

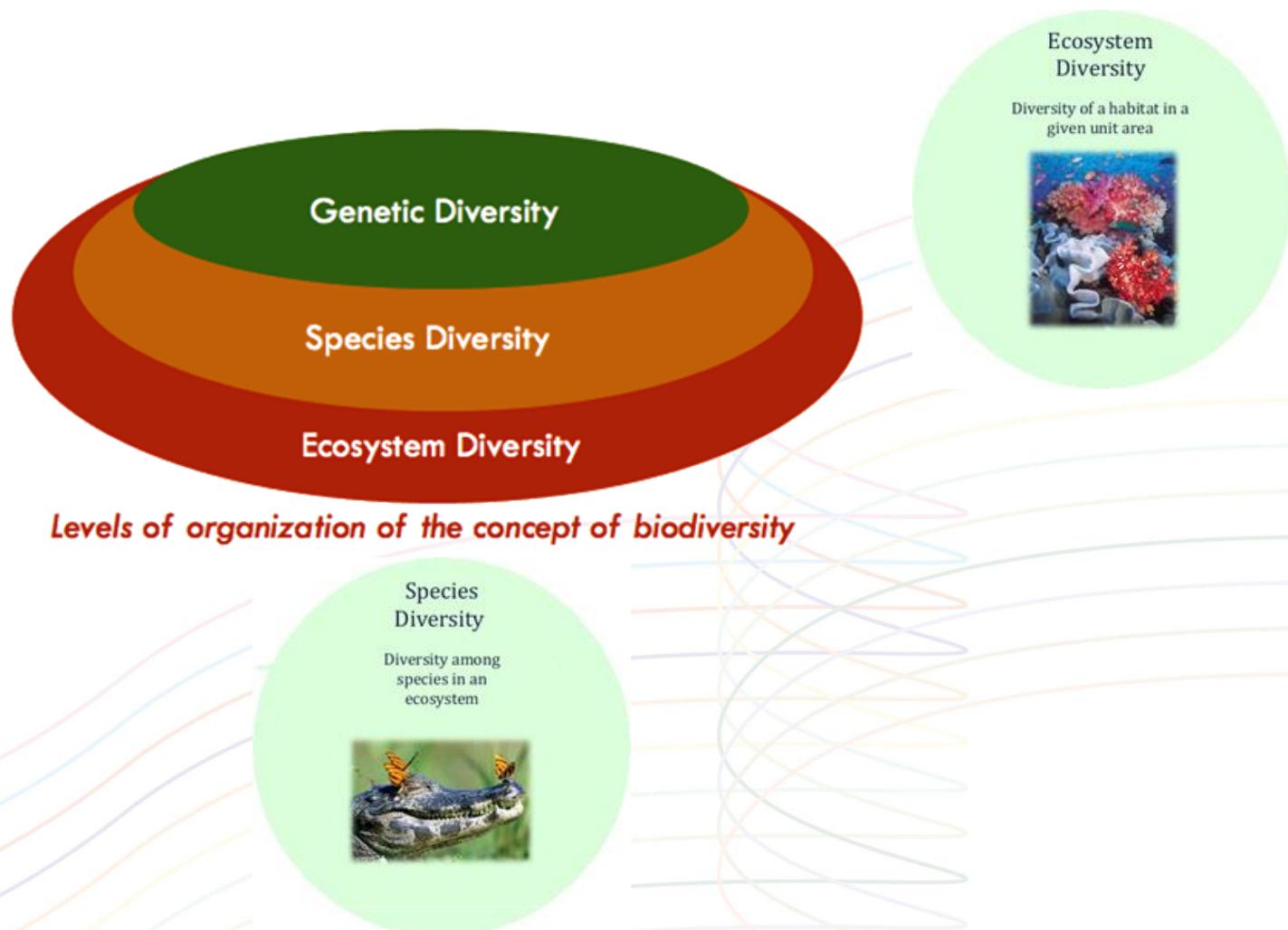
## Conservare, Caratterizzare e Valorizzare: le collezioni del CREA per rispondere alle nuove sfide dell'agricoltura



<https://www.crea.gov.it/en/home>

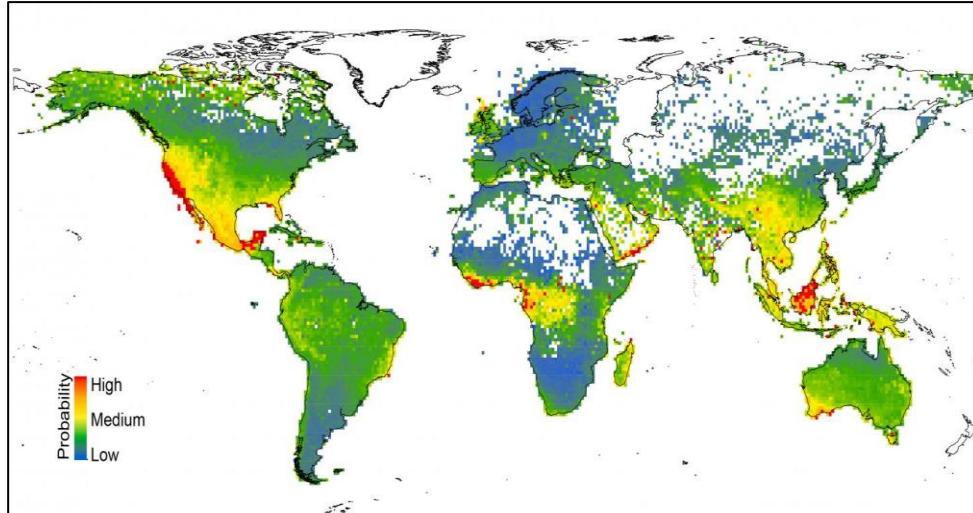
Ignazio Verde ignazio.verde@crea.gov.it

# Biodiversità vs Agrobiodiversità

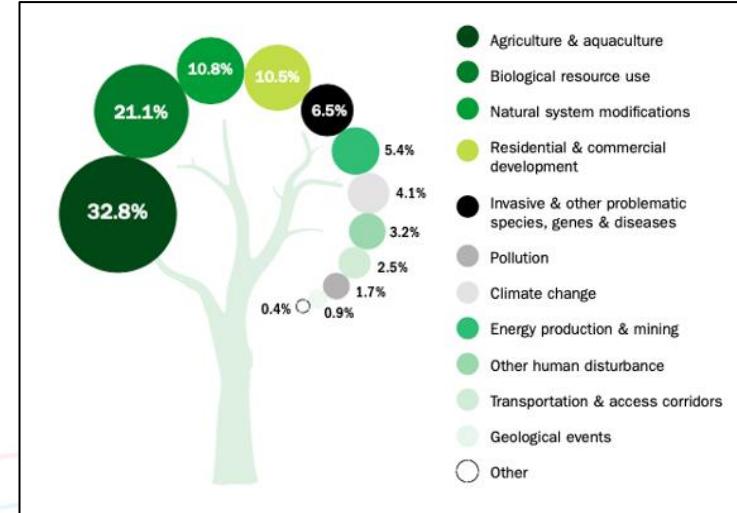


**Agrobiodiversità: Diversità genetica** delle specie coltivate e parenti affini (Crop Wild Relatives, CWR)

# Perdita di Biodiversità globale



Worldwide distribution of plant species at risk of extinction

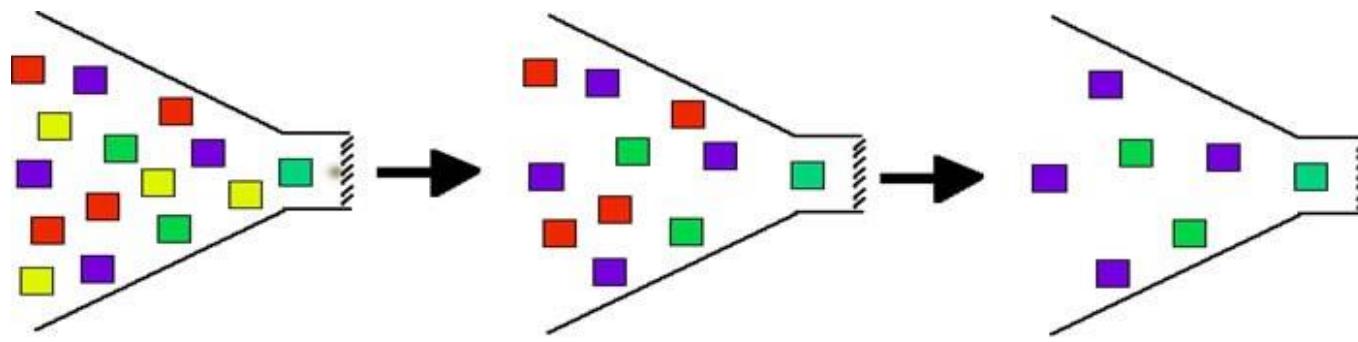


Main causes of plant extinction (1)

- Around 40% of plant species are at risk of extinction globally.
- This figure fluctuates between 10%-45% for crop wild relatives, and reaches 60% for some groups (cycads, epiphytes, orchids)

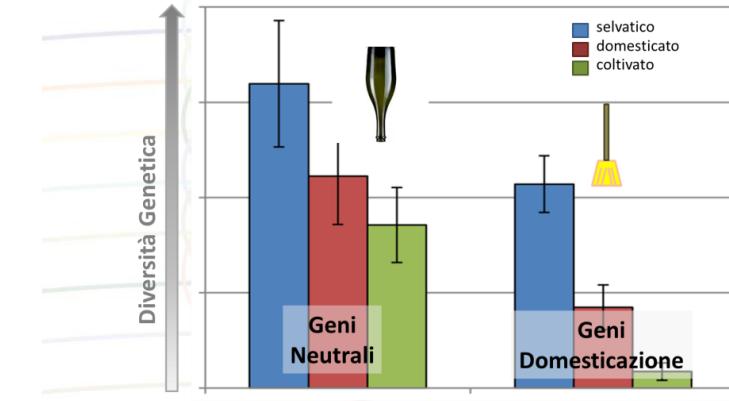
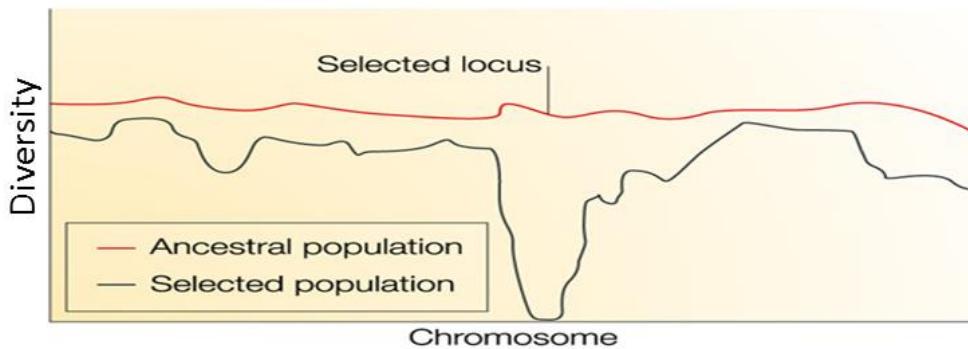
(1) Kew Gardens «State of the World's Plants and Fungi»

# Perdita di diversità genetica a causa di selezione e breeding

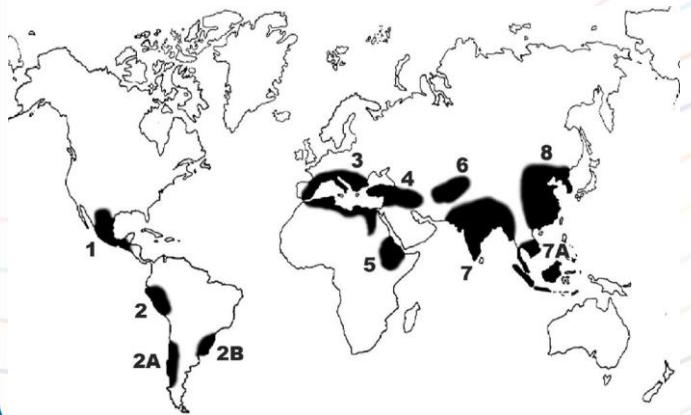


Wild species → Early domesticates → Modern varieties

Erosione Genetica: la FAO stima che il 75% della diversità genetica delle specie coltivate sia stata persa durante i processi di selezione



Adattata da: Chapman MA (2013) PLoS ONE



## Le spedizioni di Vavilov alla ricerca dei semi



Tratto da: A cena con Darwin, Jonathan Silvertown

**Centri di diversificazione di Vavilov.** (1) Messico-Guatemala, (2) Peru-Ecuador-Bolivia, (2A) Cile meridionale, (2B) Brasile meridionale, (3) Mediterraneo, (4) Medio Oriente, (5) Etiopia, (6) Asia centrale, (7) India-Birmania, (7A) Indocina, (8) Cina e Corea.[2]

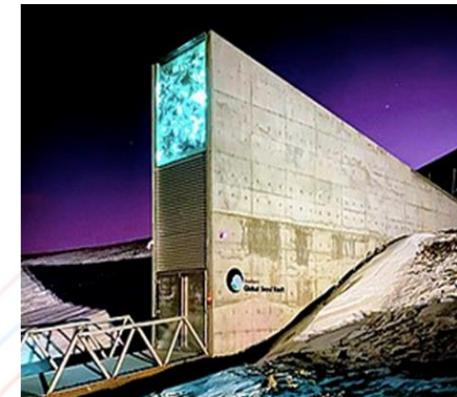
# Prevenire la perdita di agrobiodiversità



*In situ*- on farm conservation



*Ex situ* conservation (Genebanks)



Seed vaults (the last resort)

## Documentation & access

## Safety

- Around 4 M of plant accessions are conserved in worldwide genebanks (1), of which around 2 M in Europe (2)
- The European accessions are spread over 400 different institutions, with dishomogenous management standards
- International regulations, and phytosanitary hurdles prevent the effective exchange of materials
- Active *in situ* / on-farm population conservation is very limited and does not meet proposed standards (3)
- 1.3 M of accessions are conserved at the Svalbard Global Seed Vault (4)

(1) <https://www.genesys-pgr.org/>

(2) [https://eurisco.ipk-gatersleben.de/apex/eurisco\\_ws/r/eurisco/home](https://eurisco.ipk-gatersleben.de/apex/eurisco_ws/r/eurisco/home)

(3) <http://www.fao.org/agriculture/seed/sow2/en/>

(4) <https://www.croptrust.org/work/svalbard-global-seed-vault/>



Nel 2004 l'Italia ratifica il **Trattato Internazionale sulle Risorse Fitogenetiche per l'Alimentazione e l'Agricoltura (ITPGRFA)**, legge 101/2004, e istituisce un programma pluriennale, finanziato dal MASAF, con lo scopo di attuare e implementare l'articolo. 5 del Trattato stesso. Il programma è attualmente al 7 ciclo triennale.



National Programme for Conservation, Characterisation, Use and Valorisation of Plant Genetic Resources for Food and Agriculture



# Specie e accessioni conserve e scambiate dal CREA

COLTURE	SPECIE (CROP)	CROP WILD RELATIVES	N. Accessioni mantenute
Cereali	24	33	17.006
Fruttiferi (Inclusi Olivo, Citrus e Vite e piccoli frutti)	70	108	11.719
Ortive	30	9	521
Colture Industriali (inclusi legumi)	19	6	1.950
Ornamentali	197	-	974
Specie Medicinali e aromatiche	6	-	9
Colture Foraggere	70	-	7.643
Specie Forestali e da legno	2	-	733
<b>TOTALE</b>	<b>418</b>	<b>156</b>	<b>40.555</b>

Numero di accessioni  
scambiate dal CREA  
dal 2004 al 2023



Crop Group	N. of Accessions Transferred to			N. of Accessions Introduced from		
	Italy	Abroad	Total	Italy	Abroad	Total
Cereal crops	258	189	447	167	198	365
Fruit crops (including nuts, berries, citrus, olive and grape)	851	92	943	877	142	1019
Vegetable crops	87	0	87	80	152	232
Forage crops	30	50	80	71	98	169
Industrial crops (including grain legumes)	142	53	195	145	774	919
Medicinal and aromatic plants	63	10	73	13	9	22
Ornamental crops	19	1	20	11	0	11
Forest and woody crops	190	137	327	19	29	48
<b>TOTAL</b>	<b>1640</b>	<b>532</b>	<b>2172</b>	<b>1383</b>	<b>1402</b>	<b>2785</b>

## CONSERVAZIONE

### FUTURE CHALLENGES

- Climate change and new pathogens
- Population increase
- Environmental and biodiversity protection



- Identification of causal genes for important agronomic traits (as biotic and abiotic stresses) and developing of associated Molecular Markers.
- Breeding of improved cultivars using both classical and New Genomic Techniques (NGT).



## COMMON PROBLEMS ON GERMPLASM COLLECTIONS

**HOMONYMY:** two or more genotypes that should be unique but are genetically diverse.

**SYNONYMY:** two or more genotypes that should be diverse but are genetically identical.

### Molecular Markers:

- **SSR:** multiallelic, alto potere discriminante, bassa automazione
- **SNP:** biallelic, basso potere discriminante, alta automazione, high-throughput



**LABELLING ERRORS:** incorrect identification of the genotypes at the time of introduction in the collection.

Curatori banche di germoplasma  
SSR (10-20), SNP (100)

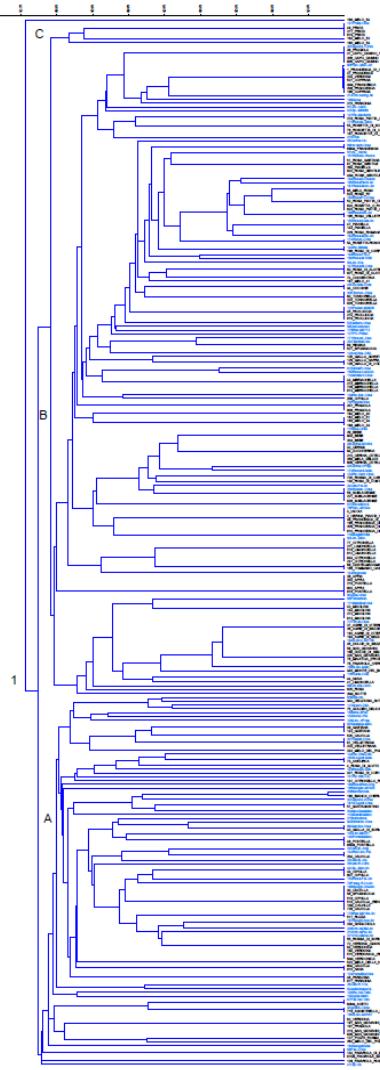


**GENETIC DIVERSITY:** estimation of heterozygosity, number of alleles, nucleotide diversity, population structure, identification of genes (GWAS)



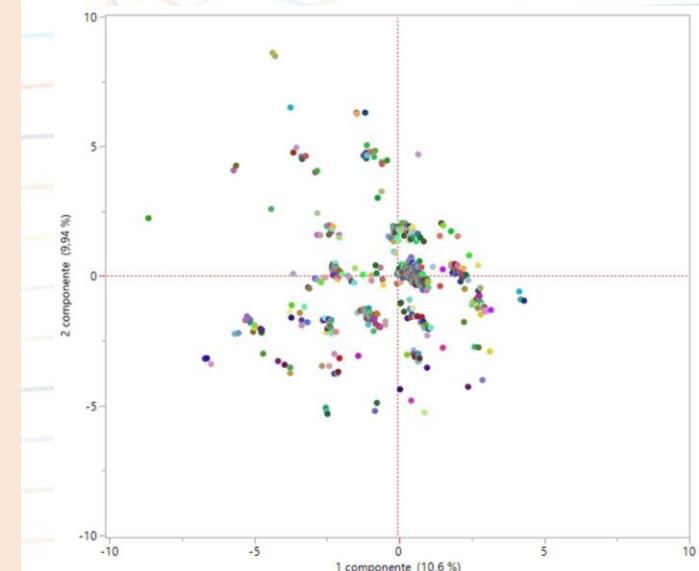
## Simple Sequence Repeat Markers:

- **Cherry** 350 genotypes by 17 SSR markers
- **Apricot** 350 genotypes by 10 SSR markers
- **Plum** 100 genotypes by 13 SSR markers
- **Peach** 970 genotypes by 14 SSR markers
- **Pistacia** 40 genotypes by 13 SSR markers
- **Pear** 280 genotypes by 7 SSR markers



## High Throughput (SNP markers)

- **Peach** 400 genotypes by 9.000 SNPs (SNP Array)
- **Apple** 200 genotypes by 10.000 SNPs (SNP Array)
- **Apricot** 144 genotypes by 25.000 SNPs (Single Primer Enrichment Technology, SPET)

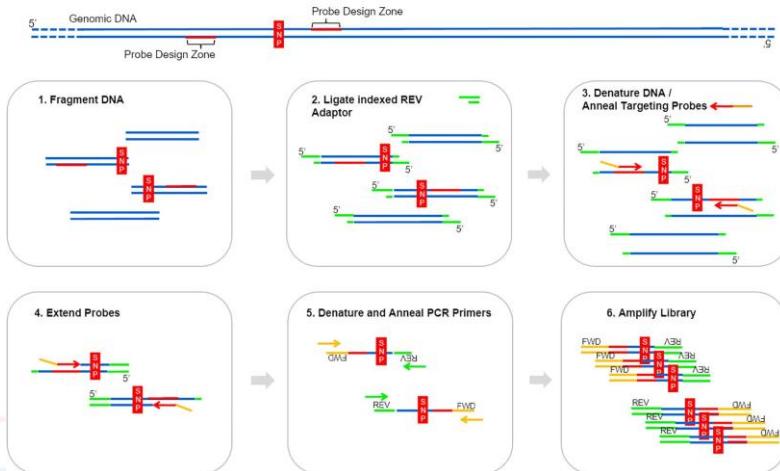


## High Throughput (SNP markers)

- **Almond** 160 genotypes by 60.000 SNPs (AxiomTM 60K SNP Array for Almond)
- **Cherry** 700 genotypes by GBS approach

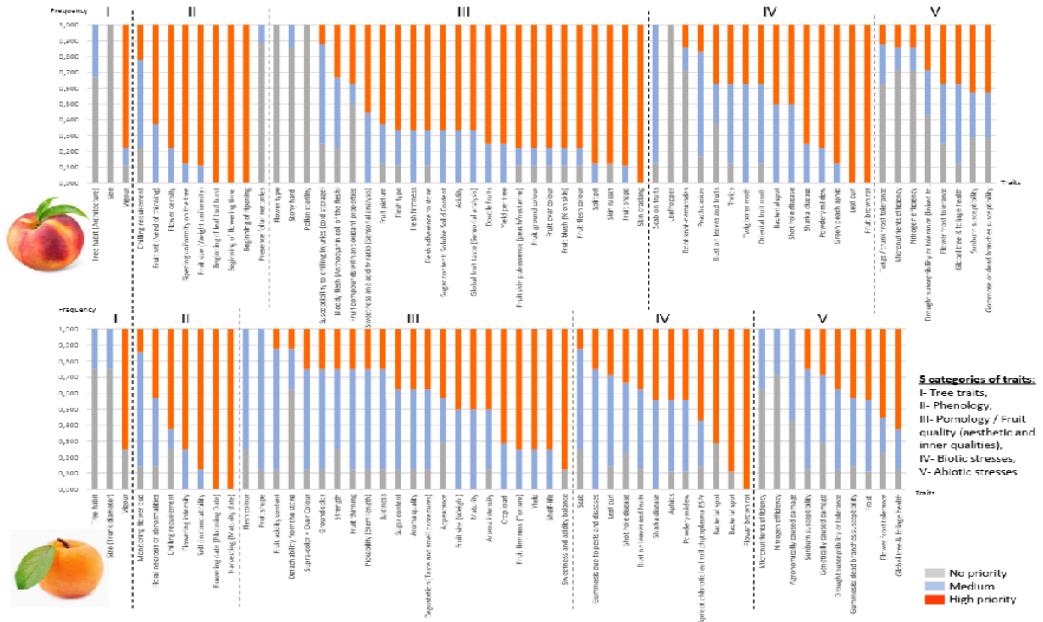


Single Primer Enrichment Technology (SPET)



# PHENOTYPIC CHARACTERIZATION

## International Standards (ECPGR, FAO, Agreed Ontologies)



# Field data collection

- Peach
  - Apricot
  - Plum
  - Cherry
  - Kiwi fruit
  - Apple

**DONE**

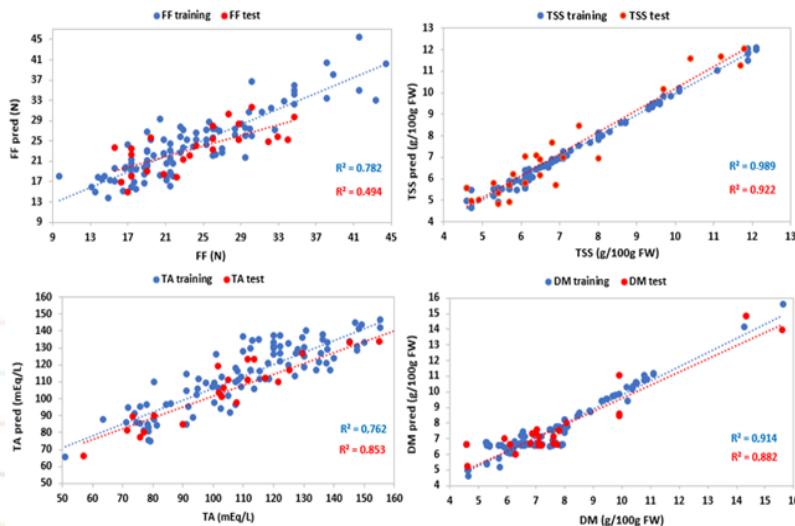
## Lab Analysis (SSC, Sugar content, Firmness,.....)

- Peach
  - Apricot
  - Cherry
  - Kiwi fruit

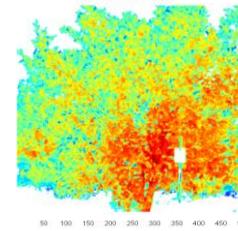


## High Throughput and Digital Phenotyping (PHENOMICS)

- Spectral and Hyperspectral cameras
- Thermal cameras
- Drones
- Robots
- Sensors
- Model construction (non disruptive analyses)

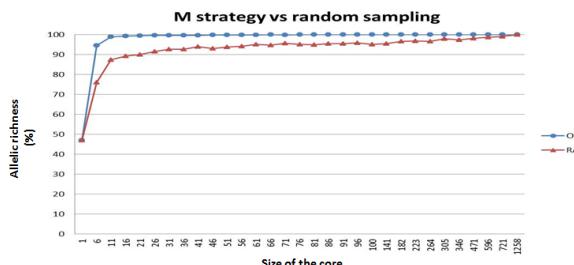


PCA Model - Contour 2D (T)  
DataSet (645)

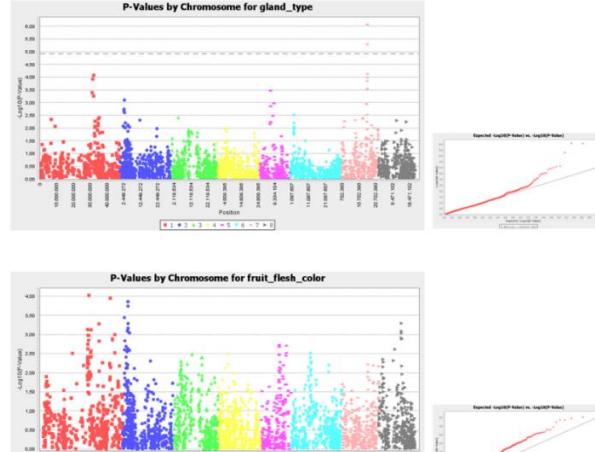


# Peach Reference Population (PeachRefPop)

## VALORIZZAZIONE



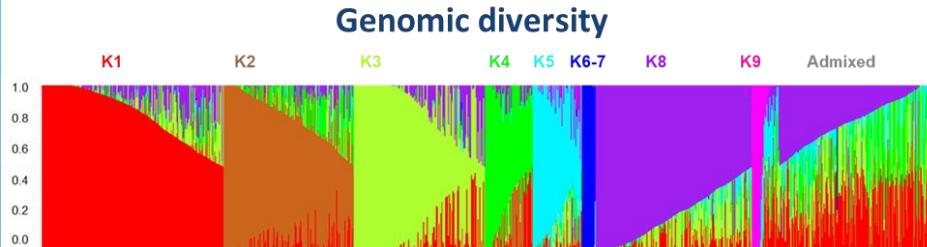
Validation through GWAS



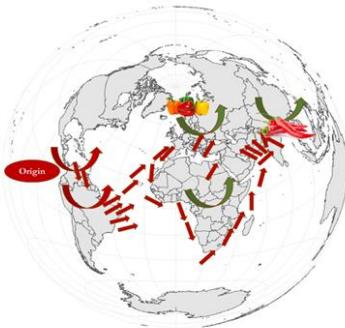
- **~1200** accessions genotyped with **9,000 SNPs**
- **4 European peach national germplasm collections**: Italy (2), France and Spain
- A core collection of **69** accessions selected
- Ref-set (**PeachRefPop**) di **150** accessions validated with genetic diversity analyses and GWAS
- The **PeachRefPop** was replicated in **5 locations** (Italy, Spain, and Greece) and is currently used to study GxE interactions in GWAS with the aim to find genetic factors involved in adaptation to climate changes as well as in the resistance to pests and diseases

# Pepper 10,000 genebank accessions

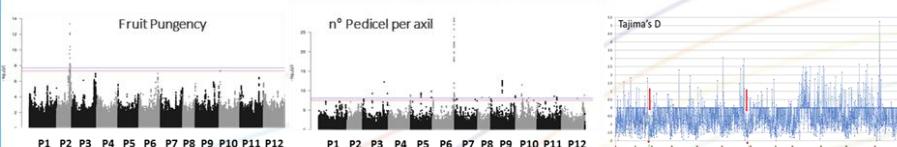
## VALORIZZAZIONE



**Routes of expansion and evolution**



**GWAS, candidate genes and selective sweeps**



- Pepper germplasm from main **International Genebanks** and Research Institutions
- Over **10,000** accessions of 14 *Capsicum* species representing **130** countries
- Genotyping by sequencing yielding  $\sim$  **27000** SNPs
- Estimation of level of duplication among genebanks and putative misclassification of the accessions (1618) (16.2%)
- **Novel centres of diversification** identified in **Eastern Europe** and **South-Southeast Asia**
- Routes of evolution from the centre of origin highlight **west–east routes of expansion**, shedding light on role of Africa forming a natural link between the Americas and the Eurasian pepper complements
- The exploration of **phenomic data** in GWAS allowed to identify **new candidate genes** as well as **selective sweeps** for key traits under selection

## VALORIZZAZIONE

### 1- Screening del germoplasma e mappaggio

**311 accessioni**

76 tropical *japonica*

224 temperate *japonica*

8 indica

2 aromatic

1 aus

**2 stagioni di crescita**

**2 condizioni**

Pieno campo

Camera di crescita

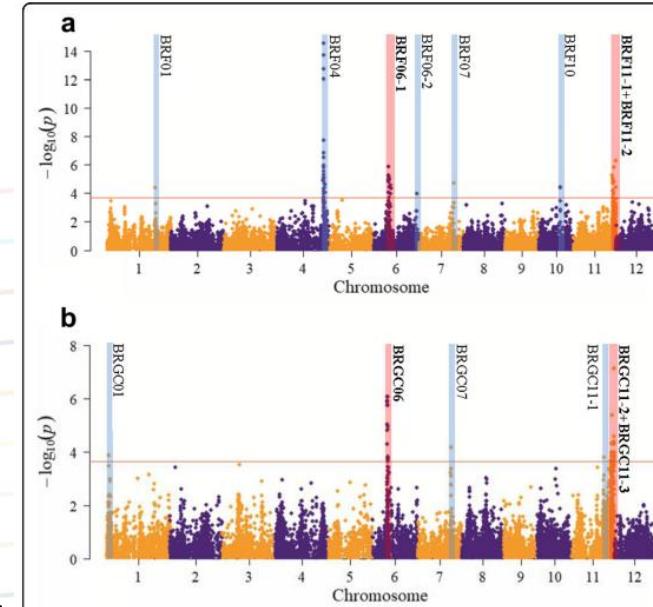
Analisi DNA

37.423 SNPs

### ANALISI DI ASSOCIAZIONE GWAS

- 14 associazioni significative (3 nuove)
- 11 accessioni ad elevato livello di R in entrambe le condizioni

## Brusone - *P. oryzae*



Rice

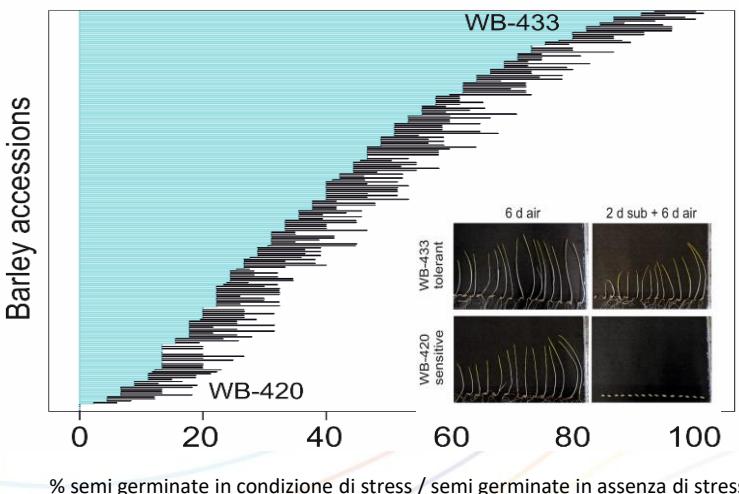
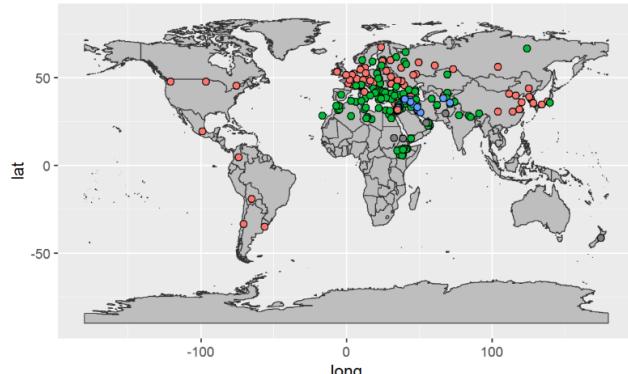
ORIGINAL ARTICLE

Genome wide association studies for *japonica* rice resistance to blast in field and controlled conditions



# La collezione WHEALBI in orzo

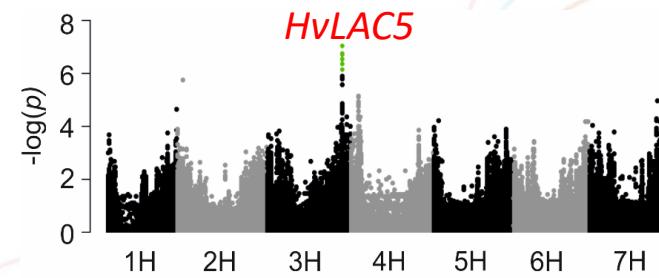
## VALORIZZAZIONE



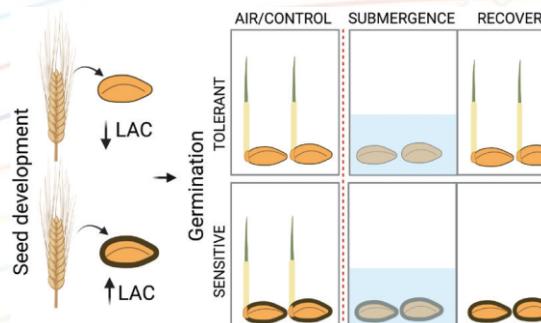
- ca. 450 accessioni di orzo di diversa origine; include ecotipi locali, varietà antiche e moderne e accessioni di *Hordeum spontaneum*, il progenitore selvatico dell'orzo coltivato

- >1 Million SNPs derivanti dal sequenziamento dell'esoma

- GWAS ad alta risoluzione



1 locus, *HvLAC5*,  
controlla la  
germinabilità  
del seme in condizioni  
di stress anossico



- Minore permeabilità del seme
- Scarsa diffusione di O<sub>2</sub> → Iporessia
- Attivazione network ABA → dormienza secondaria

## VALORIZZAZIONE

### Piante in valutazione in ambiente controllato

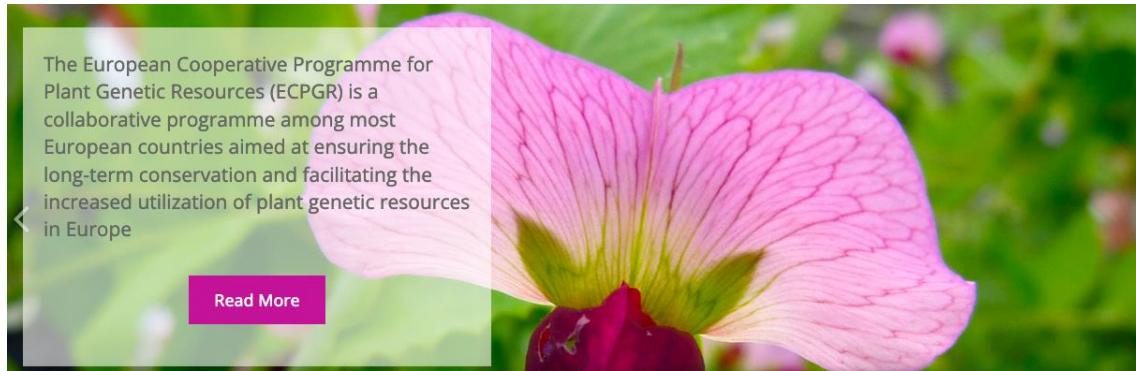
- ✓ Melo cisgenico resistente alla ticchiolatura (CREA OFA - FEM)
- ✓ Melo editato per il gene della resistenza al fuoco batterico (CREA OFA - UNIBO)
- ✓ Pero cisgenico per il gene della resistenza al fuoco batterico (CREA OFA – UNIBO)
- ✓ Kiwi editato per il gene che controlla la resistenza alla batteriosi (CREA GB - CREA OFA)
- ✓ Pompelmo editato per il gene che determina una ridotta dimensione del seme (CREA OFA)
- ✓ Pompelmo rosa cisgenico per il gene per l'accumulo di antociani (CREA OFA)
- ✓ Frumento duro editato per i geni della dimensione del seme (CREA CI)
- ✓ Riso editato per un gene che espande le radici in profondità (CREA CI)
- ✓ Pomodoro editato nei geni che controllano l'accumulo di antiossidanti e metaboliti secondari (CREA OF)
- ✓ Basilico editato per il gene di resistenza a peronospora (CREA OF)
- ✓ Melanzane con ridotto accumulo di acido clorogenico che non imbruniscono (CREA GB)

## VALORIZZAZIONE

### Piante in attesa dell'autorizzazione per le Prove di campo

- Piante di pomodoro in grado di inibire lo sviluppo delle orobanche (**notifica già presentata in attesa di risposta**) CREA OF
- Piante di arancio dolce arricchite in sostanze antiossidanti (antociani + licopene). (**Notifica in fase di preparazione**) CREA OFA
- Piante di vite resistenti alla peronospora. (**Notifica in fase di preparazione**) CREA VE
- Piante di melanzane senza semi. (**Notifica in fase di preparazione**) CREA GB

# The international arena



The European Search Catalogue for Plant Genetic Resources (EURISCO) provides passport and phenotypic data on **>2 million accessions** of crop plants and their wild relatives, preserved *ex situ* by about **400 institutes** from **43 member countries**.

**>10 EU-funded projects** have generated novel genetic resources and associated knowledge and methods on important crop plant families (Cereals, Solanaceae, Legumes, Rosaceae).



**Topic:** Research infrastructure concept development (HORIZON-INFRA-2022-DEV-01-01)

**Duration:** 1st January 2023 – 30 June 2025

**Funding:** 2.85 M€ EU + 0.37 Assoc. partners

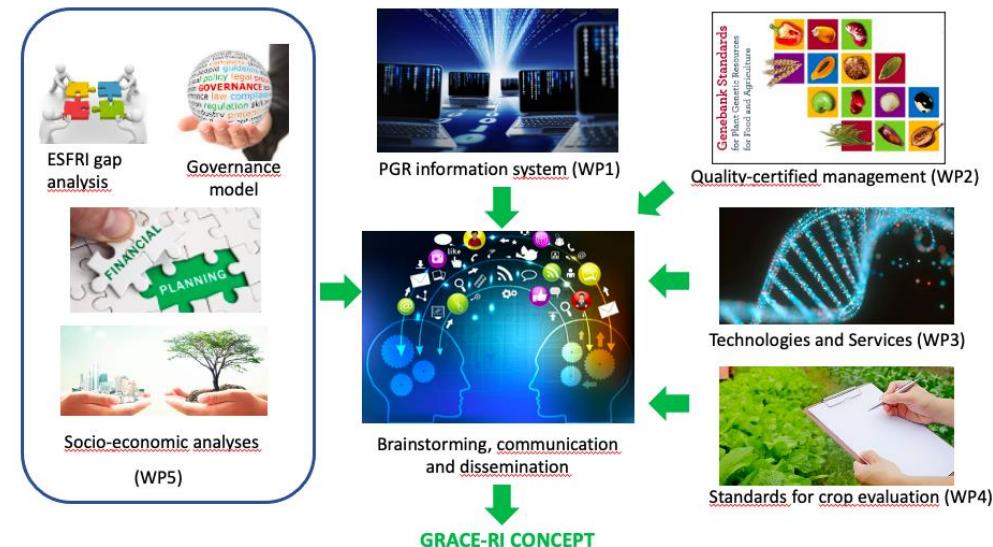
**Coordinator:** Giovanni Giuliano, ENEA; [giovanni.giuliano@enea.it](mailto:giovanni.giuliano@enea.it)

**Website:** [www.grace-ri.eu](http://www.grace-ri.eu)

## Promoting a Plant Genetic Resource Community for Europe (PRO-GRACE)



WP n.	WP title
WP1	Inventory and information system
WP2	Quality-certified ex situ and in situ management
WP3	Technologies and scientific services
WP4	Evaluation and valorisation
WP5	RI concept, social and regulatory aspects, governance and financial plan
WP6	Communication, dissemination, exploitation and training
WP7	Scientific coordination and management



THANKS FOR YOUR  
ATTENTION



Il gruppo RGV FAO



Il dott. Giovanni Giuliano, ENEA

Il dott. Vincenzo Montalbano MASAF