CREA, Research Centre for Olive, Fruit and Citrus Crops (Italy)

The role of genetics as a source of resistance to HLB.

Concetta Licciardello
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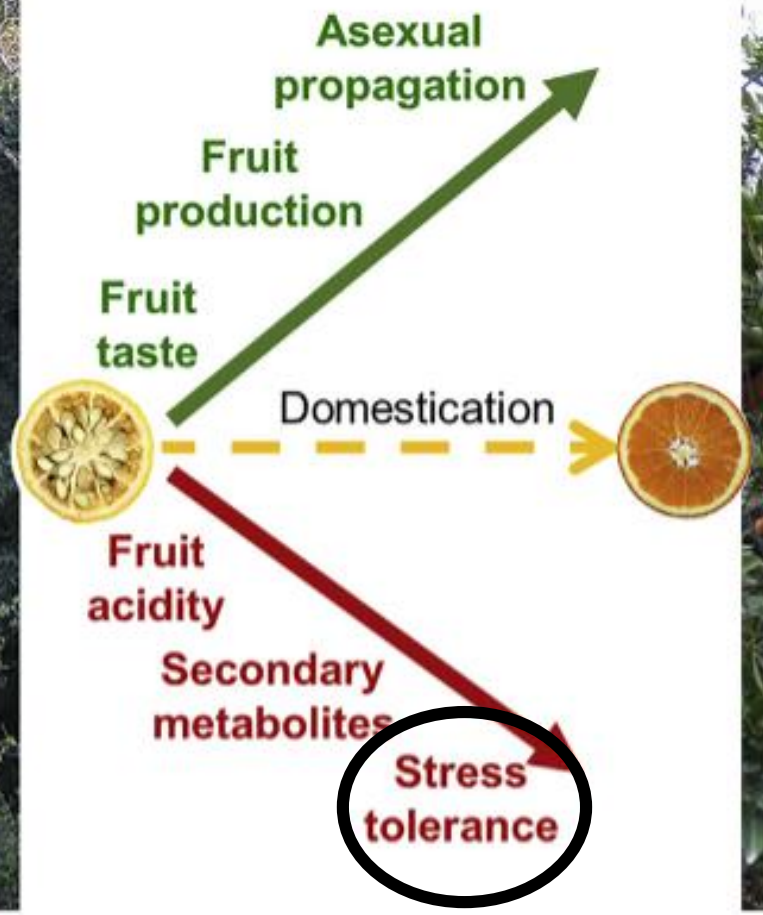
International webinar on citrus greening (HLB): experiences and lessons learned in the prevention and control of the disease and its vector in organic citrus production

February 28th, 2024

This project is supported by the Coop Sustainability Fund.

Citrus domestication

Wild Citrus in Forest

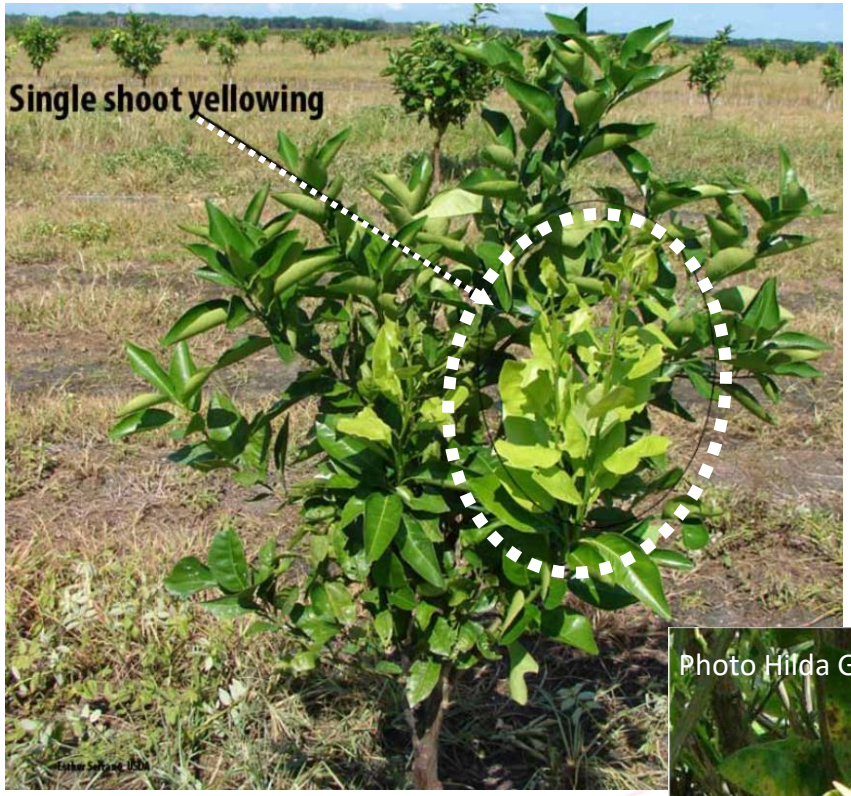


Cultivated Citrus in Orchard

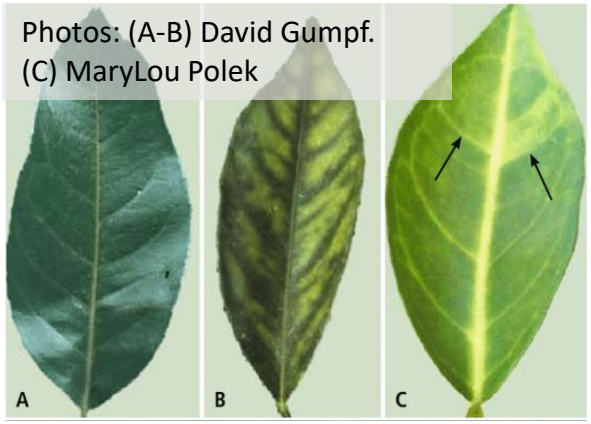
(*Properly*) **Stress resistance**

Rao et al., 2021

Huanglongbing (HLB or Greening): the most severe citrus disease



Symptoms on tree shoot.



(A): Normal leaf. (B): Leaf with zinc deficiency. (C): Yellow area on one side of the midvein and dark green area directly opposite.

Leaf symptom - green islands on *Citrus sinensis* (sweet orange)

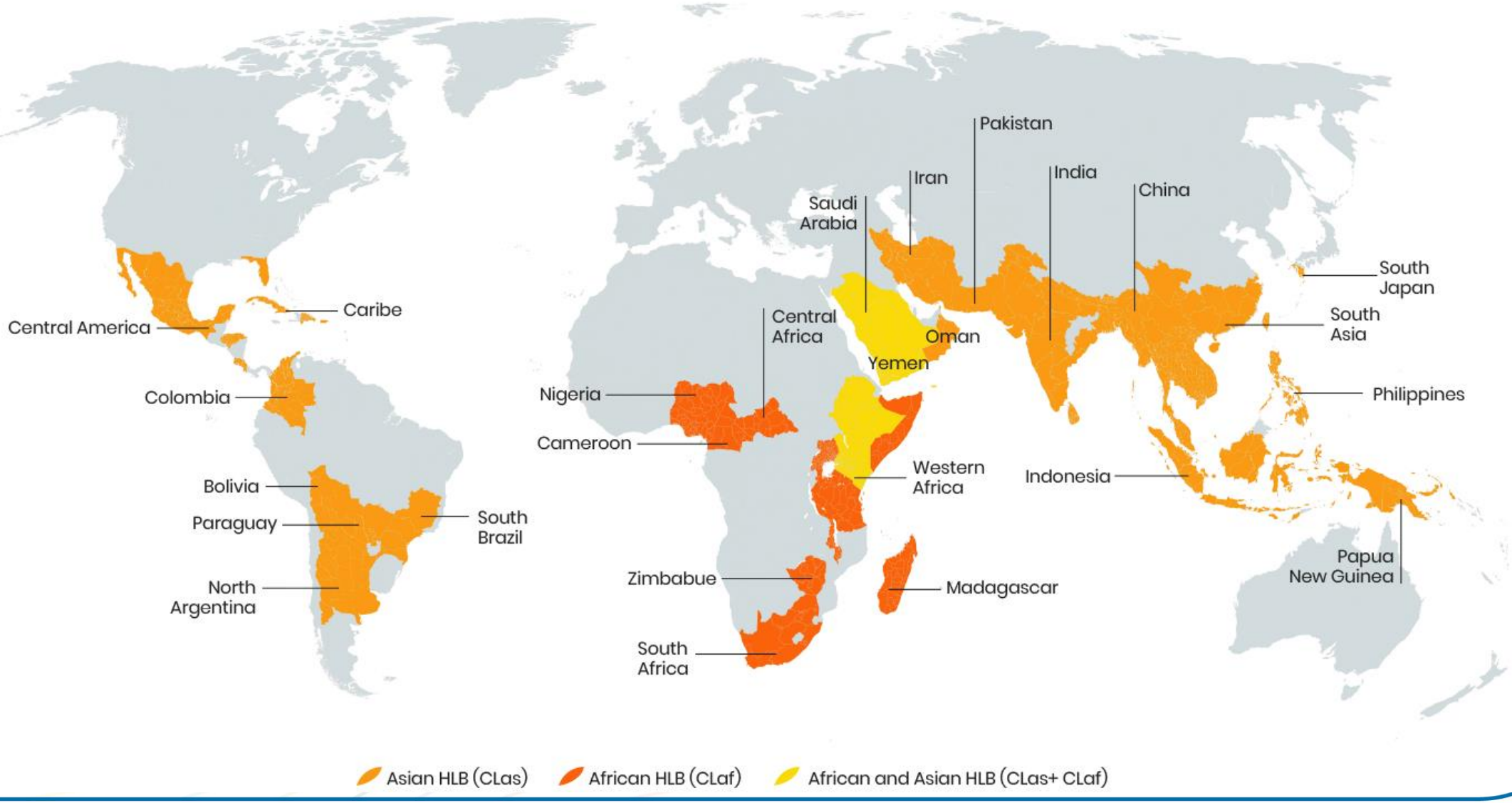
Color inversion (fruit colors opposite of healthy fruit)

Lopsided, poorly colored, aborted seeds.

Small or reduced fruit size.

Reduced fruit quality and yield

HLB in the world: the bacterium Candidatus Liberibacter *asiaticus*, *africanus*, *americanus*



The vectors: *Diaphorina citri* and *Tryoza erytreae*

(…): first detection of the vector
City, year: detection of the disease

Spain & Portugal, XXX (2014)

Cyprus, XXX (2023)

Israel, XXX (2022)

Florida, 2005 (1998)
After 7 years

California, 2012 (2008)
After 4 years

Brazil, 2004 (1942)
After 62 years

D. citri
T. erytreae
D. citri + T. erytreae

Created with mapchart.net ©

CL*as* + *D. citri*
The most aggressive combination

No cure for HLB-positive citrus trees is available (Bovè, 2006).
The **short period between the arrival of vector and the predictable appearance of *C*L** (Bovè, 2006), together with the close sightings in Europe, are helping to raise awareness that urgent and targeted measures are needed.

How difficult is to define conceptually/practically the “resistance to HLB”?

	Name of the taxa (CRC number)	Year 1	Year 2	Year 3	Year 4	Year 6
1	Category 1 (immune: no pathogen detected)					
	Glycosmis pentaphylla	40	40	40	40	40
	Orange berry (3285)	40	40	40	40	40
		40	40	40	40	40
		40	40	40	40	40
2	Category 2 (resistant: transient replication)					
	Eremocitrus glauca hybrid	38	40	40	40	36
	Australian desert lime (4105)	39	40	40	40	D*
		36	35	40	40	40
		40	40	40	40	40
3	Category 3 (tolerant: seedling variation)					
	Microcitrus australasica	30	40	36	31	30
	Australian finger lime (1484)	36	40	40	40	40
		40	35	40	40	40
		40	40	40	40	35
4	Category 4 (tolerant: recovery from infection)					
	Balsamocitrus daweii	40	30	40	40	40
	Uganda powder flask (3514)	40	33	40	40	40
		28	30	D*		
		40	35	40	40	40
5	Category 5 (tolerant: delayed infections)					
	Poncirus trifoliata	40	40	26	28	30
	var. Little-leaf (4007)	37	40	40	40	34
		40	38	30	30	29
		40	40	30	28	29
6	Category 6 (susceptible, retained leaves)					
	Citrus medica	24	32	27	29	30
	‘Diamante citron (3523)	27	26	31	29	33
		28	26	28	30	30
		24	29	29	29	28
7	Category 7 (susceptible, died after four years)					
	Citrus sinensis	40	28	23	23	D**
	Pineapple sweet orange (3858)	40	31	26	26	D**
		40	25	25	D**	
		24	26	D**		
8	Category 8 (susceptible, died in two years)					
	Citrus reticulata	29	D**			
	Unnamed mandarin (3812)	35	24	D**		
		40	25	D**		
		31	22	D**		

Ramadugu et al., 2016

Susceptible
Tolerant
Resistant

TABLE 3 | *‘Candidatus* Liberibacter asiaticus’ infection in the Citrinae genotypes re-evaluated at 24 months after inoculation, as determined through detection of the 16S rDNA by qPCR.

Category	Accession	Freq.[a]	Scion		Rootstock	
			Leaves		Bark	
			Ct avg[b] ± SEM[c]	Log avg[d] ± SEM	Ct avg ± SEM	Log avg ± SEM
1	*Citrus* × *sinensis* ‘Pera’	15/15	25.5 ± 0.7	4.7 ± 0.2	27.9 ± 0.5	4.0 ± 0.1
	C. × *sinensis* ‘Tobias’	09/09	23.8 ± 1.4	5.2 ± 0.4	29.1 ± 0.5	3.7 ± 0.2
2	*M. australasica* ‘True Sanguinea’	03/10*	33.0 ± 0.1	2.5 ± 0.0	30.9 ± 0.7	3.1 ± 0.2
	Faustrimedin hybrid; *C.* × *oliveri*	04/10*	30.3 ± 1.1	3.3 ± 0.3	31.2 ± 1.1	3.0 ± 0.3
	Microcitrus inodora	03/07*	25.3 ± 1.7	4.8 ± 0.5	27.4 ± 1.3	4.2 ± 0.4
	Microcitrus virgata hybrid	00/09*	nd[e]	nd	29.0 ± 0.4	3.7 ± 0.1
3	*Microcitrus warburgiana*	00/06	nd	nd	30.1 ± 0.0	3.4 ± 0.0
	Microcitrus papuana	00/04	nd	nd	31.2 ± 0.8	3.0 ± 0.2
	Microcitrus australis	00/08	nd	nd	30.3 ± 0.1	3.3 ± 0.0
	Microcitrus × *Eremocitrus* hybrid	00/07	nd	nd	31.0 ± 0.9	3.1 ± 0.3
	E. glauca × *C.* × *sinensis* hybrid	00/11	nd	nd	28.5 ± 0.4	3.8 ± 0.1
	Eremocitrus × *Microcitrus* hybrid	00/08	nd	nd	33.4 ± 0.1	2.4 ± 0.0

Alvés et al., 2021; Alvés et al., 2022

In most of cases the «resistance to HLB» depends by the **hybrid or pure origin** of the accession…

How difficult is to define conceptually/practically the “resistance to HLB”?

In the field, in Florida, HLB endemic region

	Name of the taxa (CRC number)	Year 1	Year 2	Year 3	Year 4	Year 6
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		40	25	D**		
		31	22	D**		

Ramadugu et al., 2016

Susceptible
Tolerant
Resistant

Under controlled experimental conditions

Partial resistance to *Diaphorina citri* in ***Poncirus*** (Westbrook et al., 2011; Richardson and Hall, 2013; Hall et al., 2015; Felisberto et al., 2019) and Oceanian *Microcitrus* and *Eremocitrus* species (Eduardo et al., 2022).

In most of cases the «resistance to HLB» depends by the **hybrid or pure origin** of the accession…

… in addition to the **natural or controlled conditions** of the infection.

PreHLB - PREVENTING HLB EPIDEMICS FOR ENSURING CITRUS SURVIVAL IN EUROPE (Horizon 2020, 2018-2023)

9 Countries (24 Institutions)

1. Spain (#10)
2. France (#1)
3. Italy (#3)
4. Portugal (#3)
5. Netherlands (#1)
6. United kingdom (#2)
7. Israel (#1)
8. China (#1)
9. Brazil (#2)

Short term	Medium term	Long term
Preventive measures	Psyllid biology in EU	Identification of resistant genotypes and traits
	Pest risk assesment	
	Interaction between Plat/bacteria/psyllis	
	Chemical, biological, management measures	
	Diagnostic kit	
	Development of effectors/metabolites/biostimulants/biopesticides	

The geographic & phylogenetic distribution of the "resistance" to HLB

Generally, Aurantioideae species **resistant** are more attractive for *D. citri* – but resistant to CL – and can be used in the *trap-and-kill* approaches, applying chemical substances or engeenering to be letal to psillids.

Partial resistance and **susceptibility** to *CLas* start to appear in the species belonging to Citrineae subtribe, being all *Citrus* susceptible to the bacterium and sexual compatible with the genotypes of asias origin.

Diverse rutaceae (Clauseneae tribe) - ornamental

Murraya

Murraya paniculata, *D. citri* host, CLas transient host

Bergera

Bergera *koenigii* *D. citri* host CLas non-host

Glycosmis

Clauseneae tribe

***Bergera koenigii* L.**
***Murraya paniculata* (L.) Jack**

Swinglea glutinosa (Blanco) Merr
Naringi crenulata (Roxb.) Nicolson
Atalantia
Citrus
Fortunella
Poncirus

Citreae tribe
Citrinae subtribe

Commercial edible citrus

Orange
Mandarin
Lime
Lemon
Grapefruit
Pummelo
Hybrid

Diverse rutaceae (Citreae tribe) - rootstocks

Poncirus
Atalantia
Severinia
Fortunella
C. latipes

Eremocitrus glauca (Lindl.) Swingle
Microcitrus australasica (F. Muell) Swing.
Microcitrus australis (Cunn Ex. Mudie) Swing.
Microcitrus inodora (FM Bail) Swing.
Microcitrus papuana Winters
Microcitrus warbugiana (FM Bail) Swing.

All Citrus parents of Oceanean origin are **partially** o **totally resistant** to CL. Alternatively, susceptible traits can be missing because never exposed to Clas and vector.

Rutaceae

Eremocitrus

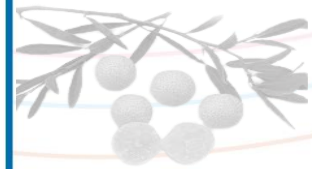
Microcitrus

INTRODUCTION OF RESISTANCE to HLB through traditional and novel breeding approaches.

Diverse rutaceae (Clauseneae tribe) - ornamental



Murraya Bergera Glycosmis




Eremocitrus Microcitrus

No sexually compatible with *Citrus*

Commercial edible citrus

Orange
Mandarin
Lime
Lemon
Grapefruit
Pummelo
Hybrid

Diverse rutaceae (Citreae tribe) - rootstocks



Poncirus
Atalantia
Severinia
Fortunella
C. latipes

INTRODUCTION OF RESISTANCE to HLB through traditional and novel breeding approaches.

New Genomic Techniques

E. glauca x *Citrus*
Microcitrus spp x *Citrus*

Genome editing
of susceptible genes in susceptible varieties.

Cisgenesis
of resistance genes from a resistant «donor» to a susceptible «acceptor» variety.

Commercial edible citrus

Orange
Mandarin
Lime
Lemon
Grapefruit
Pummelo
Hybrid

Diverse rutaceae (Citreae tribe) - rootstocks

Poncirus
Atalantia
Severinia
Fortunella
C. latipes

Rutaceae

Eremocitrus

Microcitrus

YES sexually compatible with *Citrus*

The New Genomic Techniques

Traditional Breeding

- Many economic resources;
- Large field experiments;
- Genotyping & Phenotyping
- Long juvenility

Pollination

Breeding population & gene selection

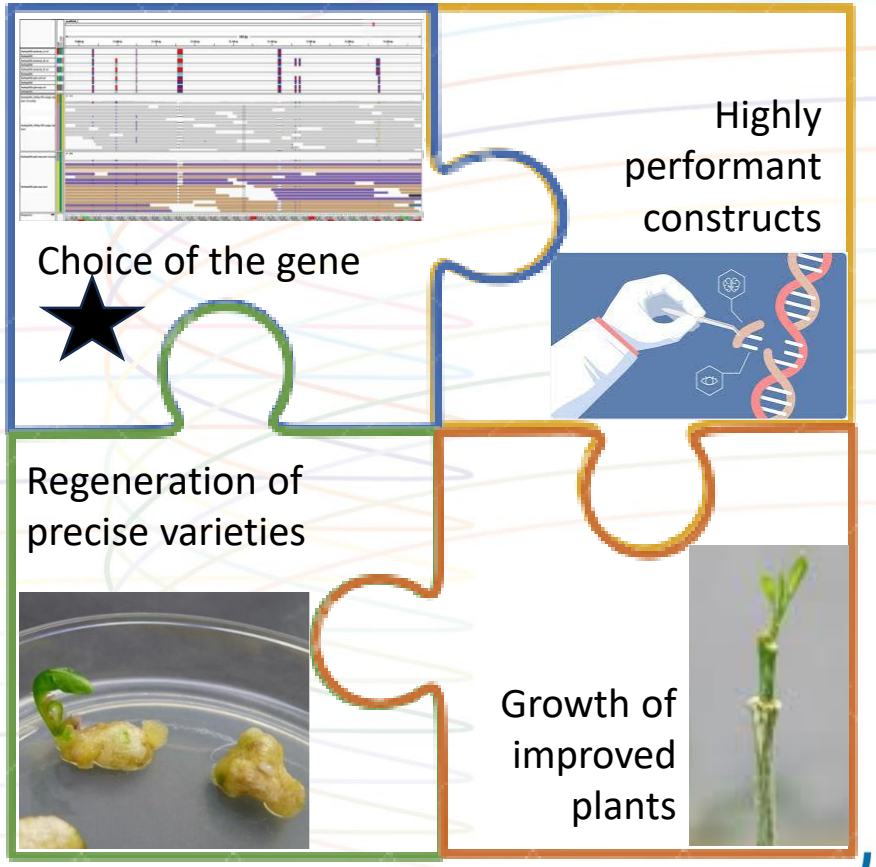
Growth of plants

More than 20 years

New Breeding Technologies

- (Relatively) Few economic resources;
- (Relatively) Rapid result

Less than 5 years



The genomes of *Citrus* and relatives

Relatives

Quality & resistance

S 2011 Pineapple *Citrus sinensis*

S 2013 Valencia *Citrus sinensis*

S 2014 Pineapple *Citrus sinensis*

S 2014 *Clementine Citrus clementine*

S 2017 Cedro *Citrus medica*

S 2017 Pummelo *Citrus maxima*

S 2017 Papeda *Citrus ichangensis*

S 2017 *Atalantia buxifolia*

S 2017 Mandarino Satsuma *Citrus unshiu*

S 2018 Mandarin Mangshan *Citrus reticulata*

S 2019 Kumquat *Fortunella hindsii*

S 2021 Limone *Citrus limon*

S 2021 Valencia **LONG READS**

R 2023 Fingerlime *Microcitrus australis*

S 2023 Arancio dolce **DIAPLOIDE**

S 2023 Arancio dolce **DIPLOIDE**

S 2023 Arancio dolce **PANGENOMA**

R 2023 *Eremocitrus glauca*

Origin & domestication

Diversity & advancement in technology

R Resistant

S Susceptible

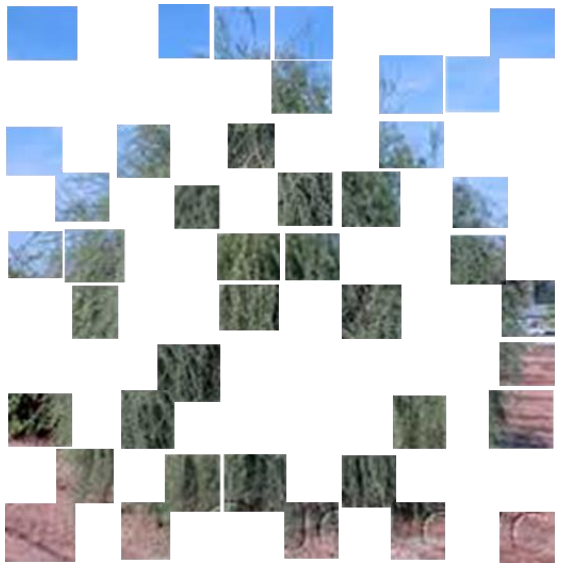
Citrus

The de novo genome of *E. glauca* through an integrated sequencing approach

illumina®

NovaSeq6000 150PE

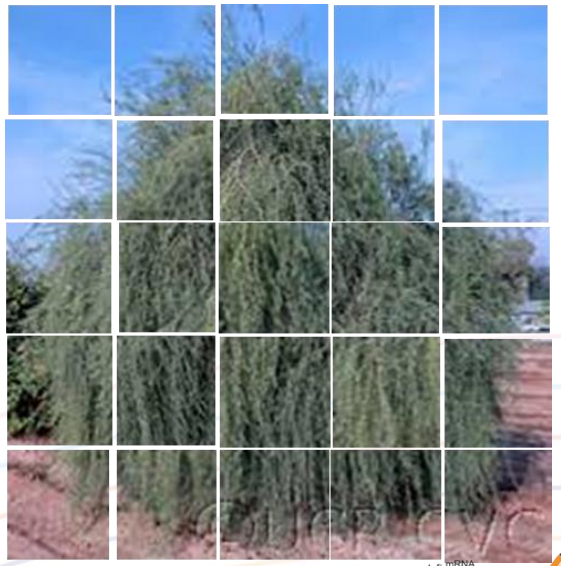
150x

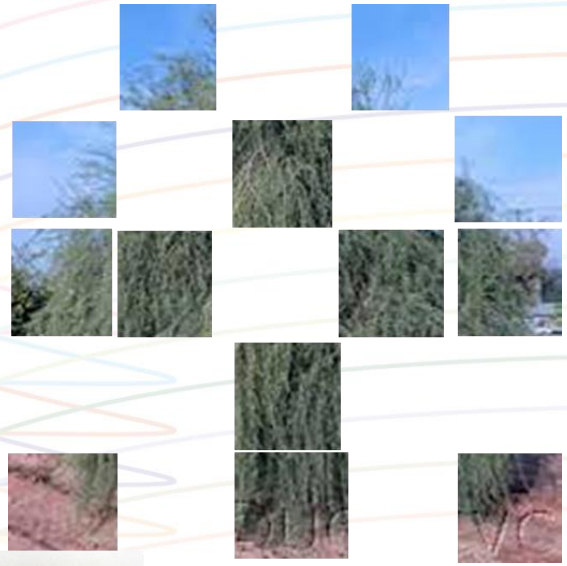


Oxford NANOPORE Technologies

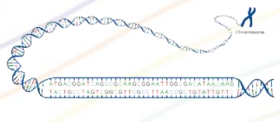
PromethION & MinION

>300x



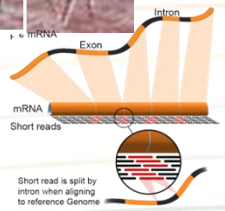


GENOME



	E. glauca (primary)
Number of scaffolds	28
Total size of scaffolds	**270 Mb**
Longest scaffold	43.84 Mb
Shortest scaffold	10 kb
N50 scaffold length	**29.79 Mb**

TRANSCRIPTOME
Gene annotation & prediction



Leaves

Peel

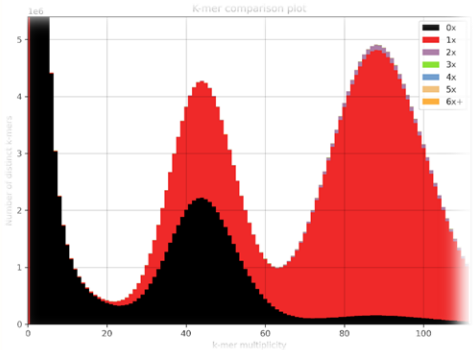
	E. glauca
Genes	42,561
Exons	243,415
Coding regions (CDSs)	233,057
Five prime UTRs	20,736
Introns	200,854
Three prime UTRs	21,113

Cortex

Petals & other parts of flowers

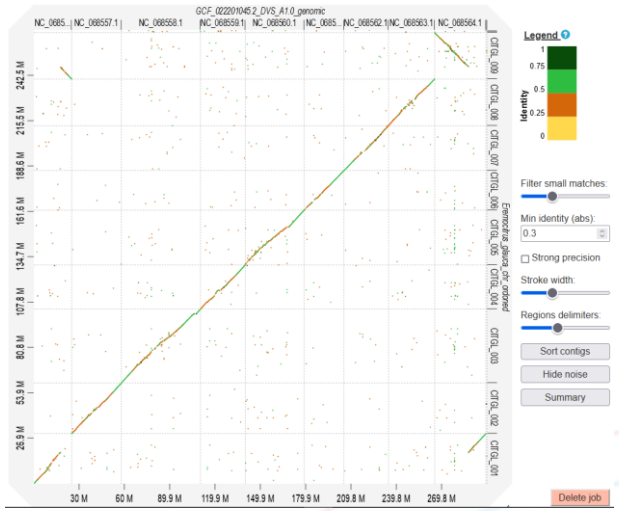
IGATech

High level of synteny with *C. sinensis* diploid genome

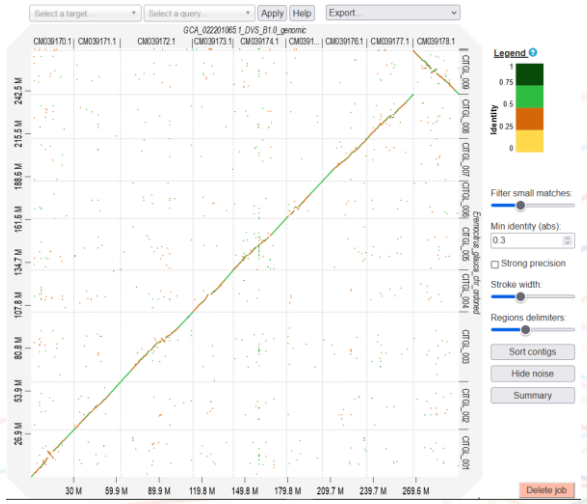


Genome with a high level of heterozygosity

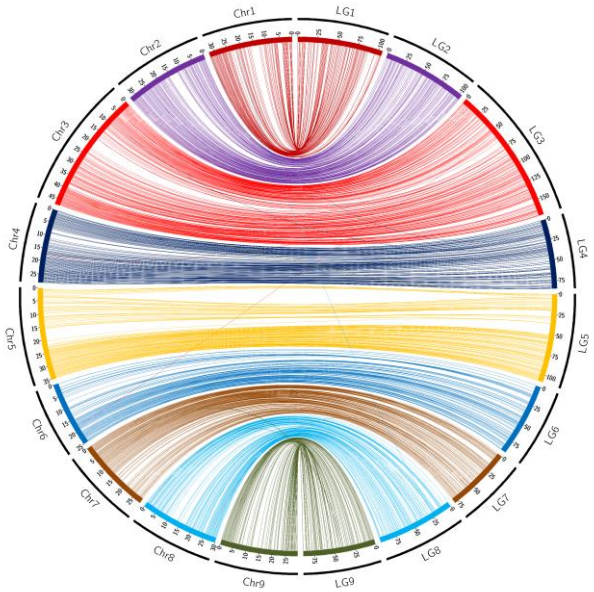
E. glauca ordered versus *C. sinensis* Haplo *C. reticulata*



E. glauca ordered versus *C. sinensis* Haplo *C. maxima*



Genome of high quality compared with other citrus.



Link between physical (Chrs) & genetic map position (LGs)

Thanks to Patrick Ollitrault, CIRAD

A transcriptomic approach to individuate the candidate genes responsible for the resistance of E. glauca

Thanks to M. Alvés

NO bacterium in the **leaves** from ***E. glauca***

YES bacterium in the **bark** from ***E. glauca*** **5 cm above the grafting point with sweet orange (SUSCEPTIBLE) infected (Clas+) budsticks**

Bark and root from the 'Rangpur' lime (SUSCETIBLE) rootstock was full of Las

Most of upgulated genes are:

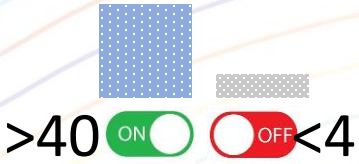
- Positive regulators
- Target of miRNAs
- Cytokinin crosstalk

1 Original dataset

Padj ≤ 0,01
Log2FC >|1|
-9……..+9

2.045 DEGs
(861 Up, 1184 down)

1 Log2FC & normalized counts (TPM)



32 DEGs
(13 Up, 19 down)

2 Filtering for «GO-related stress defence»

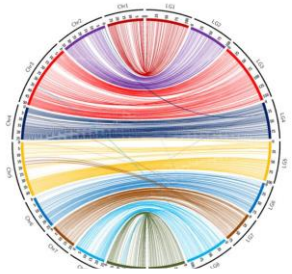
JAR4 JASMONATE-RESISTANT4
(*GO: Induced Systemic Resistance - ISR)*

30 DEGs
(4 Up, 26 down)

3 Filtering for padj of «ISR»

363 DEGs
(98 Up, 265 down)

Final considerations & Take home message



The availability of the genome of ***E. glauca*** is essential to furthermore investigate the resistance to HLB.

It is not yet enough.

crea
Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria

Thanks to

Commissione Europea

PreHLB

crea
Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria

Giusi D'Amante

Angelo Ciacciulli

IGATech

Michele Vidotto

Vera Vendramin

Davide Scaglione

cirad

Patrick Ollitrault

Fundecitrus
CIÊNCIA E SUSTENTABILIDADE PARA A CITRICULTURA

Monica Alvés

Nelson Wulff

ibmcp
Instituto de Biología Molecular y Celular de Plantas

Leandro Peña

Thank you!