

Genomic tools for berry pre-breeding material: from genome to new berry cultivars

The basis for utilizing molecular markers in breeding

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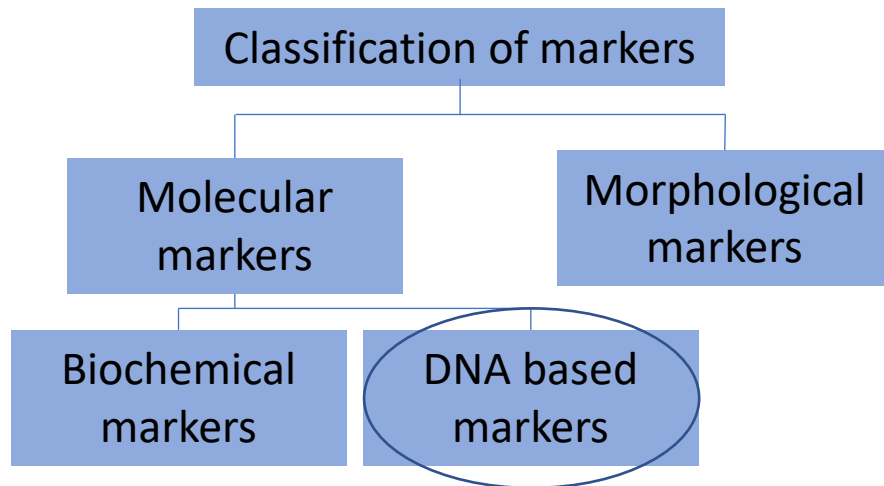
14.02.2024 - WORKSHOP

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Molecular markers

Béatrice Denoyes (INRAE)

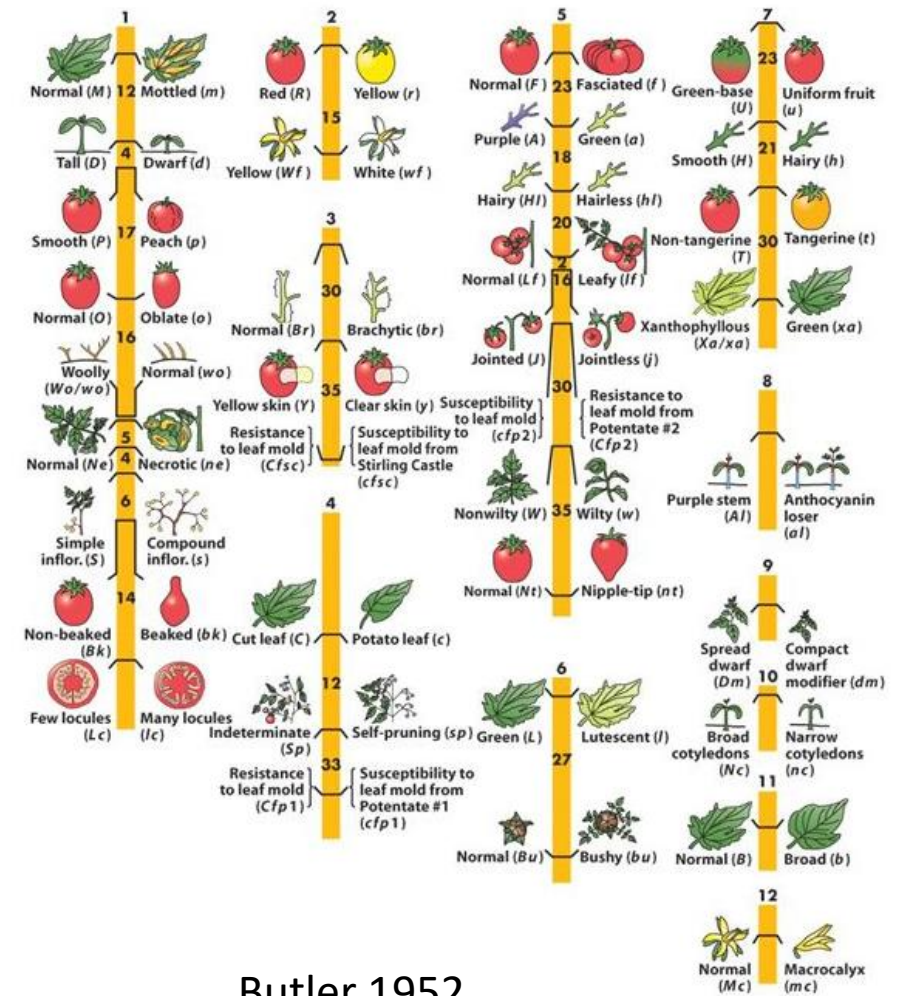
Types of markers



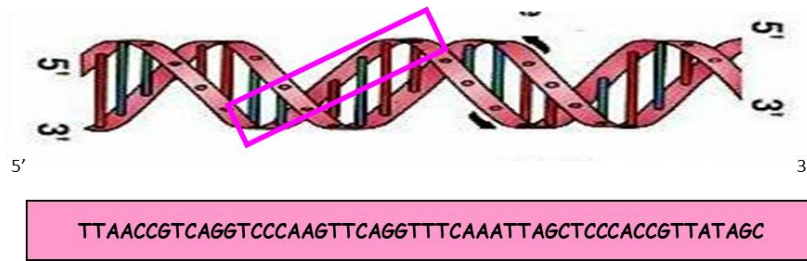
First molecular markers

Molecular marker is a piece of DNA molecule that is associated with a certain trait of a organism

For mapping genes -> need of markers



Molecular marker

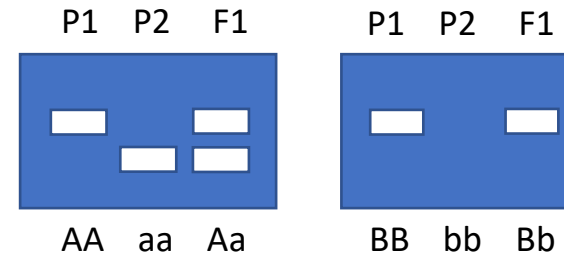


Specific fragment of DNA used to 'flag' the position of a particular gene or the inheritance of a particular characteristic

(1) co-dominant or dominant markers

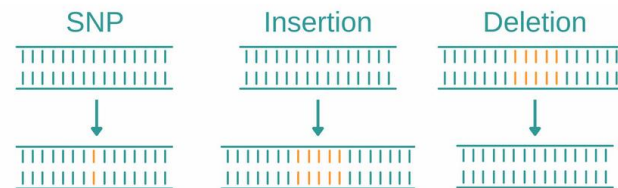
(2) method of detection (array, PCR...)

(3) mode of transmission (bi-parental nuclear inheritance or maternal nuclear inheritance, paternal or maternal organelle inheritance)



Allele: different form of the genomic region

Polymorphism between the nucleotide sequence of different genotypes: single-nucleotide polymorphism (SNP), insertion, deletion, translocation...



Credit to <https://www.integrabiosciences.com/canada/en/blog/article/genotyping>

An ideal DNA marker should be co-dominant, evenly distributed throughout genome, highly reproducible and having ability to detect higher level of polymorphism

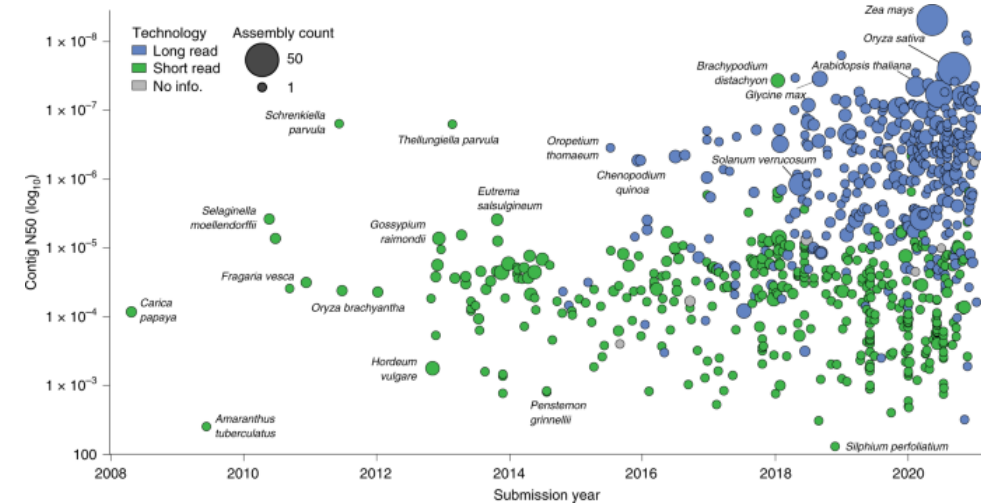
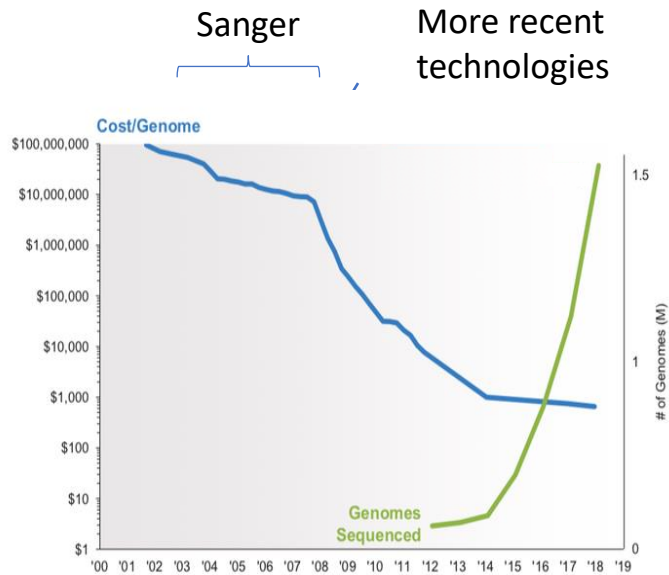
➡ **Sequence-based marker, SNP**

Sequencing's contribution

First DNA markers at the end of 80s:

- 1977: DNA sequencing (Sanger)
- 1978: Discovery of restriction enzymes -> RFLP markers
- 1983: Kary Mullis: PCR (targeted amplification of DNA) -> new markers, 1st linkage maps with all linkage groups, 1st QTLs

Plant genomes sequenced



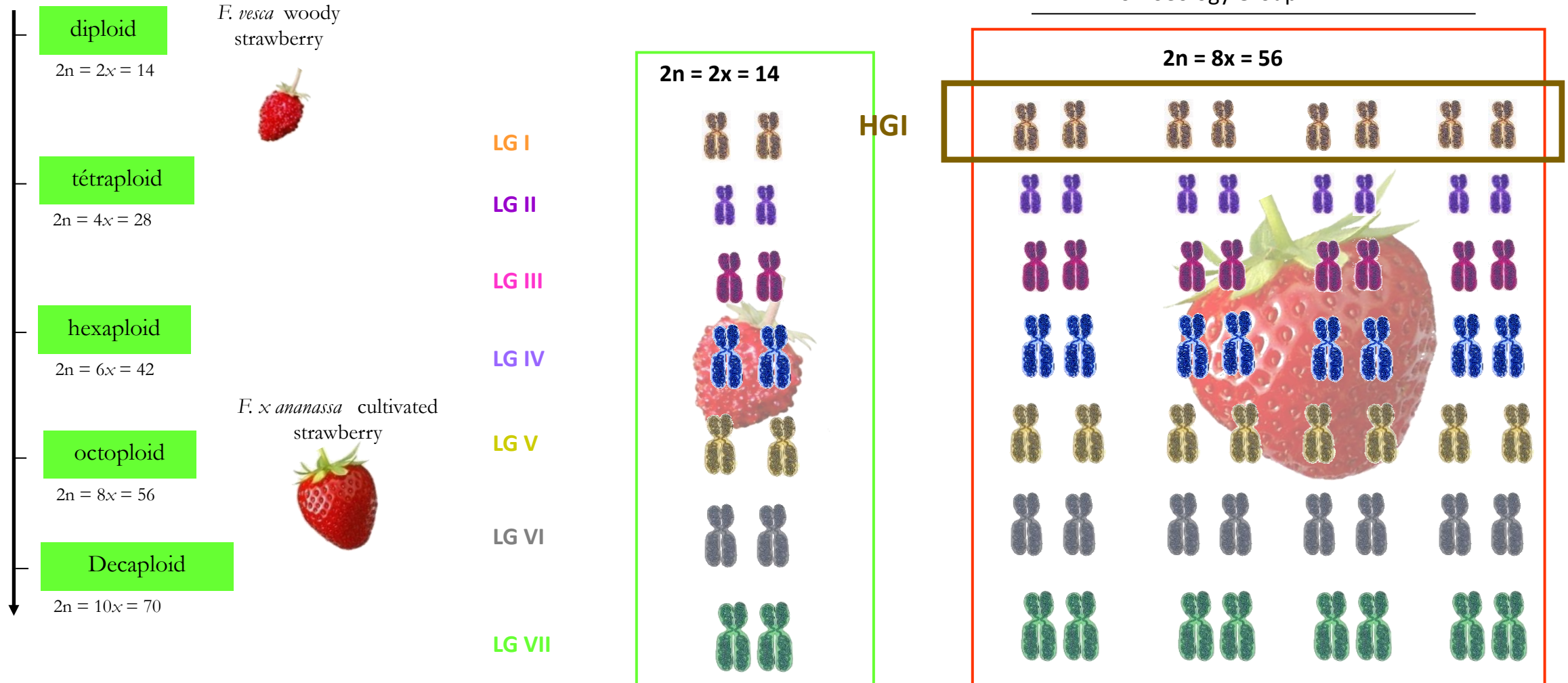
Marks et al., 2021, Nature Plants

Sequencing has always been the cornerstone of genomic science

The goal of a **reference genome** is telomere-to-telomere contiguity to fully characterize a genome sequence (Purugganan, Jackson, 2021)

Unraveling the organisation of the cultivated strawberry genome

Karyotype of *Fragaria*

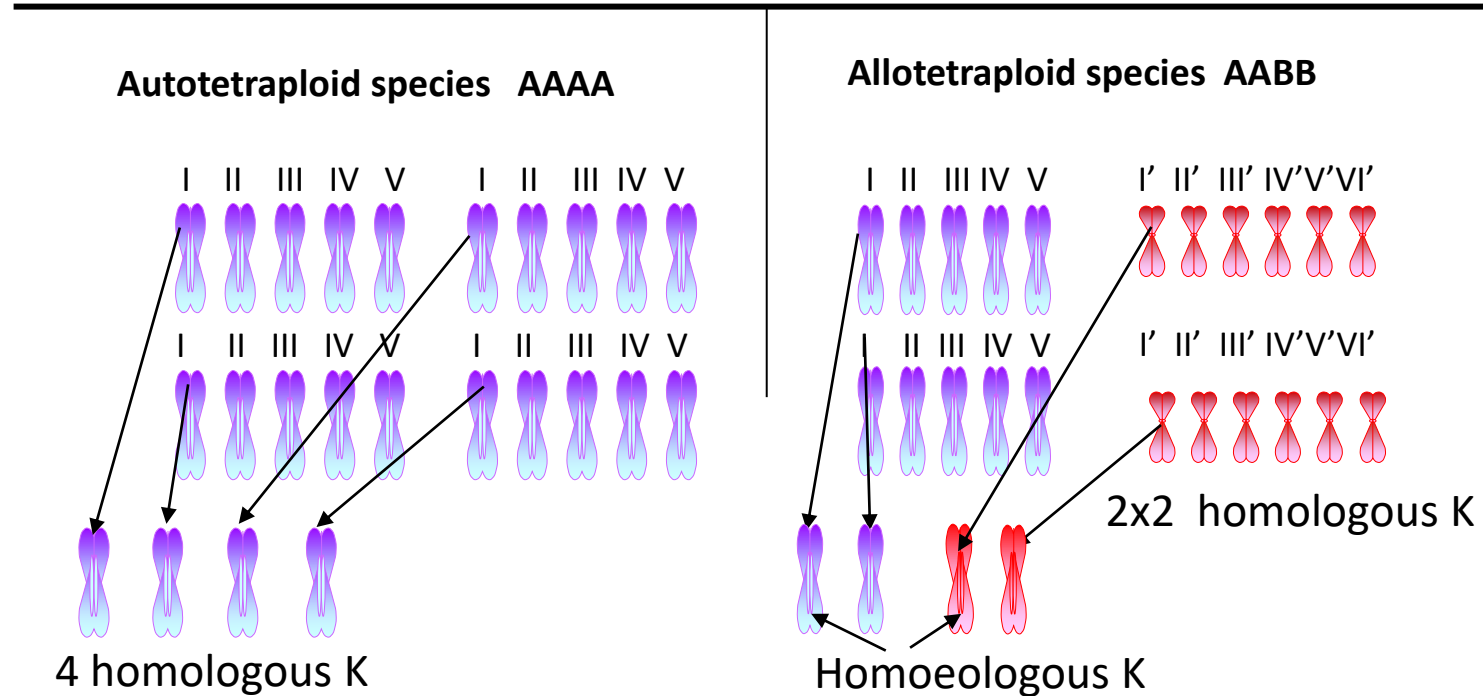


Consequence of polyploidization

Polyploid more than two sets of chromosomes

Homology vs. Homoeology

Autopolyploidy vs. Allopolyploidy



Mecanism for fertility restauration as gene controlling pairing at meiosis

Bivalent at meiosis

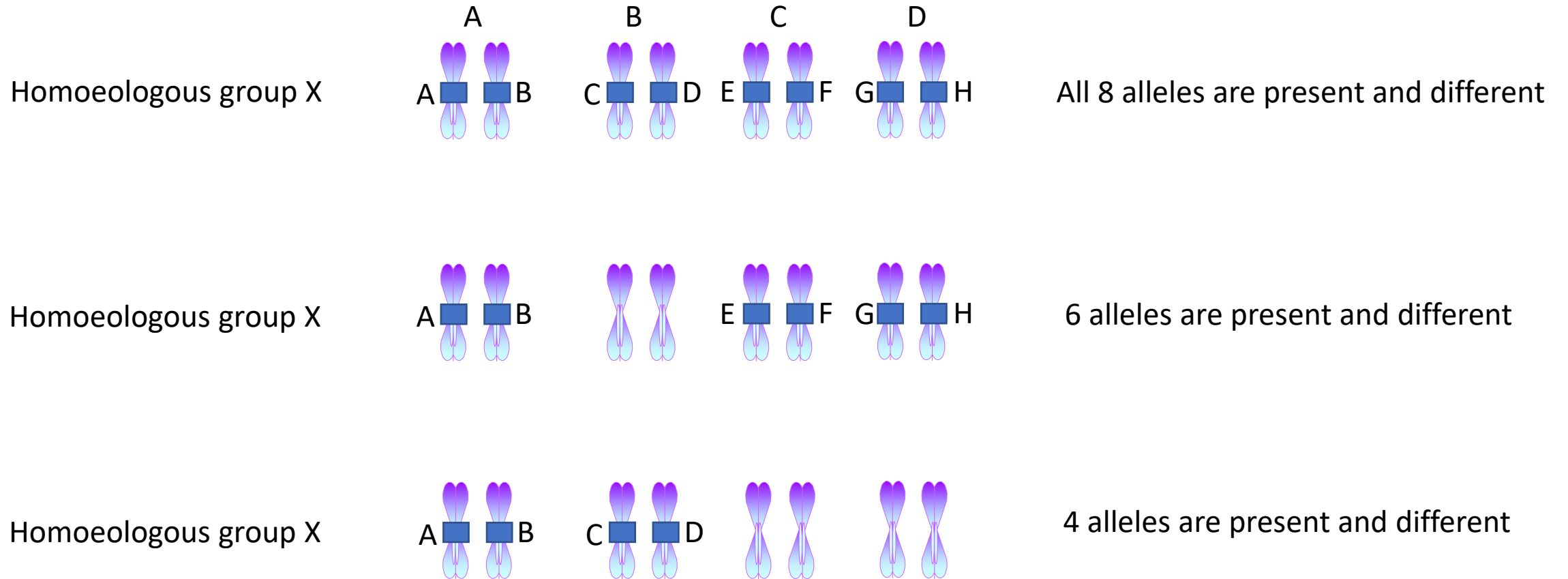
Morphological changes



Genetic and epigenetic changes

Maintained copies of a gene in the octoploid strawberry

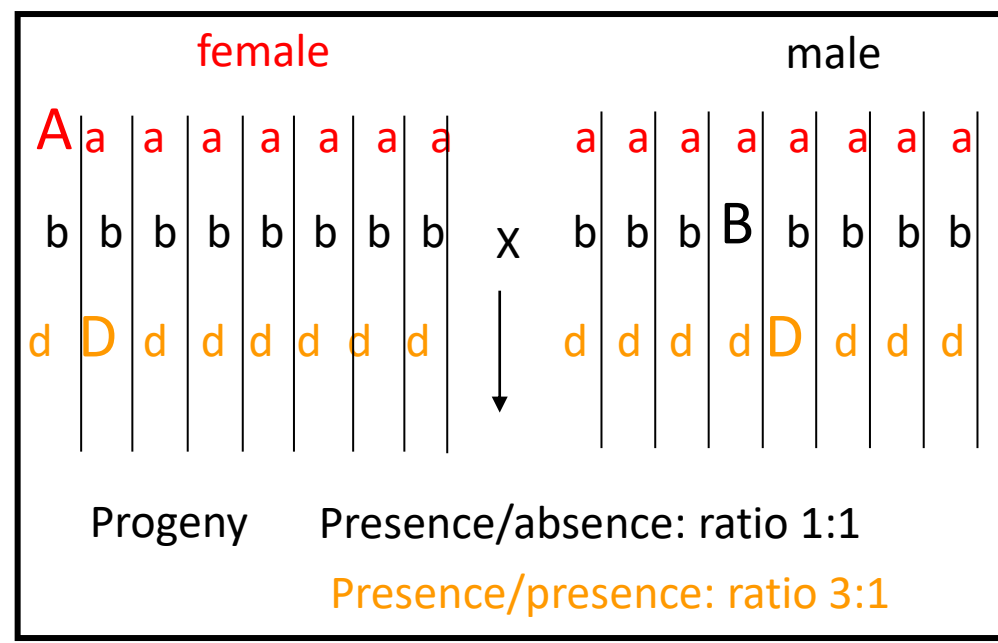
Sub-genomes



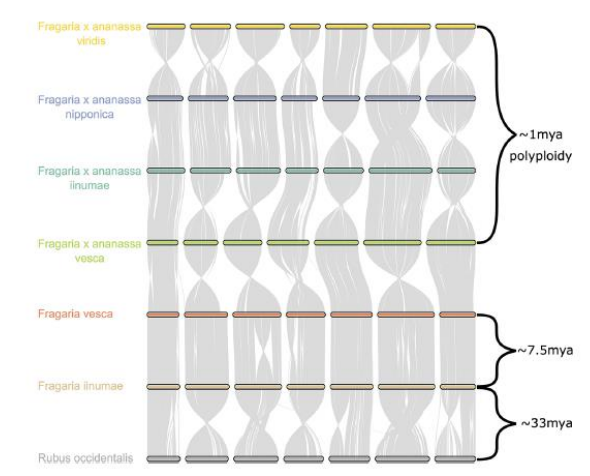
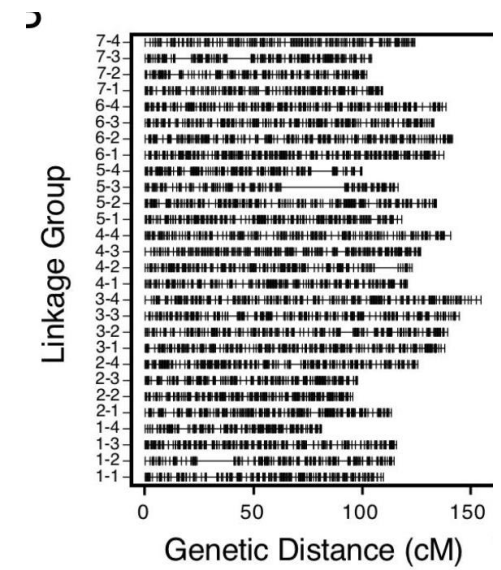
~46% of the annotated genes maintained all 4 copies (Jin et al., 2023, NP)

Polypoidy and linkage maps

Two-step process (Wu et al 1992, and then Ripoll et al 1999 and Qu and Hancock 2001) based on the analysis of single-dose alleles
Analysis of coupling phase



- marker A : Female map (SD*ND)
- marker B : Male map (ND*SD)
- marker D : Association female and male maps (SD*SD)



Hardigan et al., 2021 MBE

Cultivated strawberry sequencing

1st *Fragaria × ananassa* genome

<i>Fragaria × ananassa</i> Camarosa Genome Assembly v1.0	combination of short and long read approaches, including Illumina (455-fold coverage), 10X Genomics (117-fold coverage), and PacBio, totaling 615-fold coverage of the genome	Edger et al., 2019, Nat. Genetics
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Phased genomes available including in complex polyploid genomes such as *Fragaria × ananassa*

<i>Fragaria × ananassa</i> RoyalRoyce Genome Assembly v1.0	PacBio HiFi, nomenclature and physical orientations proposed by Hardigan et al. (2021)	Hardigan et al., 2021, bioRxiv; Pincot et al., 2021, G3
<i>Fragaria × ananassa</i> FL15.89-25 Genome v1.0	Pacbio HiFi for FL15.89-25, Illumina for parents	Han et al., 2022, bioRxiv
<i>Fragaria × ananassa</i> Wongyo 3115	Hi-C library, N50 of 27.3Kb (after correction of gaps)	Lee et al., 2021, FiPS
<i>Fragaria × ananassa</i> Yanli	Pacbio, HiFi and Illumina, N50 of 26.70Mb	Mao et al., 2023, Hort. Res.



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Molecular markers in breeding

Aurélie Petit (INVENIO)

Experimentation station and R&D toolkit for Vegetables et Fruits producers

Private research

Based in Southwestern France

Research for members (growers...)
Breeding, Development
Diffusion

3 main activities

13 studied species

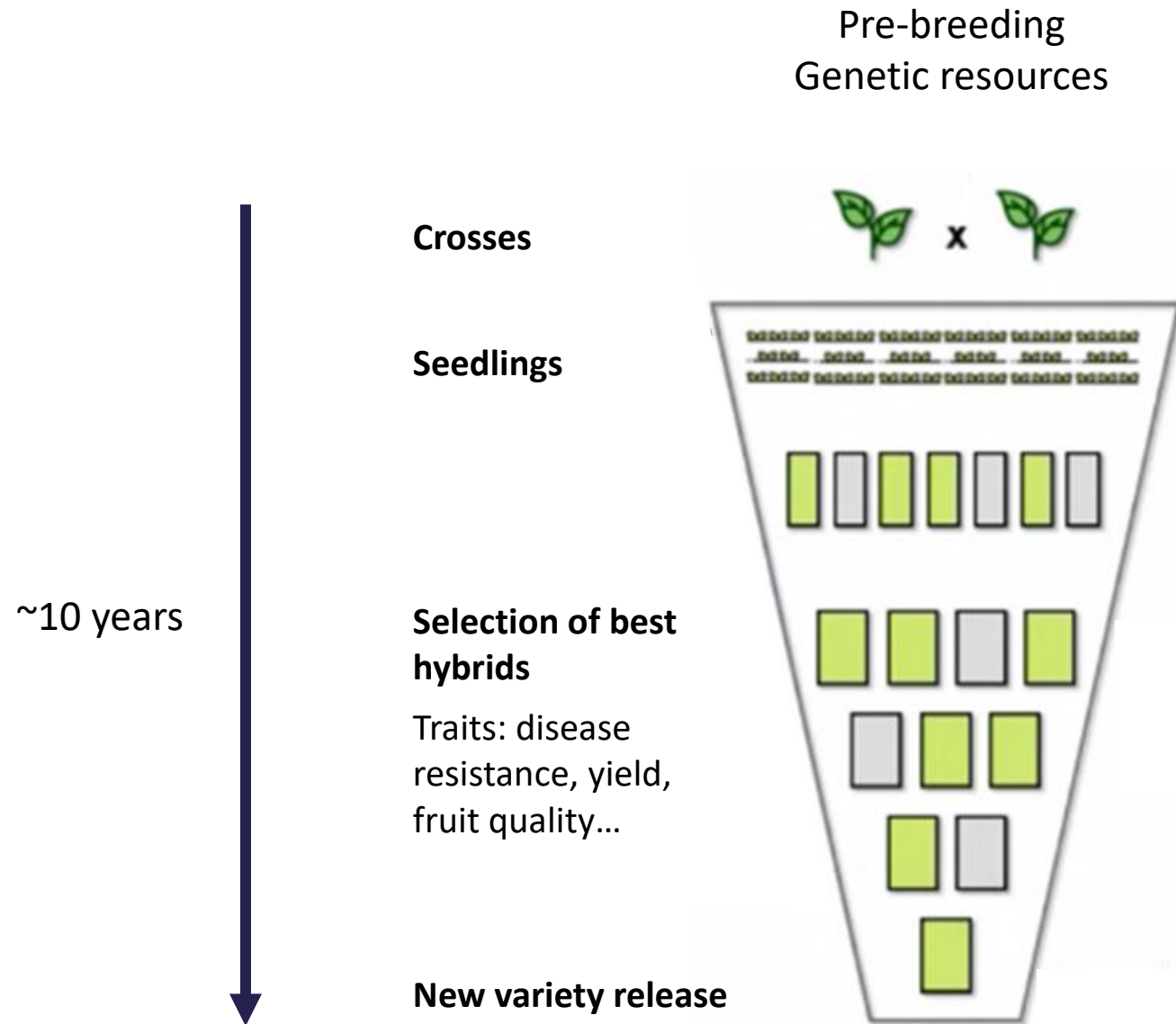
Fruits, vegetables and plants production



Facilities

- 21 ha of orchards
- 14 ha of vegetables productions
- 2 ha of greenhouses et 1 ha of tunnels
- in-vitro laboratory
- farm machinery conception center

Traditional strawberry breeding program



Molecular marker use in Invenio's strawberry breeding program

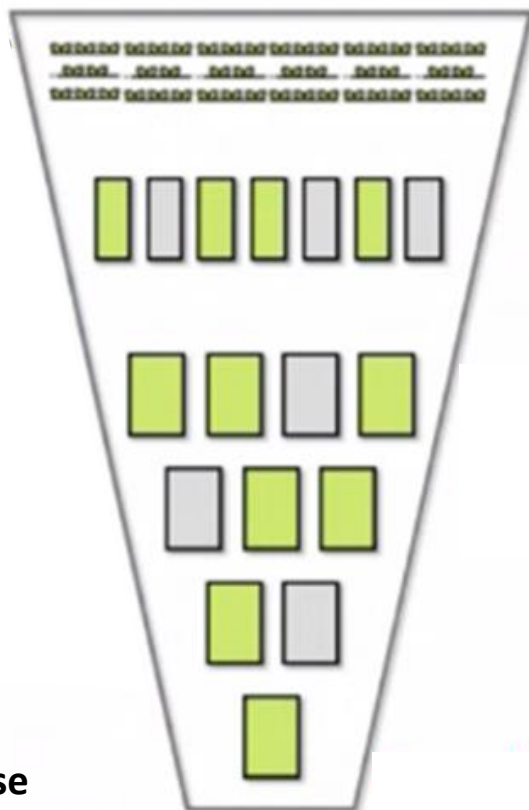
Collab. INRAE

Pre-breeding
Genetic resources



Crosses

Seedlings



Selection of best
hybrids

New variety release

Genetic characterization to better manage GR collection:
diversity study, genetic structure analysis, core collection establishment
for GWAS and to reduce the collection maintenance costs



Genomic prediction to better choose parents

Marker-Assisted Selection (MAS) to select earlier the best hybrids
for monogenic traits (aroma, disease resistance...)
⇒ QTL analysis and GWAS, SNP array design



Genomic Selection (GS) to select earlier the best hybrids
for polygenic traits (yield, disease resistance...)



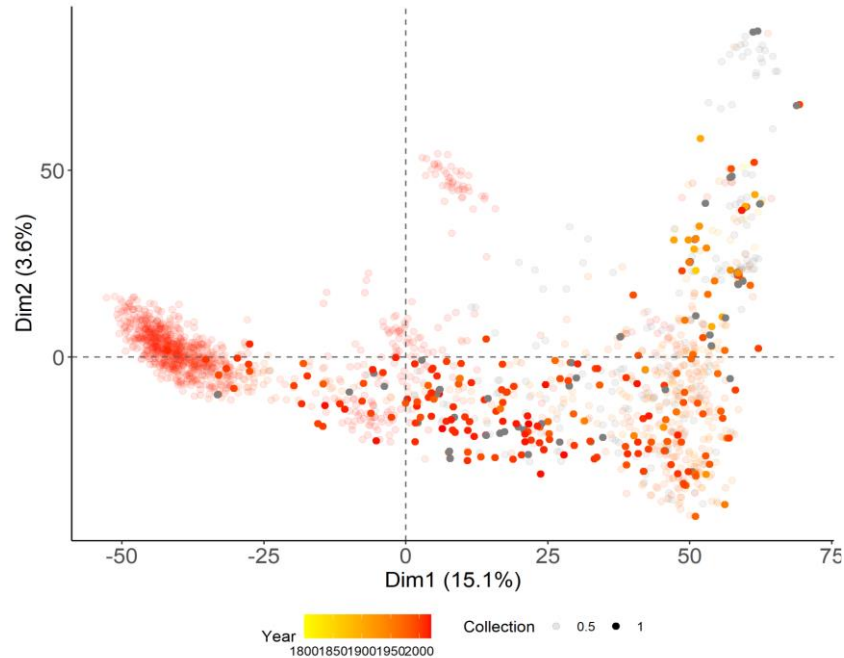
Genetic fingerprints to check variety = variety control for maintenance
and commercial plant production (micropropagation, nursery)

Invenio's genetic resources characterization

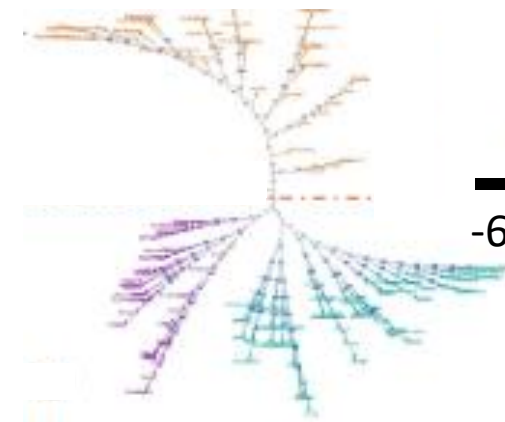
Genetic structure of a collection of 220 cultivated strawberry individuals



Genetic diversity

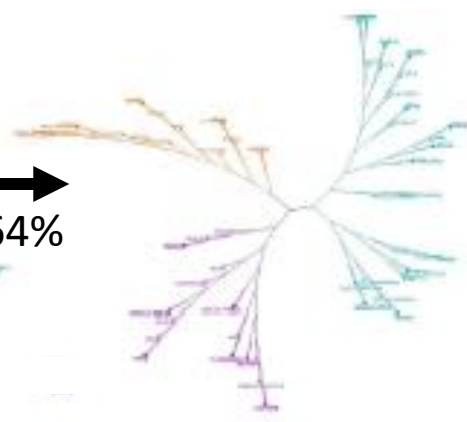


Collection



Core collection

-64%



Poster at IHC2022
Alexandre Prohaska's thesis

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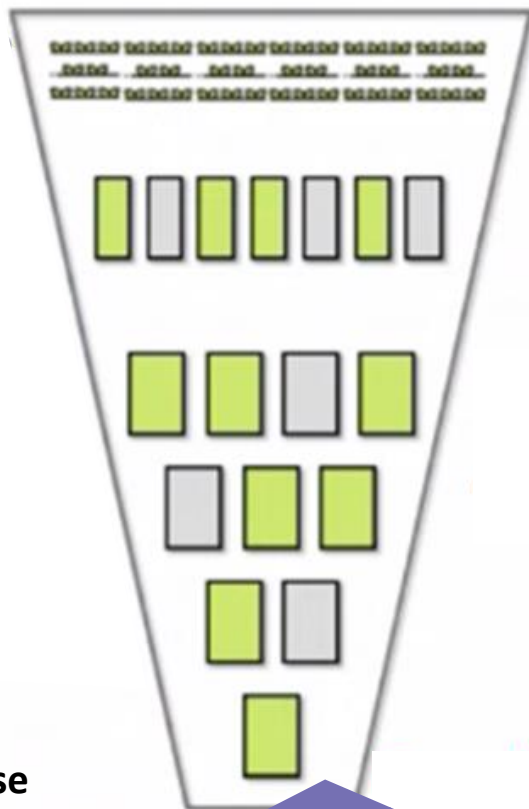
Pre-breeding
Genetic resources

Phenotypic and genetic characterization ⇒ **Nahla Bassil & Steven Knapp's talks**
Data integration ⇒ **Björn Usadel & Elisa Senger's talks**
Linkage & association mapping for MAS dvpt ⇒ **Dan Sargent & Jahn Davik's talks**



Crosses

Seedlings



Marker-Assisted Selection (MAS) ⇒ **Iraida Amaya's talk**

Genomic prediction ⇒ **Juliette Bénéjam's talk**

Selection of best
hybrids

New variety release

New Breeding Techniques
⇒ **Bruno Mezzetti's talk**



UMR 1332, FDFE team
Strawberry group



BreedingValue project



Strawberry breeding team

**INRAE and Invenio's trainees,
PhD students and post-doctoral fellows**