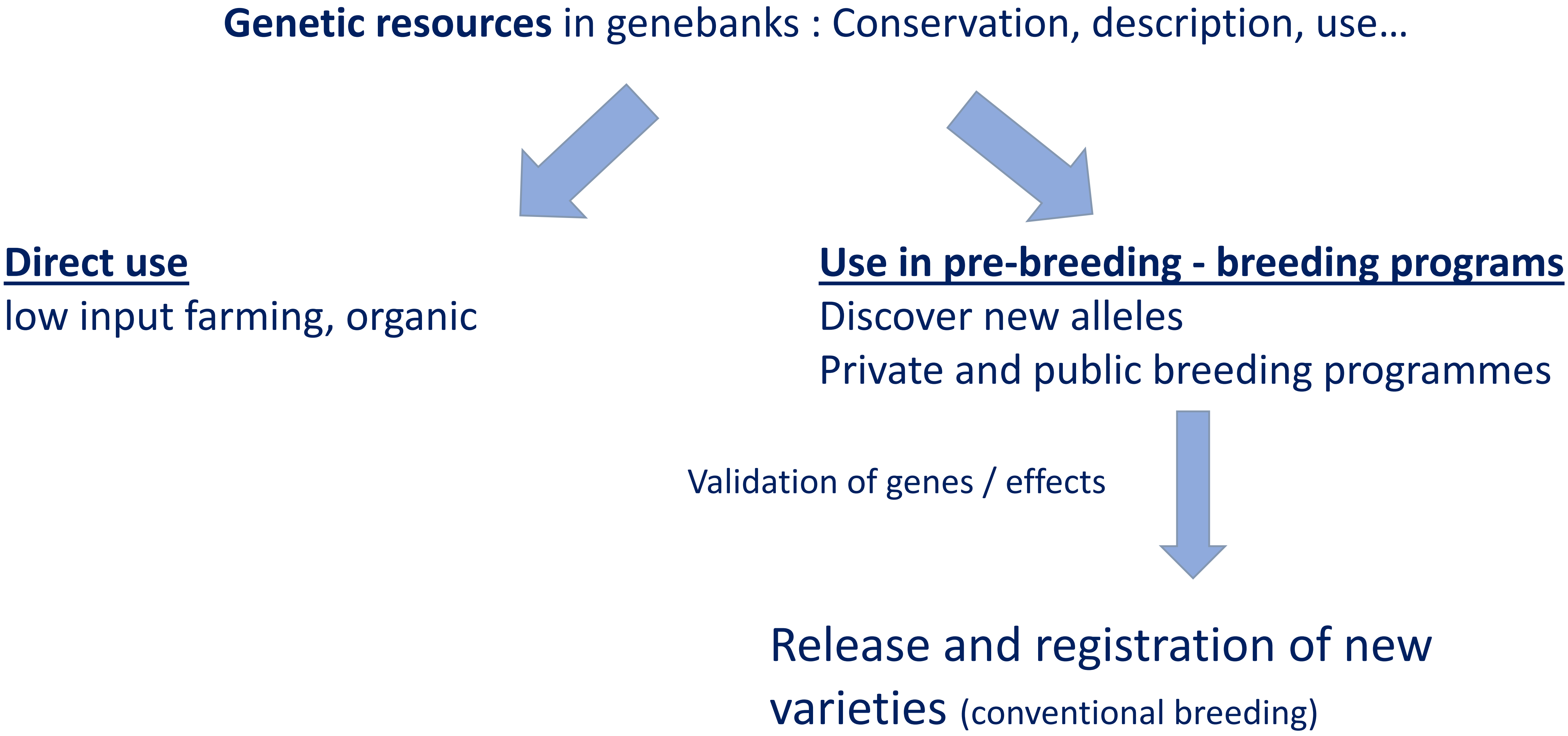
It is desirable to maintain, particularly of domesticated cultivars and their ancestors, a wide **genetic base**.

The **wider the genetic base**, the greater the capacity for adaptation to particular environmental conditions.

National and international **genebanks** (also known as seedbanks) conserve and distribute plant genetic resources for food and agriculture

Many valuable genes and alleles are stored in seedbank collections

Next generation breeding



Genetic resources in genebanks : Conservation, description, use…

Direct use

low input farming, organic

Use in pre-breeding - breeding programs

Discover new alleles

Private and public breeding programmes

Validation of genes / effects

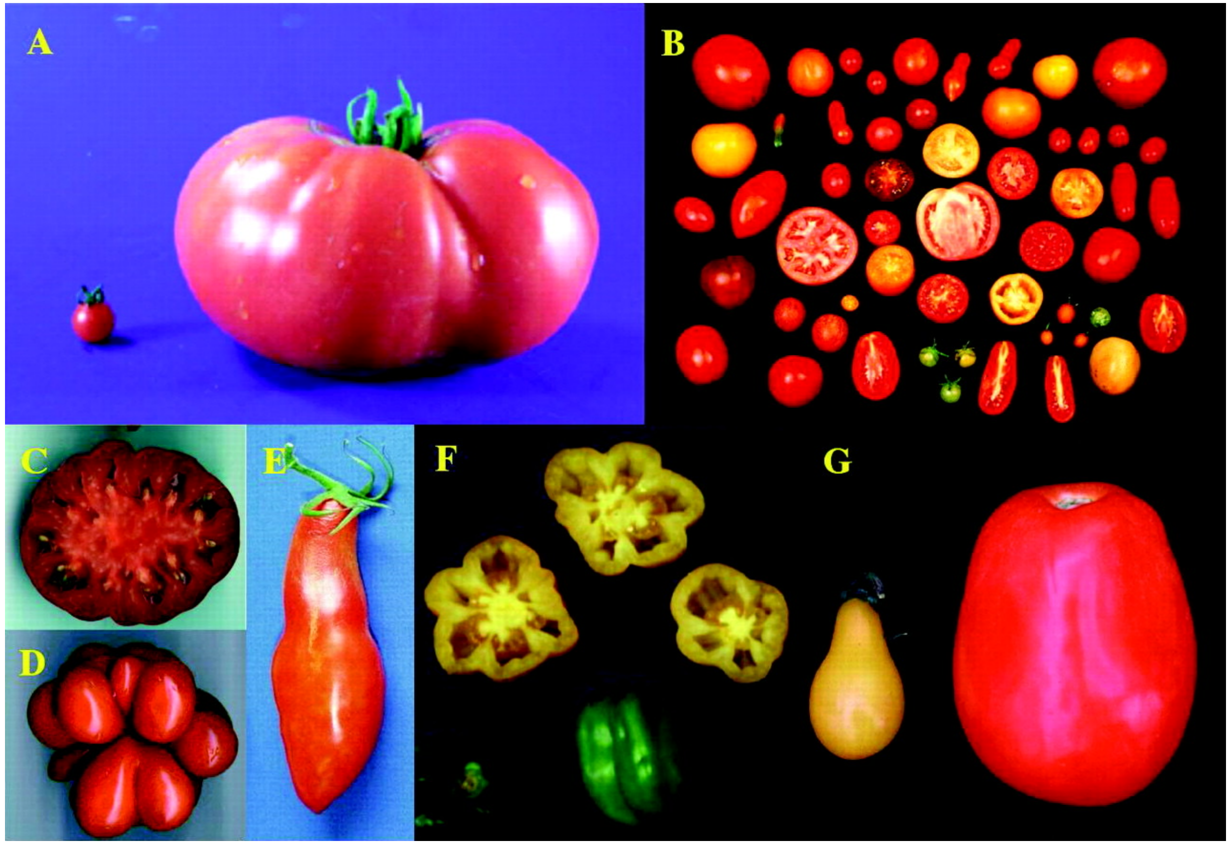
Release and registration of new varieties (conventional breeding)

Next generation breeding

- Crop evolution and domestication have drastically **reduced** genetic diversity and intensive breeding could further worsen the situation.
- **Crop wild relatives (CWRs)** is wild plant taxon that has an indirect use derived from its relatively close genetic relationship to a crop
- **Crop wild relatives,** which are known to possess genes for several important traits like tolerance to various stresses that have been lost during domestication or breeding processes.
- **CWRs** can be exploited for introgression of new traits in cultivated lines to expand their genetic base.

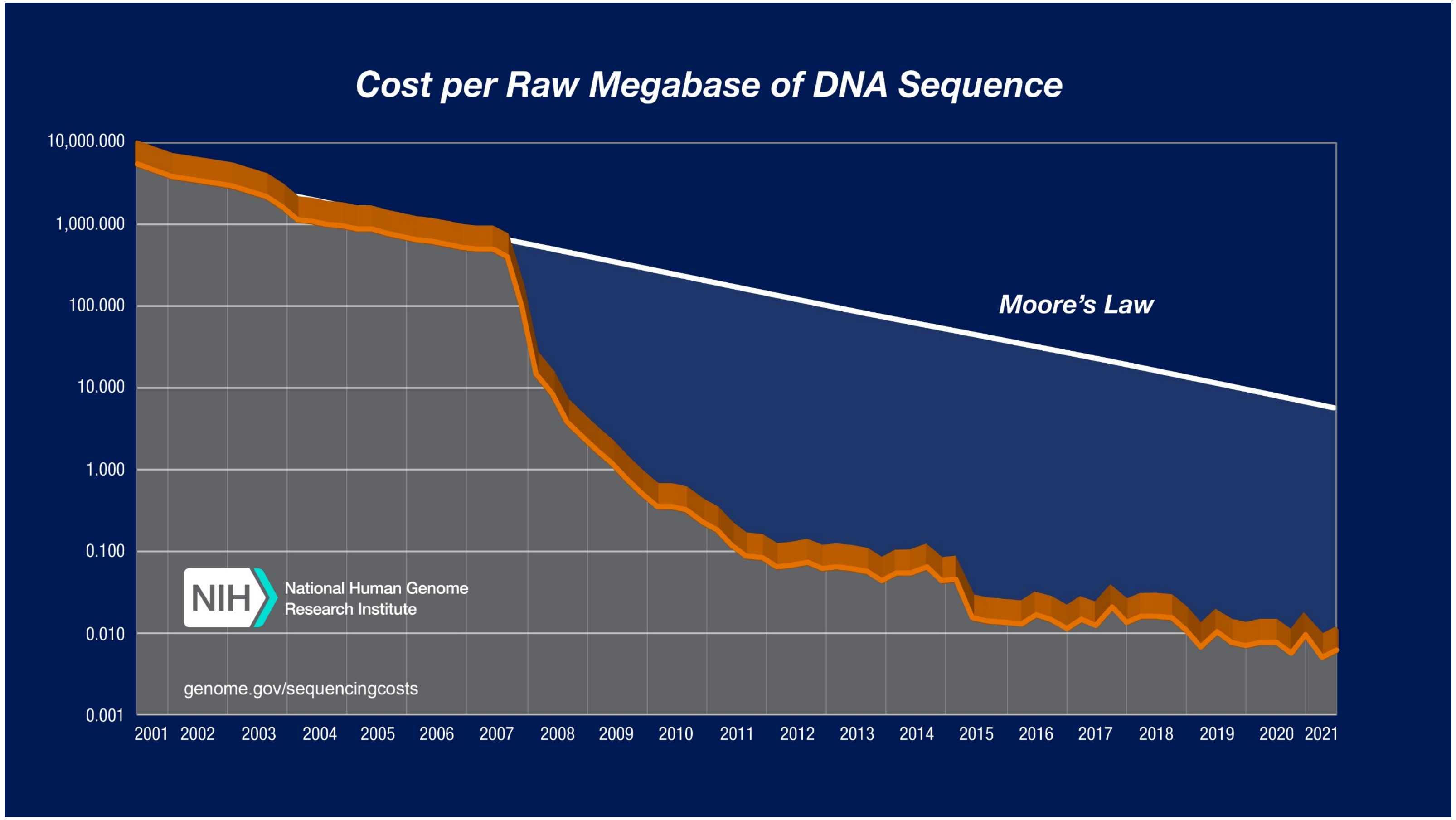
Genebanks have been an important component in the conservation and availability of wild species useful for plant breeding

Next generation breeding



Genotyping

- Genotyping is the process of determining the DNA sequence or polimorphism, i.e. genotype, at specific positions within the genome. Sequence variations (**SNPs**) can be used, for example, to identify relevant genes to specific traits or disease.



Cost per Raw Megabase of DNA Sequence

genome.gov/sequencingcosts

Biodiversity in the Solanaceae

G2P-SOL (**G**enotype to **P**henotype) is an EU-funded project (March 2016-February 2021) to preserve and revive the genetic resources of the main four **Solanaceous crops: potato, tomato, pepper and eggplant**.

G2P-SOL main objectives:

1) **Inventory, describe and conservation** of the accessions (65,000 in G2P consortium) for the four crops.
2) **Building of core collections of 450 accessions for each crop**. High density genotyping and growth of the core collections in different locations for two years in order to perform **GWA** studies
3) **Make available** genotypic and phenotypic data to breeders **to released new varieties with improved agronomic traits**

Genotyping

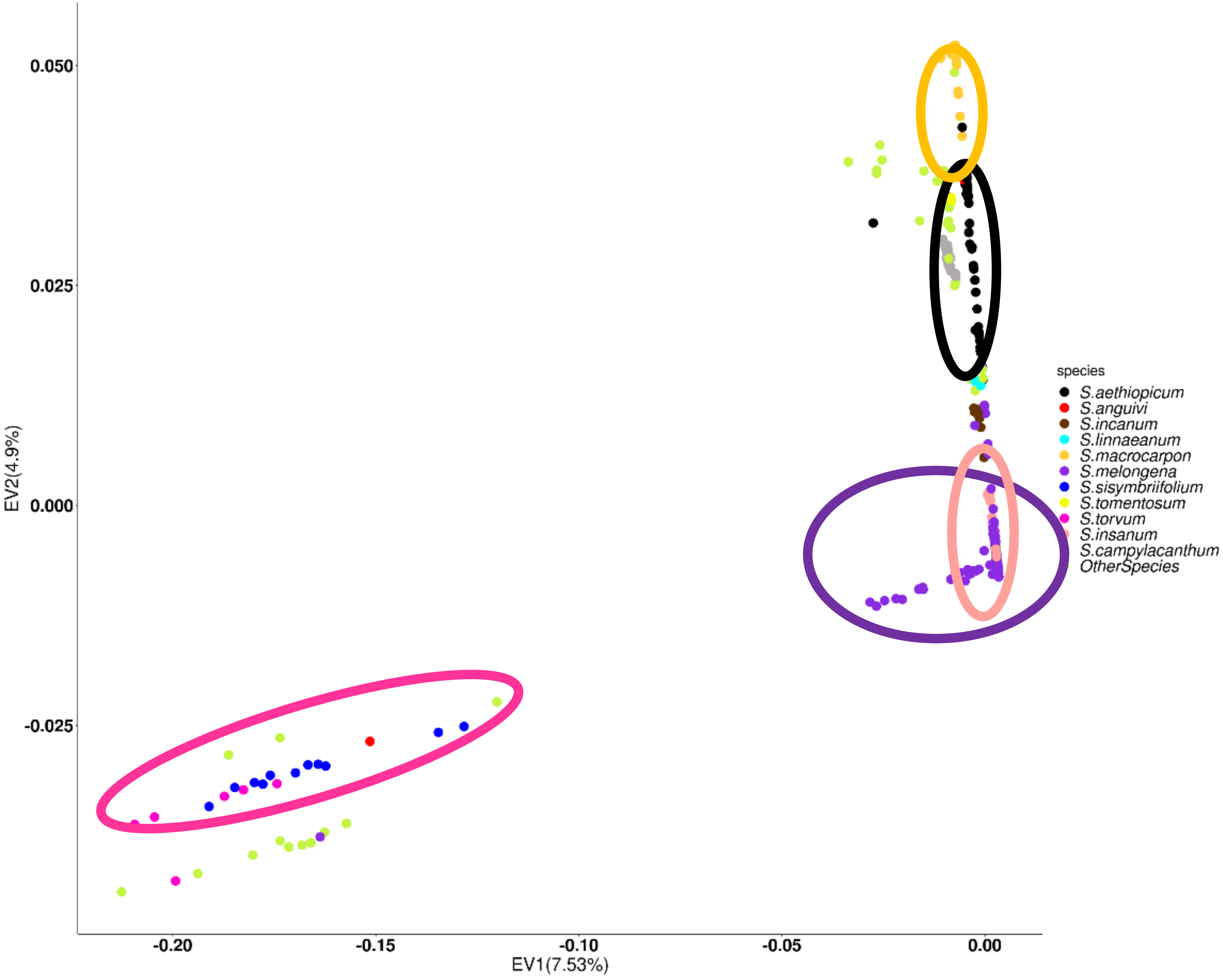
Species	count
S. aethiopicum	313
S. anguivi	9
S. burchellii	1
S. campylacanthum	40
S. cerasiferum	2
S. dasyphyllum	4
S. humile/S.rigescens	10
S. incanum	17
S. indicum	3
S. insanum	22
S. lichtensteinii	11
S. lidii	3
S. linnaeanum	8
S. macrocarpon	123
S. melongena	2896
S. melongena_F1	3
S. myoxotrichum	1
S. paniculatum	2
S. prinophyllum	1
S. pseudolulo	2
S. pyracanthos	1
S. renschii	1
S. richardii	1
S. rostratum	2
S. schimperianum	2
S. sisymbriifolium	11
S. torvum	8
S. viarum	2

Genotyping

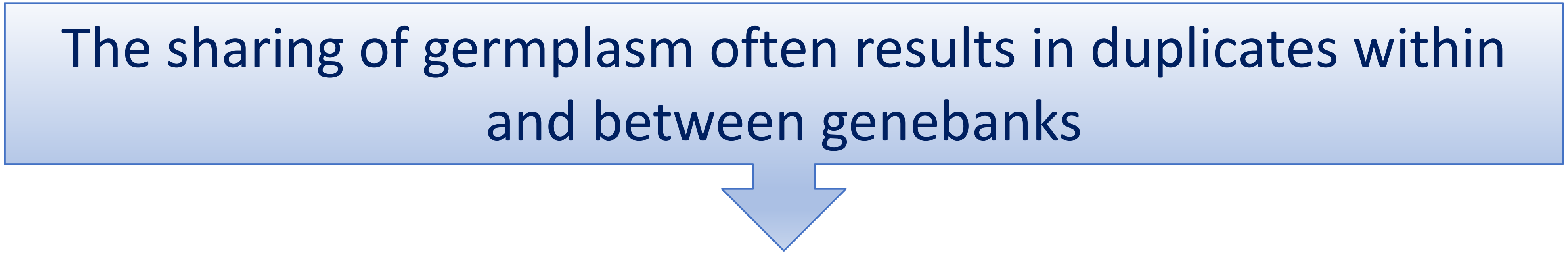
3,532 accessions genotyped with 5k probes

3,412 final eggplant accessions after quality filters with more than 5K SNPs

Population structure: PCA



Searching for duplicates and mislabelled



The sharing of germplasm often results in duplicates within and between genebanks

FAO estimated that, of 6 million accessions conserved worldwide, only **1 – 2** million were distinct

The majority of duplicates from *S. melongena* (262), followed by *S. aethiopicum* (217) and *S. macrocarpon* (103)

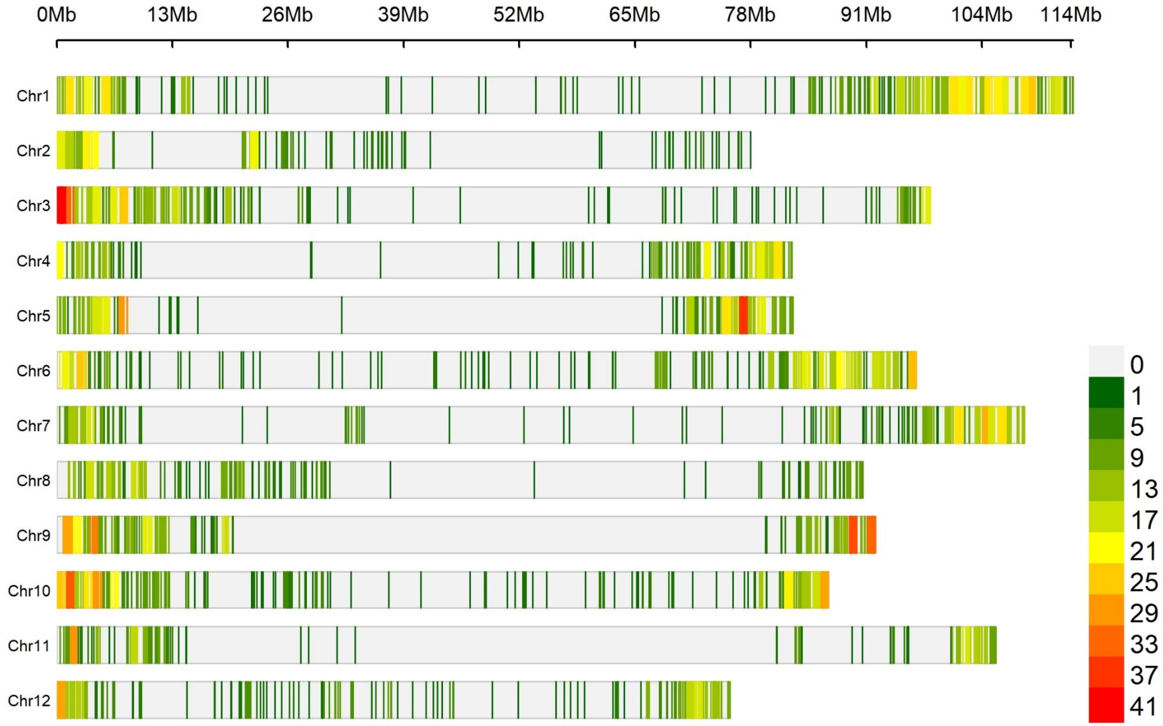
A similar approach could be used to spot mislabelled accessions

GWA: phenotypic data retrieving of historical traits

18 qualitative/pseudoqualitative descriptors for plant, flower, and fruit trait categories useful for **breeding purposes**

TRAIT	MEAN	STANDARD DEVIATION	MAX	MIN
Average Color Of The Flesh	1.35	0.85	3	0
Calyx Prickliness	2.23	2.35	9	0
Corolla Color At Anthesis	5.72	1.52	9	1
Cultivation Type	3.96	1	7	1
Flowering Earliness From Sowing	4.39	1.19	7	3
Fruit Apex Shape	5.67	1.35	7	3
Fruit Colour At Commercial Ripeness	3.67	2.94	9	1
Fruit Colour Distribution	4.22	2.72	7	1
Fruit Cross Section	2.26	1.97	9	1
Fruit Curvature	2.14	1.76	9	1
Fruit Shape	4.94	1.25	7	3
Fruit Shape Lb Ratio	5.33	2.6	9	1
Fruit Size	5.25	1	9	1
Fruit Yield Per Plant	5.02	2.11	9	1
General Anthocyanin Distribution	3	2.26	7	0
Growth Habit	4.54	1.19	7	1
Leaf Blade Lobes	4.37	1.28	9	1
Leaf Prickliness	1.46	1.75	9	0

About 4K markers: target SNPs



Leaf prickliness

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(Barchi

Genetic resources, Genetic Improvement and Genomics: The case study of durum wheat

- **Worldwide relevance of common wheat to durum wheat = 95: 5**
- **Durum wheat is an "orphan crop“** with lower investments in research, pre-breeding, extension and training as compared to bread wheat
- However:
- It is a main and characterizing crop for Italy, the Mediterranean countries and other cereal countries
- Generate a respectful agro-food chain
- The genetic resources of tetraploid wheats are extensive and very rich, also for common wheat improvement

There is a need to reverse the trend, promote, organize and coordinate research and exchanges at an international level

The Global Durum Genomic Resource (TGC + GDP)

Svevo genome sequencing consortium + Germplasm Banks: USDA-ARS, IPK, CNR-Bari, EIAR Ethiopia

(Maccaferri et al. 2019, *NatGenet*, 51, 885-895)

Tetraploid wheat Global Collection (TGC)

1,800 *turgidum* accessions

DW-Expert Working Group + durum wheat breeders world-wide

(Mazzucotelli et al. 2020, FPlS vol11 art 569905)

Global Durum Panel (GDP)

1,000 cultivars and landraces

- **Both collections Genotyped with the Illumina 90K *iSelect* SNP array.**

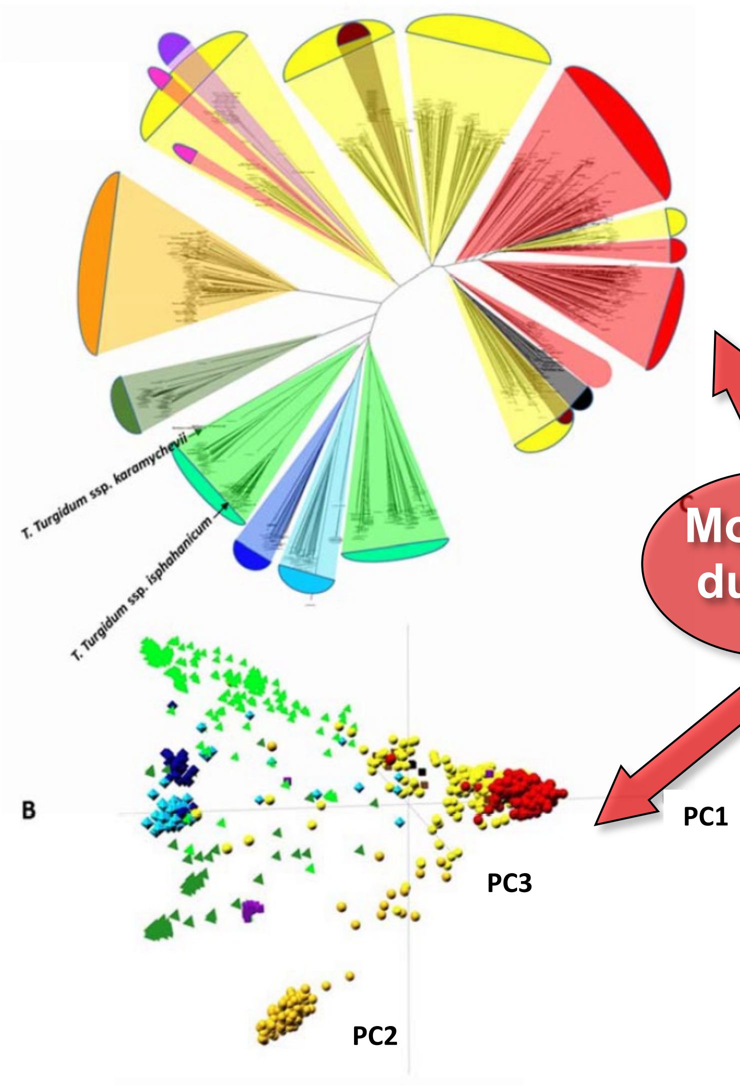
TGC phylogeny and population structure obtained with three independent analysis methods

WEW

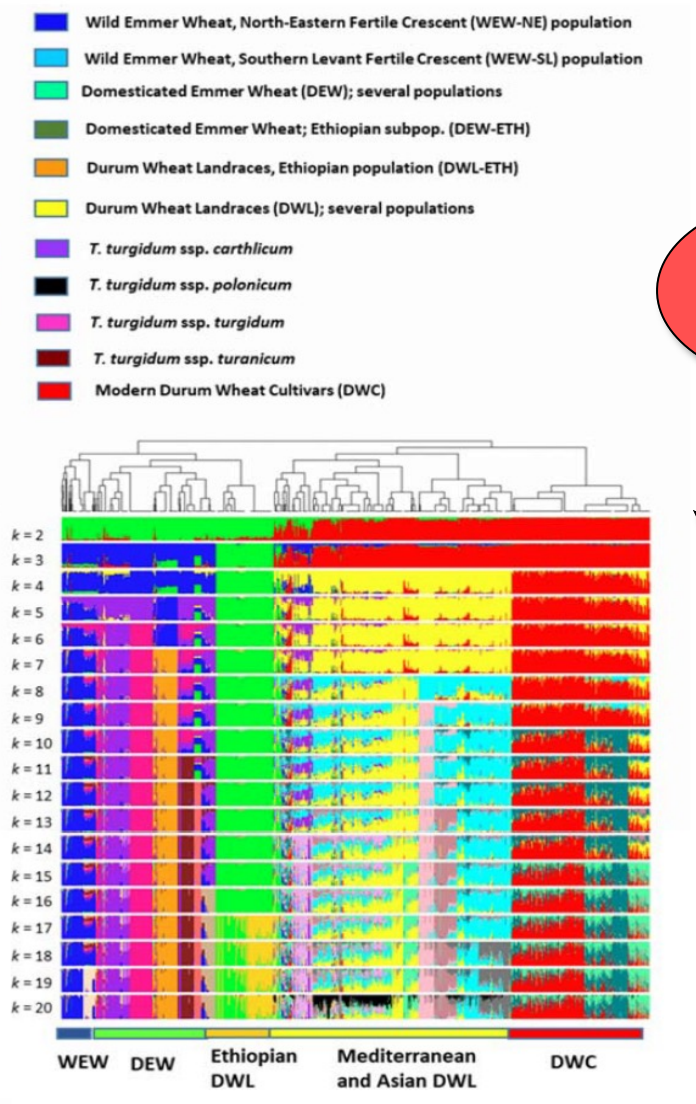
DEW

DWL

DWC

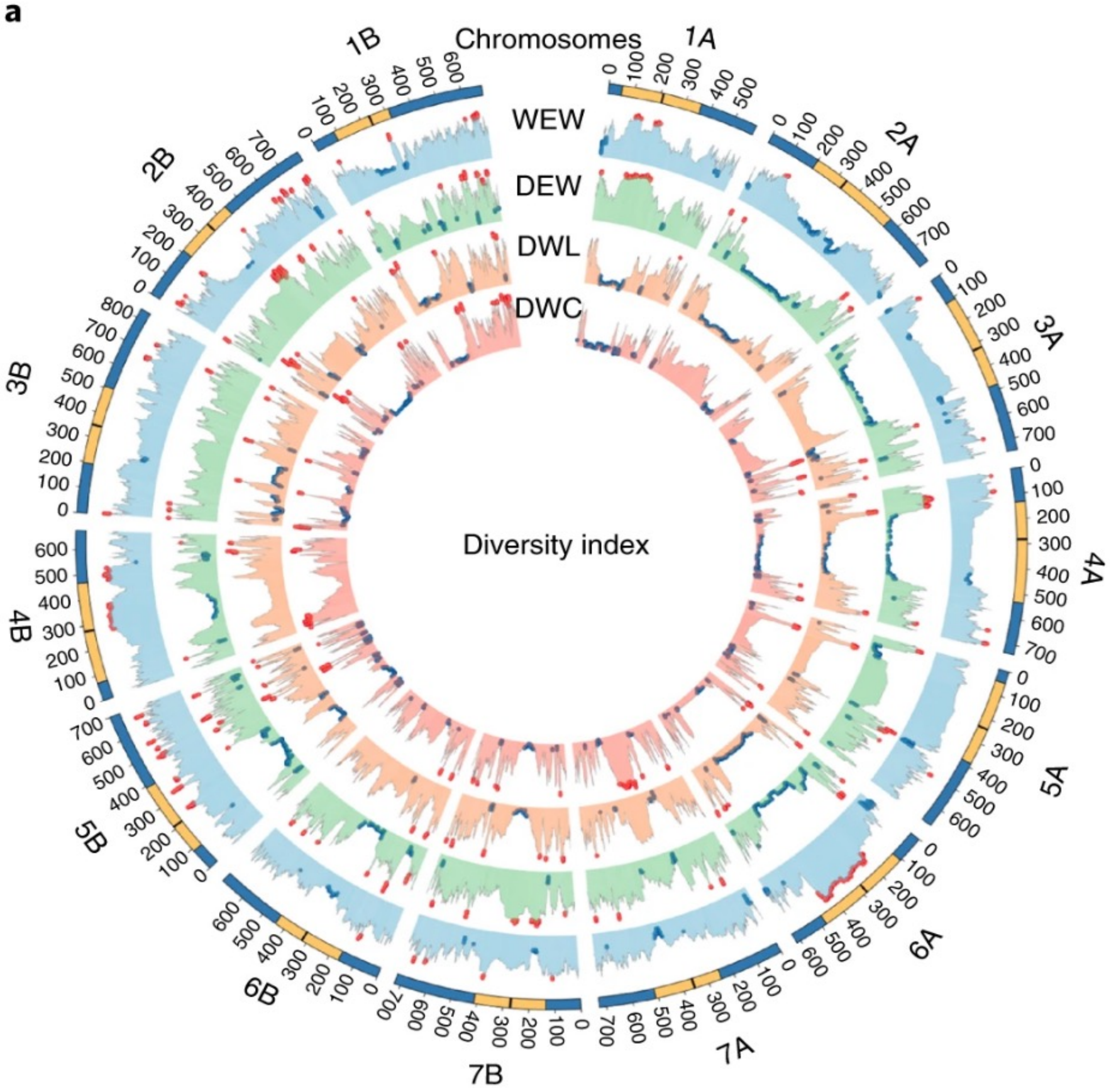


Modern durum



Complex population structure

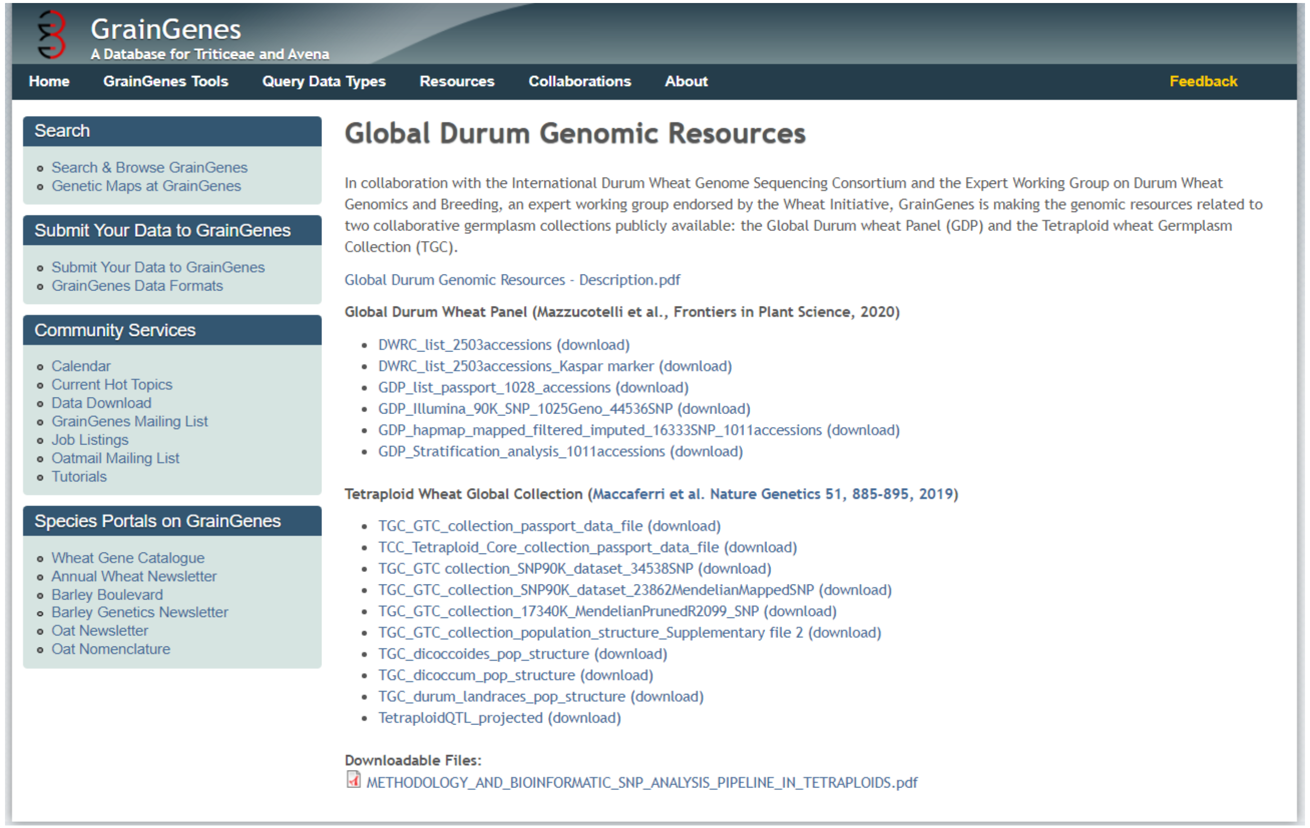
What the genomic tools and the Svevo genome sequence allow us to do?



- The Svevo genome sequence allowed to **map directly on the genome the SNP polymorphism level**

- the reduction of genetic diversity (**Reduction of Diversity, ROD**) through domestication and selection.

The Global durum wheat genomic resources
https://wheat.pw.usda.gov/GG3/global_durum_genomic_resources



Global Durum Genomic Resources

In collaboration with the International Durum Wheat Genome Sequencing Consortium and the Expert Working Group on Durum Wheat Genomics and Breeding, an expert working group endorsed by the Wheat Initiative, GrainGenes is making the genomic resources related to two collaborative germplasm collections publicly available: the Global Durum wheat Panel (GDP) and the Tetraploid wheat Germplasm Collection (TGC).

Global Durum Genomic Resources - Description.pdf

Global Durum Wheat Panel (Mazzucotelli et al., Frontiers in Plant Science, 2020)

- DWRC_list_2503accessions (download)
- DWRC_list_2503accessions_Kaspar marker (download)
- GDP_list_passport_1028_accessions (download)
- GDP_Illumina_90K_SNP_1025Geno_44536SNP (download)
- GDP_hapmap_mapped_filtered_imputed_16333SNP_1011accessions (download)
- GDP_Stratification_analysis_1011accessions (download)

Tetraploid Wheat Global Collection (Maccaferri et al. Nature Genetics 51, 885-895, 2019)

- TGC_GTC_collection_passport_data_file (download)
- TCC_Tetraploid_Core_collection_passport_data_file (download)
- TGC_GTC collection_SNP90K_dataset_34538SNP (download)
- TGC_GTC_collection_SNP90K_dataset_23862MendelianMappedSNP (download)
- TGC_GTC_collection_17340K_MendelianPrunedR2099_SNP (download)
- TGC_GTC_collection_population_structure_Supplementary file 2 (download)
- TGC_dicoccoides_pop_structure (download)
- TGC_dicoccum_pop_structure (download)
- TGC_durum_landraces_pop_structure (download)
- TetraploidQTL_projected (download)

Downloadable Files:
METHODOLOGY_AND_BIOINFORMATIC_SNP_ANALYSIS_PIPELINE_IN_TETRAPLOIDS.pdf

- Collections available on the basis of the FAO Treaty
- with the commitment to publish / return results

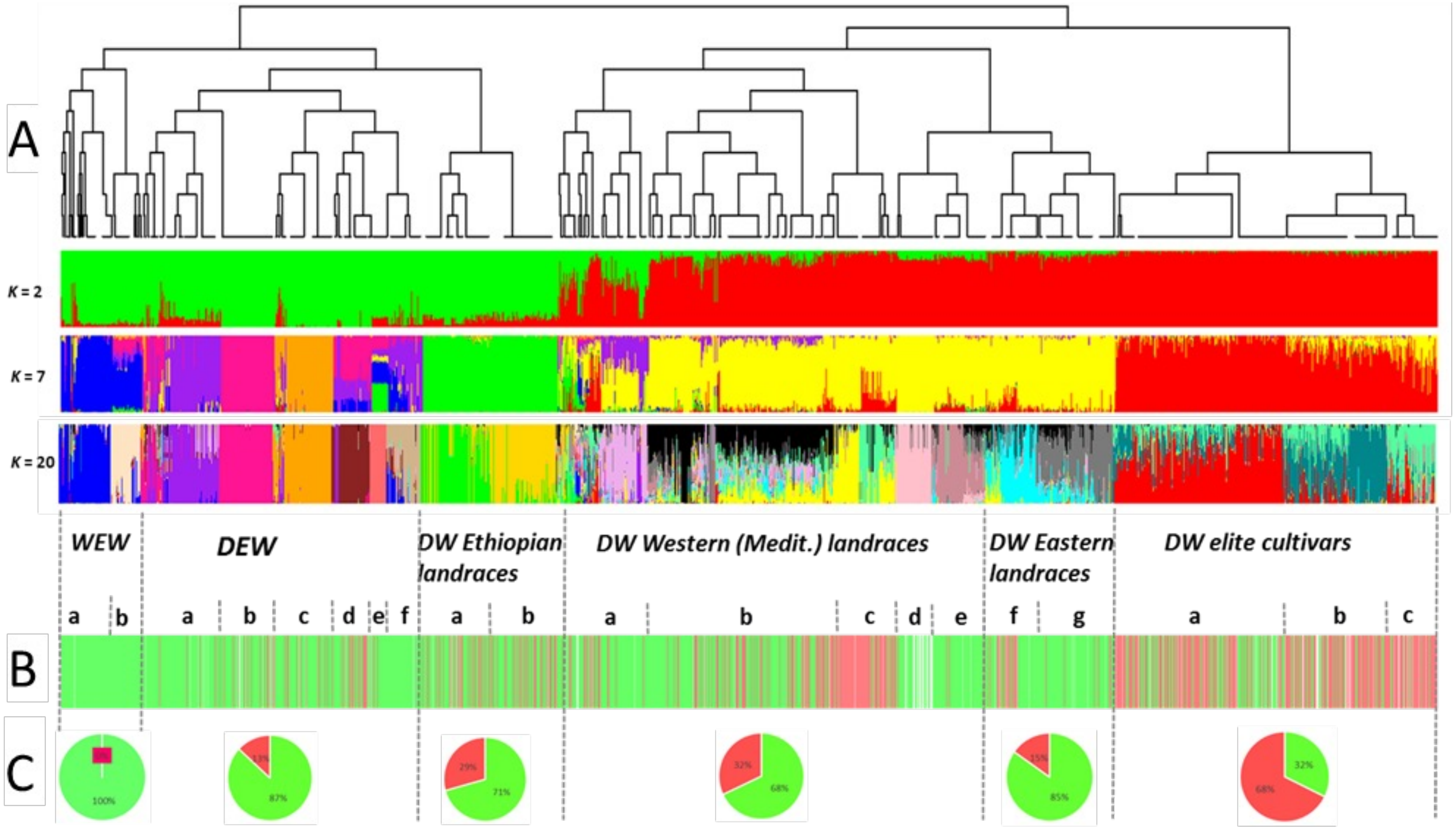
- GDP has been multiplied by ICARDA (Filippo Bassi) and is available at c / o ICARDA genebank http://indms.icarda.org/
- TGC and TCC have been multiplied by UNIBO and CREA (M. Maccaferri, E. Mazzucotelli)

The case of *Cdu1* locus for cadmium accumulation in durum wheat grains

Allelic variation at *HMA3-B1* = *Cdu1*

🟩 **Functional allele *HMA3-B1a***
Low cadmium

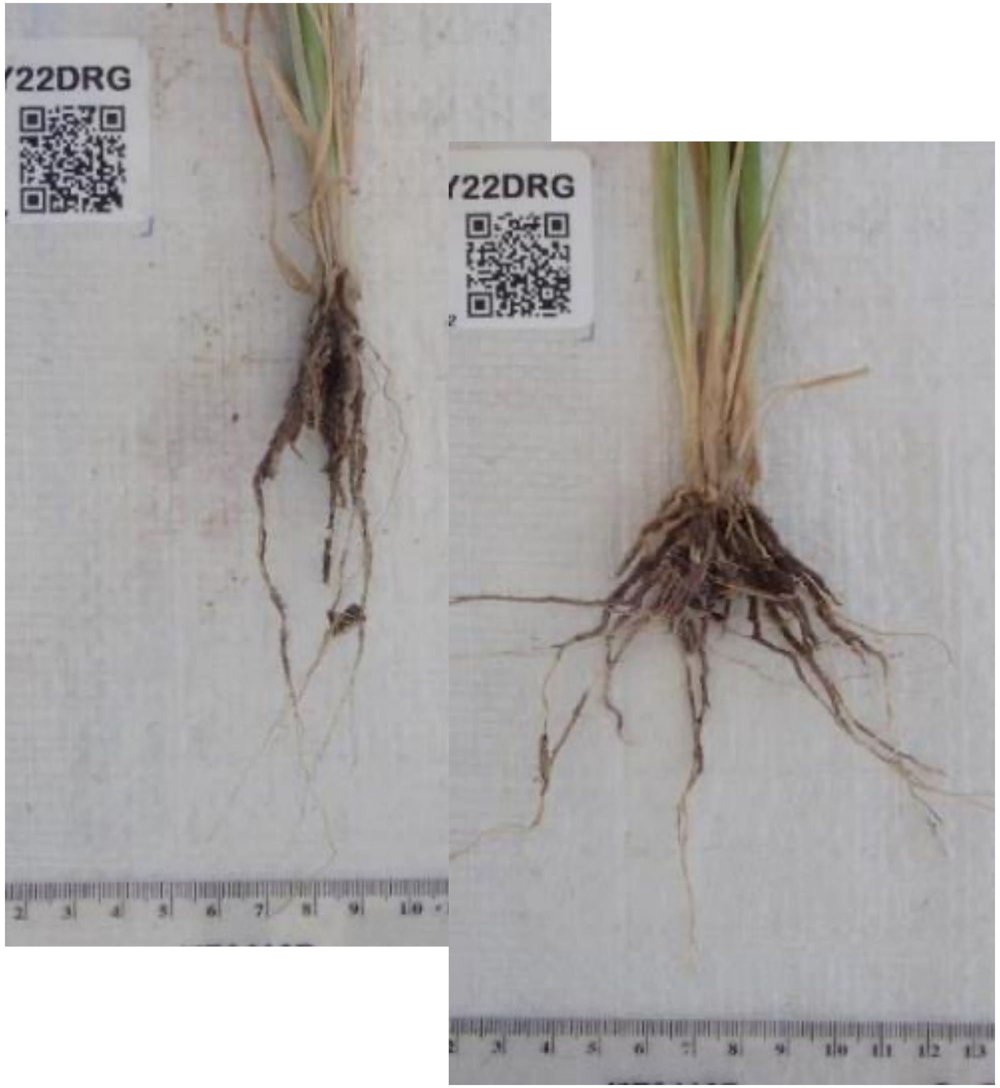
🟥 **Non-functional allele *HMA3-B1b***
High cadmium



The case of natural variation for Root Growth Angle in the durum wheat germplasm

Cultivars cumulating contrasting shallow or narrow haplotype alleles at 3 QTLs were already present in cultivated germplasm.

Divergent GDP elite cultivars were selected for field validation of RGA phenotype and field trials for yield evaluation under well-watered and drought conditions.



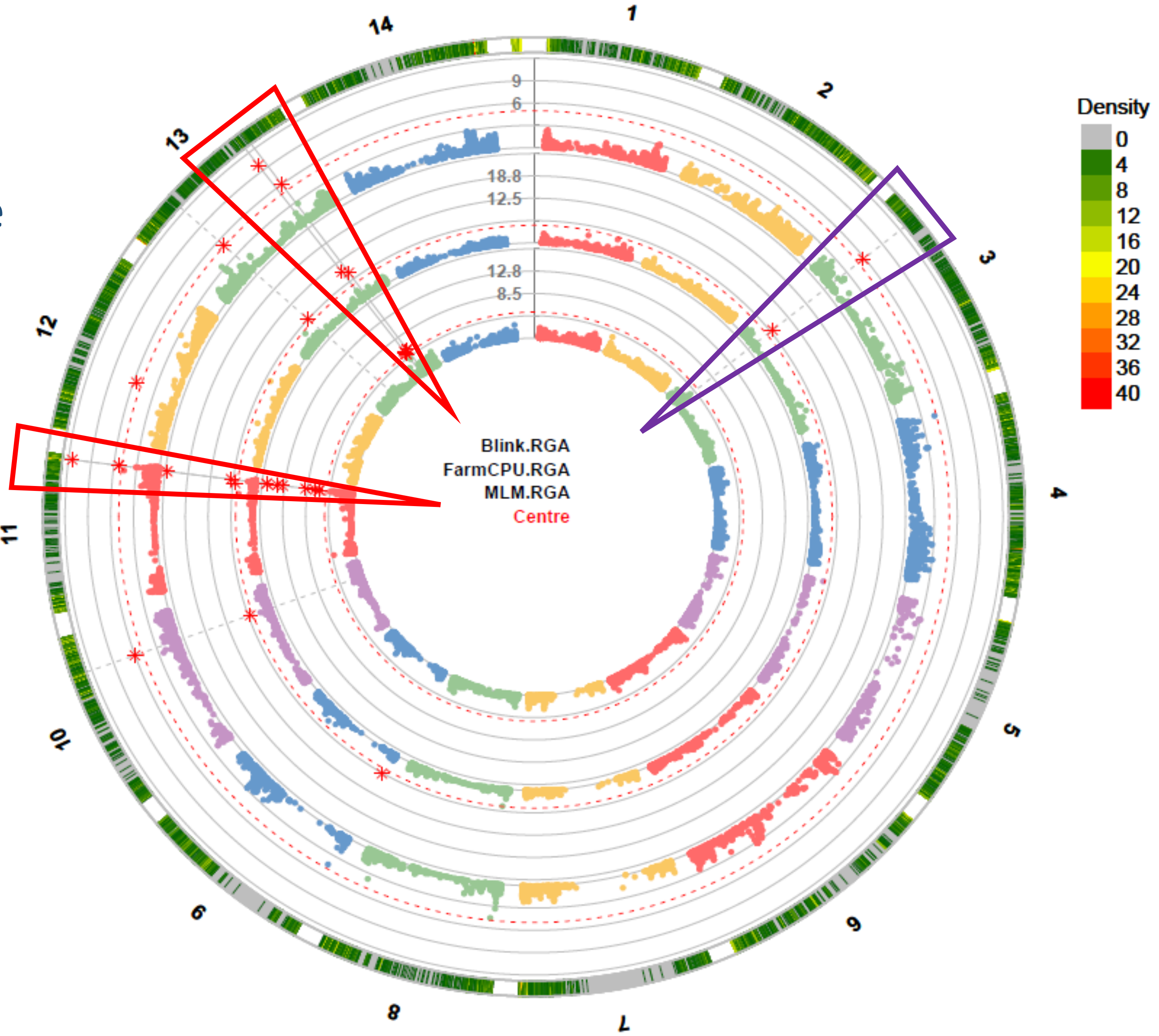
Name	Norm QRGA_2A HapEffect	Norm QRGA_6A HapEffect	Norm QRGA_7A HapEffect	RGA
BRAVADUR	-1.606	-7.255	-2.855	37.96
IDSN46-7104	-1.606	-7.255	-2.855	53.61
Zeina1	-1.606	-7.255	-2.855	53.13
Normanno	-1.606	-7.255	-2.855	50.38
Balloran	-1.606	-7.255	-2.855	53.08
Sebatel2	-1.606	-7.255	-2.855	42.38
Icambel_ICARDA1	-1.606	-7.255	-2.855	46.81
Bani_Suef4	-1.606	-7.255	1.816	48.21
Don_Valent√≠n	-1.606	-7.255	1.816	45.69
YAVAROS_79	-1.606	-7.255	1.816	64.39
Miki3	-1.606	-7.255	1.816	53.68
Geromtel_3	-1.606	-7.255	1.816	61.79
Jordan	-1.606	-7.255	1.816	44.92
Odisseo	-1.606	-7.255	1.816	62.88
SVEVO	-1.606	-7.255	3.785	50.93
PLATANI	-1.606	-4.01	-2.855	46.87
MEXICALI_75	-1.606	-4.01	-2.855	51.97
JUPARE_C2001	-1.606	-4.01	-2.855	57.68
IDSN46-7037	-1.606	-4.01	1.816	55.06
LAGONIL2_ICARDA	-1.606	-4.01	1.816	82.08
OUASERL1	-1.606	-4.01	1.816	43.38
MERIDIANO	-1.606	5.248	3.785	64.08
Calero	-1.606	7.899	1.816	90.67
Margherita	-1.606	7.899	1.816	76.65
IDSN46-7052	-1.606	10.038	-2.855	79.98
Ouassara1	-1.606	10.038	-2.855	84.15
Icacube	-1.606	10.038	-2.855	77.00
IcaKader2	-1.606	10.038	-2.855	112.99
Icamoram 7	-1.606	10.038	-2.855	57.47
Trouve' = Nachit	-1.606	10.038	1.816	77.02
Ammar10	-1.606	10.038	1.816	82.06
KOFA	-0.99	7.899	-2.855	107.20
IRIDE	7.115	-7.255	-2.855	70.20
Saragolla	7.115	-7.255	-2.855	62.03
ATILC_2000	7.115	-4.01	3.785	67.31
ESDCB-2015/2016-27	7.115	10.038	-2.855	85.98
PLATA_16	7.115	10.038	-2.855	72.36
ALTAR_84	7.115	10.038	-2.855	75.54
DBA-Aurora_(ex:UAD0951096)	7.115	10.038	-2.855	85.02
MARJANA	7.115	10.038	1.816	88.99
Caparoi	7.115	10.038	1.816	77.11

Identification of major QTLs for Root Growth Angle in durum wheat

QRGA.ubo on chr. 6A, 7A and 2A were confirmed using different GWAS tools (GAPIT3) and in GDP and TGC.

Haplotype analysis was then conducted in GDP.

The narrow and shallow alleles at the three QTLs are detectable in various combinations in the GDP

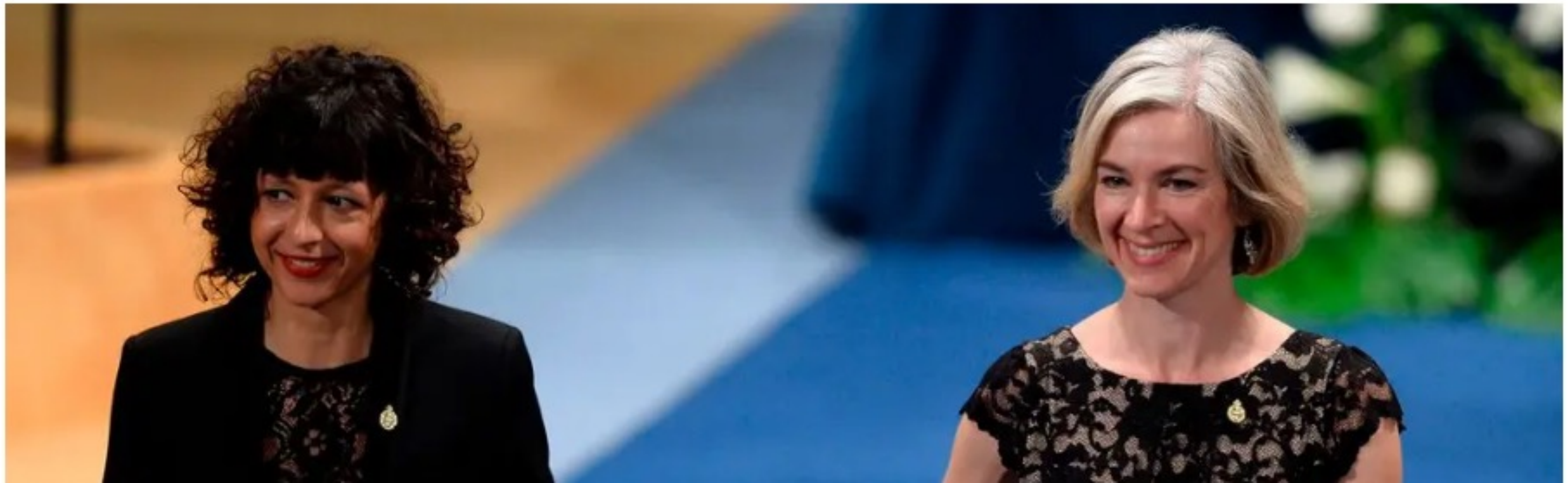


The New York Times

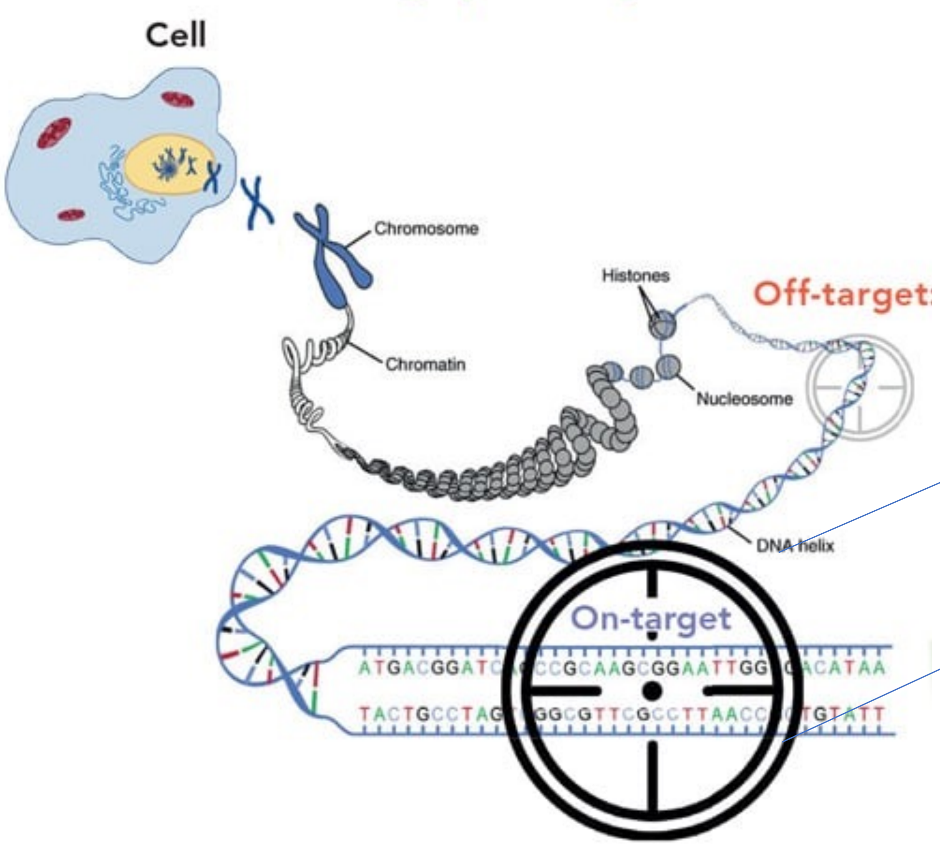
This Year's Nobel Prize in Chemistry Honors a Revolution

With Crispr, two scientists turned a curiosity of nature into an invention that will transform the human race.

Oct. 7, 2020



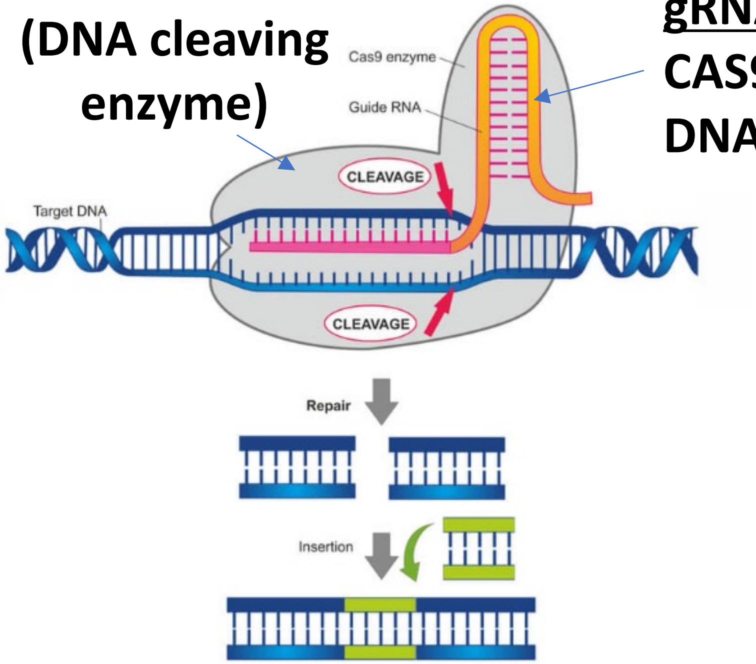
Genome editing enables targeted gene modification



CRISPR-CAS9

<u>CAS9</u>
(DNA cleaving enzyme)

<u>gRNA</u> (RNA guiding CAS9 at the target DNA position)



- **Mutation at a targeted single gene or even nucleotide in the gene**

i.e. targeted mutagenesis

- **Gene substitution or insertion deletion at targeted positions (eg. *Improved cis-genesis*)**

Several names for the same thing

- **Gene or Genome editing**

- **CRISPR-CAS9 (ie. the main technology components – CRISPR or gRNA and the DNA nuclease CAS)**

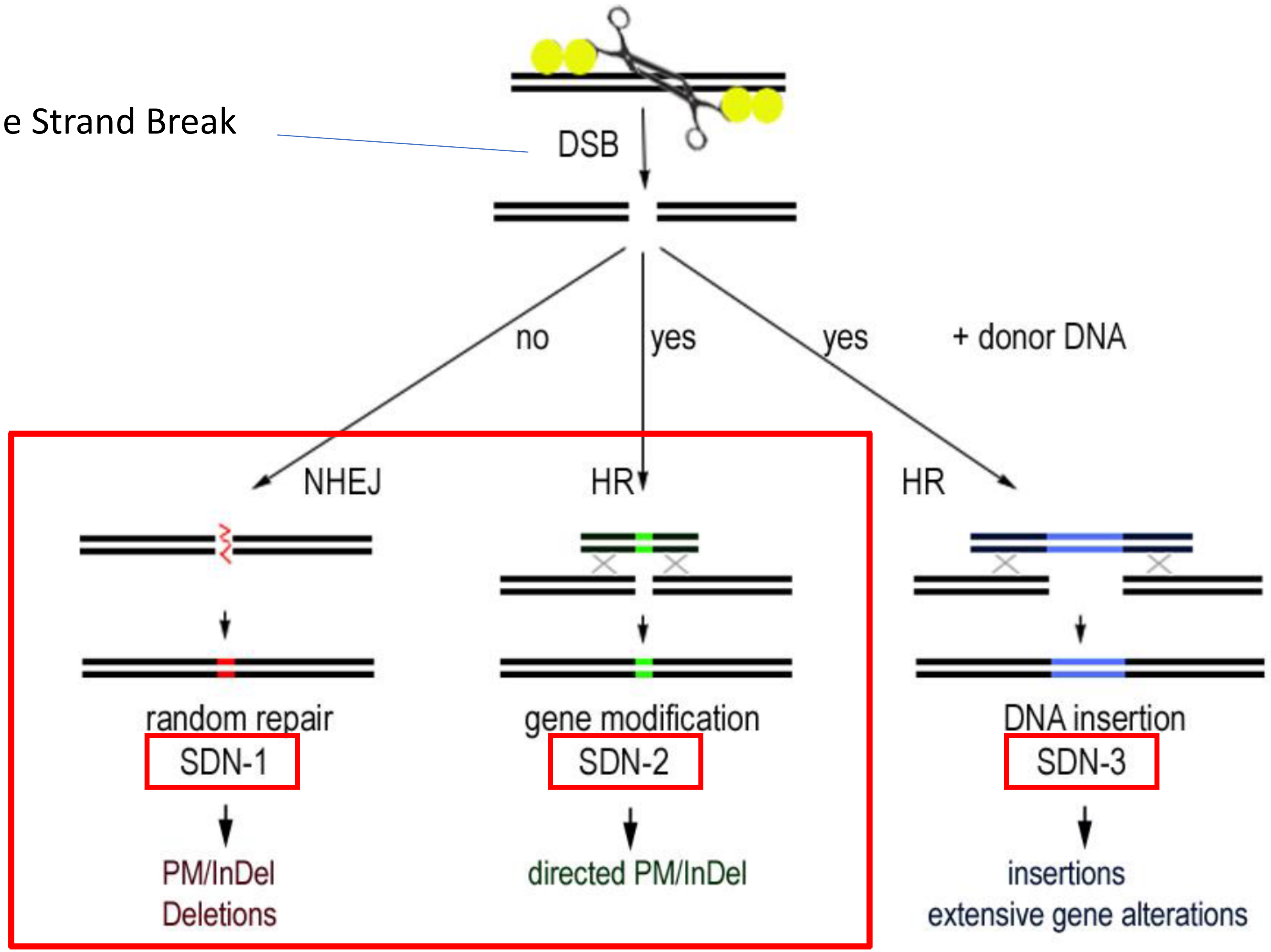
- **Targeted mutagenesis (SDN-1 o 2, not SDN-3)**

- **NBT o NGT = New Breeding Techniques o New Genetic/Genomic Techniques**

- In Italy: **TEA = Tecniche di Evoluzione Assistita, proposed by Società Italiana di Genetica Agraria (SIGA)**

Main modification types by Genome Editing

DSB = Double Strand Break



SDN = Site-Directed Nuclease technologies, 1-3

Genome edited crops in the field

Specie	Tipo di modifica	Metodo	Azienda	Status
Brassica juncea	Ridotto contenuto di tiocianati	CRISPR-CAS	Pairwise	Completa approvazione, test pieno campo, USA
Camelina	Alto contenuto di olio e tolleranza ad erbicidi	CRISPR-CAS	Yield10 Bioscience Inc.	Completa approvazione, test di pieno campo, USA
Colza	Resistenza a erbicida	ODM	Cibus	In coltivazione, USA e Canada
Colza	Alto contenuto di olio	CRISPR-CAS	Yield10 Bioscience Inc.	Completa approvazione, test pieno campo. USA e Canada
Mais	Mais con amido waxy (solo amilopectina) per uso industriale	CRISPR-CAS	CORTEVA-DUPONT	In coltivazione, USA
Pomodoro	Frutto nutraceutico ad alto GABA ad effetto ipotensivo	CRISPR-CAS	SANATECH-Seeds	In coltivazione, Giappone
Riso	Tolleranza alla siccità	CRISPR-CAS	Enti pubblici	Completa approvazione, test pieno campo, India
Soia	Alto acido oleico	TALEN	Calyxt	In coltivazione, USA

Salvi S. 2022 Genome editing, se non ora quando? https://terraevita.edagricole.it/biotecnologie/genome-editing-se-non-ora-quando/

Genome editing and Bioeconomy – the legislation bootleneck

Trends in Biotechnology

Science & Society

The future of genome editing innovations in the EU

Stuart J. Smyth[1,*,@] and Justus Wesseler[2]

ELSEVIER

EFB Bioeconomy Journal

journal homepage: www.elsevier.com/locate/bioeco

European genome editing regulations: threats to the European bioeconomy and unfit for purpose

Carsten Hjort[a], Jeff Cole[b,*], Ivo Frébort[c]

Detrimental effects of unfit regulation

- **Field trials of NGT products developed by SMEs (and by scientists in Italy!) almost impossible**
- **Decreasing number of patent applications from SMEs.**
- **Comply with the EU GMO legislation is too cumbersome and too expensive for SMEs.**
- **Increased difference with Americas and Asia (competitions, environmental benefits, etc.)**
- **Existing plans to establish SMEs to develop improved varieties for world market abandoned.**
- **Overall - Maintaining the current regulatory situation would have effects that are contrary to what the EU would like to achieve by means of the Green Deal.**

European community main pronouncements about GMO and NGTs (New Genetic/Genomic Techniques)

- **The main EU regulatory framework for genetically modified organisms is the so-called 'GMO Release Directive', i.e. Directive 2001/18/EC. 1,** it regulates the deliberate release into the environment of GMOs and the placing on the market of GMOs as or in products.

- **European Court of Justice, July 25th, 2018**

Gene editing come OGM - Based on EC regulation of 2001 (Illogical decision as they cannot be distinguished by natural or chemically induced mutations)

- **EC study on new genomic techniques** – *April 29, 2021 NBTs provide many opportunities to farmers…*

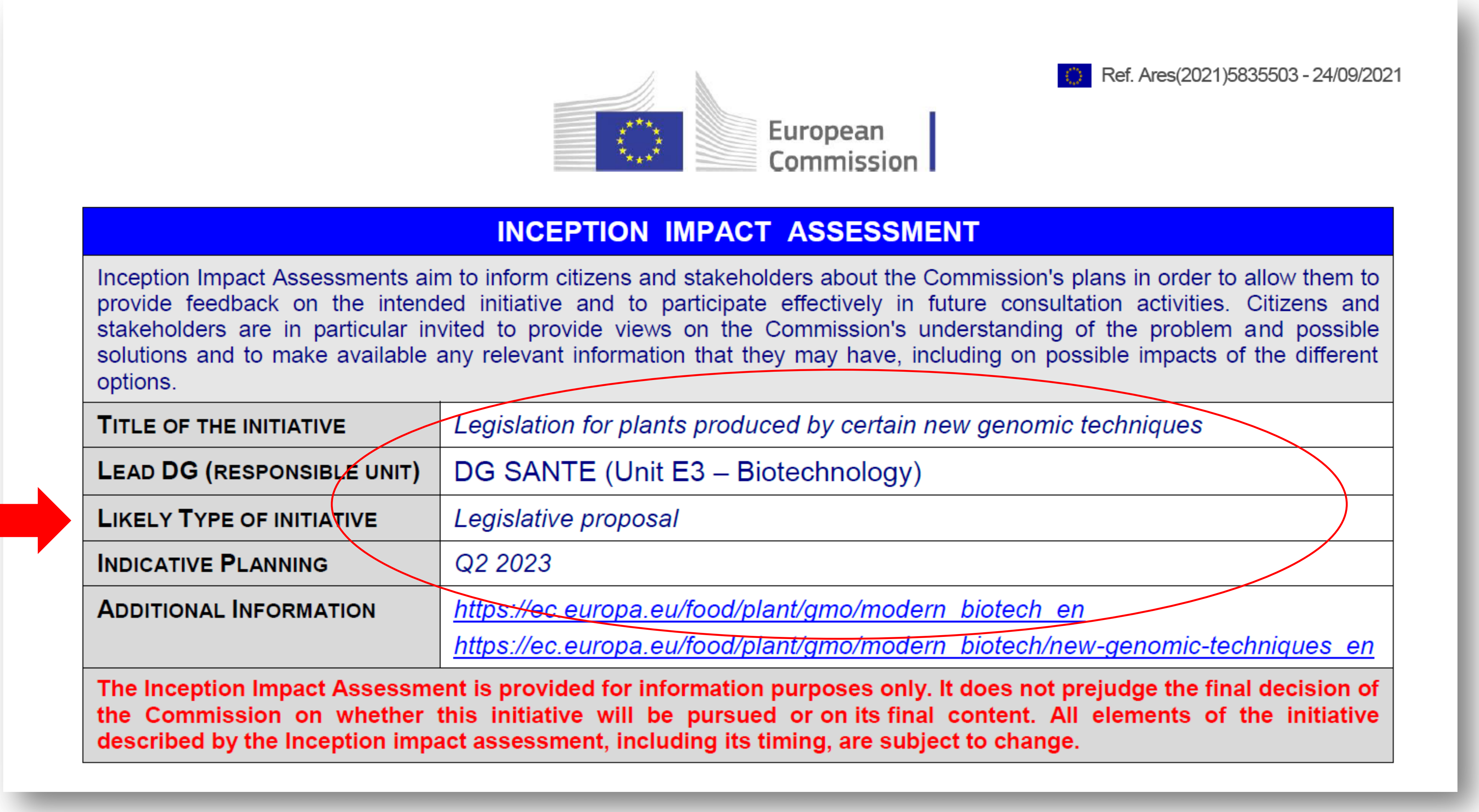
- **G20 Agriculture Ministers' Meeting Florence 17-18 Sept, 2021.** *Research about NBT and Genome editing is encouraged…*

- **Public consultation for legislation for plants produced by certain new genomic techniques April-July 2022.** Results available in September 2022

- **EFSA document, "Updated scientific opinion on plants developed through cisgenesis and intragenesis"** 18 October 2022. …the use of NGTs reduces the risks associated with potential unintended modifications of the host genome. Thus, fewer requirements may be needed for the assessment of cisgenic and intragenic plants obtained through NGTs

- **EFSA document "Criteria for risk assessment of plants produced by targeted mutagenesis, cisgenesis and intragenesis" 20 October 2022 ….** criteria as advice for consideration for the risk assessment of plants produced by targeted mutagenesis, cisgenesis and intragenesis. …. Stress on **History of Safe Use** to be adopted when considering mutations or alleles produced by NBT

Commitment of European Commission on updating NGT regislation



Ref. Ares(2021)5835503 - 24/09/2021

European Commission

INCEPTION IMPACT ASSESSMENT

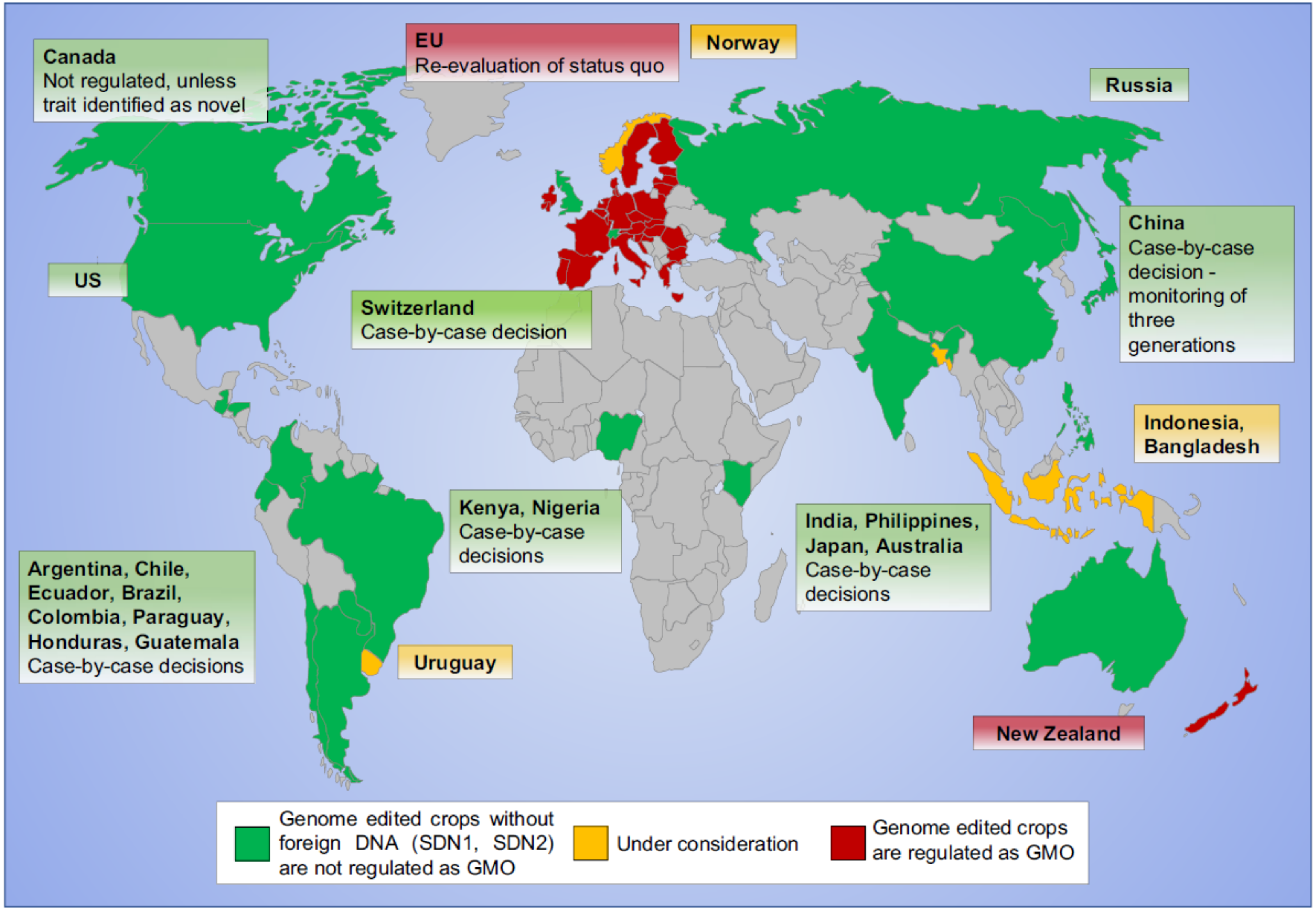
Inception Impact Assessments aim to inform citizens and stakeholders about the Commission's plans in order to allow them to provide feedback on the intended initiative and to participate effectively in future consultation activities. Citizens and stakeholders are in particular invited to provide views on the Commission's understanding of the problem and possible solutions and to make available any relevant information that they may have, including on possible impacts of the different options.

TITLE OF THE INITIATIVE	*Legislation for plants produced by certain new genomic techniques*
LEAD DG (RESPONSIBLE UNIT)	DG SANTE (Unit E3 – Biotechnology)
LIKELY TYPE OF INITIATIVE	*Legislative proposal*
INDICATIVE PLANNING	*Q2 2023*
ADDITIONAL INFORMATION	*https://ec.europa.eu/food/plant/gmo/modern_biotech_en* *https://ec.europa.eu/food/plant/gmo/modern_biotech/new-genomic-techniques_en*

The Inception Impact Assessment is provided for information purposes only. It does not prejudge the final decision of the Commission on whether this initiative will be pursued or on its final content. All elements of the initiative described by the Inception impact assessment, including its timing, are subject to change.

https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/13119-Legislation-for-plants-produced-by-certain-new-genomic-techniques_en

Rest of the world



Buchholzer and Frommer New Phytologist (2022)

Conclusions

- Genomics tools provide opportunity to catalogue, store and utilize genetic diversity and to targeted gene modification by NGT
- Unfit regulation in EU has been recognized as major constraint in bioeconomy applications of NGT
- The EC committed to address NGT and update the 2001-legislation in 2023
- Better communication to improve trust in science
- Additional constraints are clearly in scientific knowledge and strategic choices - which trait and gene to target? Consumers vs farmers? Which cropping system?

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