

Estudio de genética de poblaciones en *Amaranthus palmeri*

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and J. Torra



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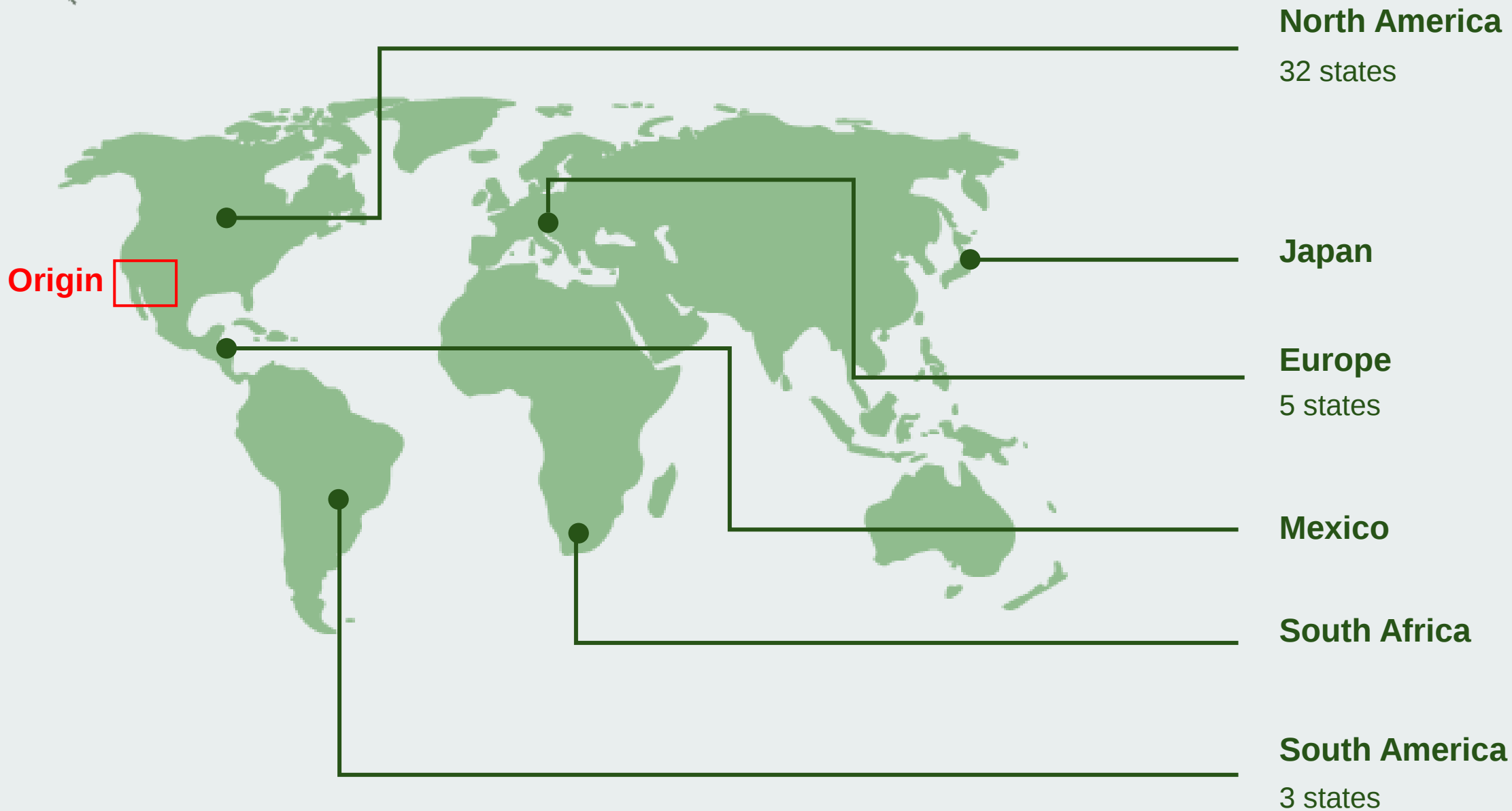


01

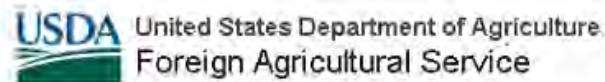
Introduction



Distribution worldwide



Distribution in Spain



Voluntary Report – Voluntary - Public Distribution

Date: June 07, 2023

Report Number: SP2023-0018

Report Name: Spain Soybean and Products Market Outlook

Country: Spain

Post: Madrid

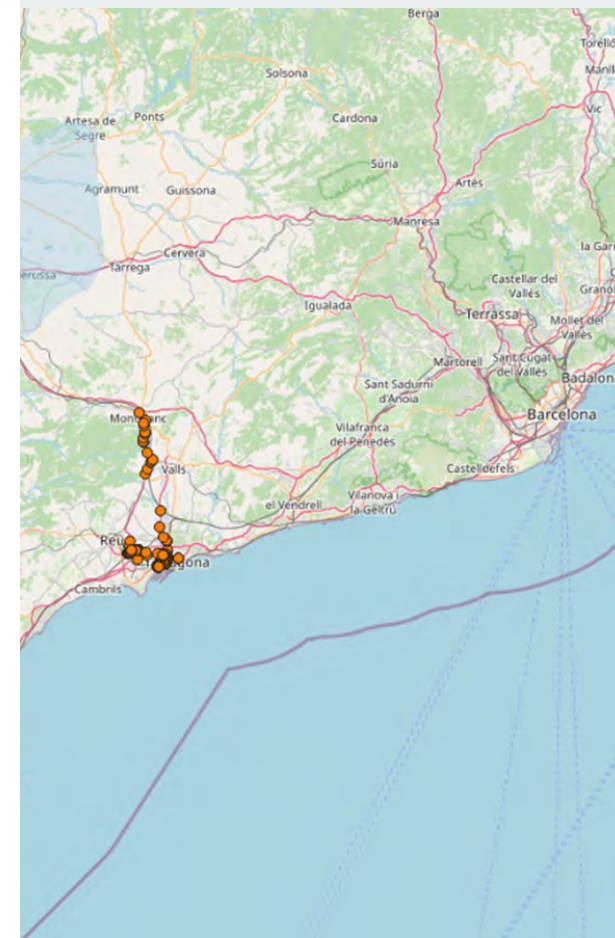
Report Category: Oilseeds and Products

Prepared By: Marta Guerrero

Approved By: Karisha Kuypers

Report Highlights:

This report presents an overview of Spain's soybean and products market. Imports of soybean and soybean products to Spain amount on average to nearly 6 million metric tons (MT) per year. The robust, dynamic, and export-oriented livestock sector drives the country's feed ingredients demand. This combined with the limited in-country oilseeds production makes Spain one of the European Union's (EU) largest oilseeds and products importers. Although Spain's soybean meal import market is largely dominated by Argentina, most of the supply of soybeans for crushing is sourced from Brazil, followed by the United States.



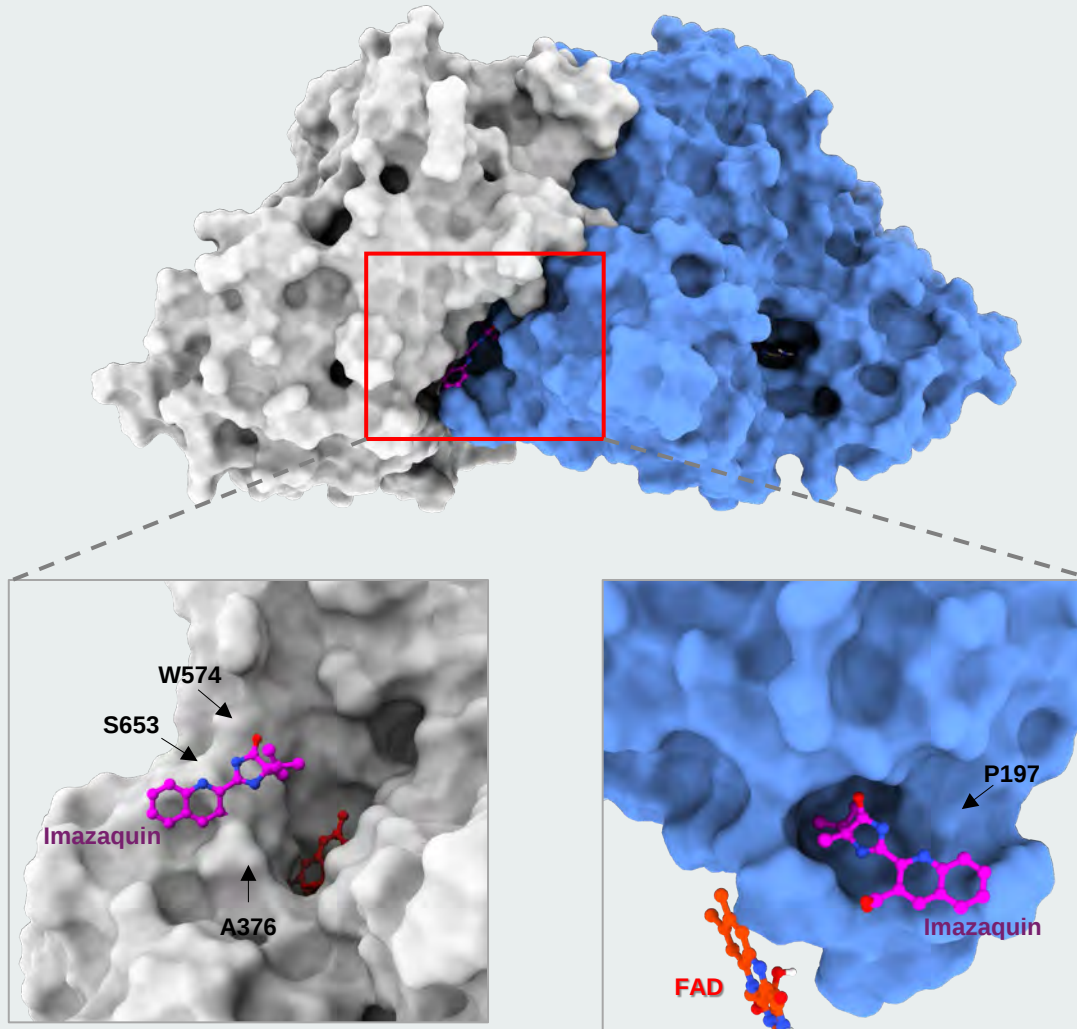




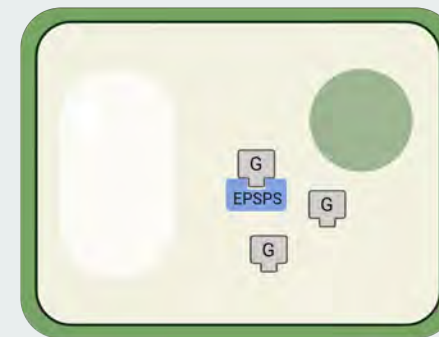
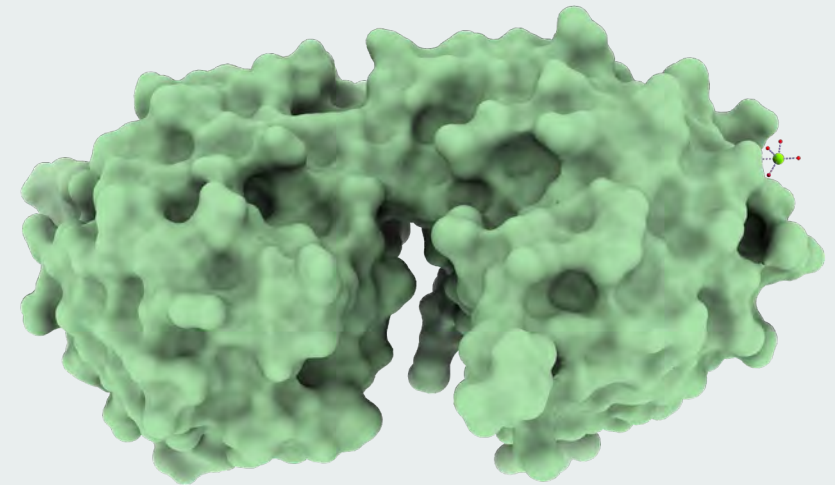


Target-site resistance

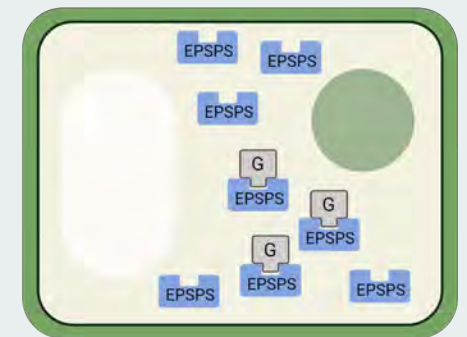
Acetolactate syntase (ALS)



5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)



Sensitive



Resistant

How resistance evolved

Research Article

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Genetic basis and origin of resistance to acetolactate synthase inhibitors in *Amaranthus palmeri* from Spain and Italy

Alfredo Manicardi,^a Laura Scarabel,^{b*} Josep Maria Llenes,^c José María Montull,^a María Dolores Osuna,^d Joel Torra Farré^a and Andrea Milani^b



Abstract

BACKGROUND: *Amaranthus palmeri* is an aggressive annual weed native to the United States, which has become invasive in some European countries. Populations resistant to acetolactate synthase (ALS) inhibitors have been recorded in Spain and Italy, but the evolutionary origin of the resistance traits remains unknown. Bioassays were conducted to identify cross-resistance to ALS inhibitors and a haplotype-based genetic approach was used to elucidate the origin and distribution of resistance in both countries.

RESULTS: *Amaranthus palmeri* populations were resistant to thifensulfuron-methyl and imazamox, and the 574-Leu mutant ALS allele was found to be the main cause of resistance among them. In two Spanish populations, 376-Glu and 197-Thr mutant ALS alleles were also found. The haplotype analyses revealed the presence of two and four distinct 574-Leu mutant haplotypes in the Italian and Spanish populations, respectively. None was common to both countries, but some mutant haplotypes were shared between geographically close populations or between populations more than 100 km apart. Wide genetic diversity was found in two very close Spanish populations.

CONCLUSION: ALS-resistant *A. palmeri* populations were introduced to Italy and Spain from outside Europe. Populations from both countries have different evolutionary histories and originate from independent introduction events. ALS resistance then spread over short and long distances by seed dispersal. The higher number and genetic diversity among mutant haplotypes from the Spanish populations indicated recurrent invasions. The implementation of control tactics to limit seed dispersal and the establishment of *A. palmeri* is recommended in both countries.

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CENTRO DE INVESTIGACIONES
CIENTÍFICAS Y TECNOLÓGICAS
DE EXTREMADURA

Analysis of multiple-herbicide resistant *Amaranthus palmeri* populations from Spain points to an introduction of the eccDNA from America

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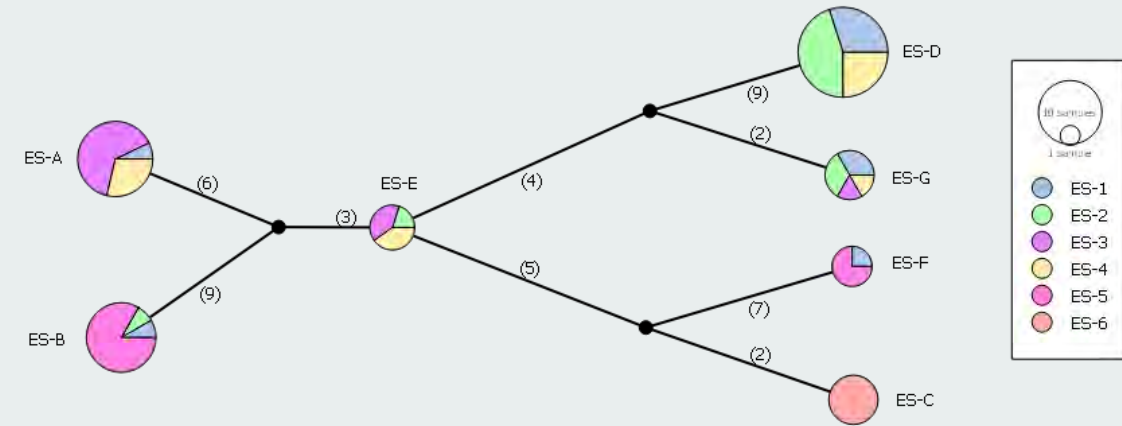
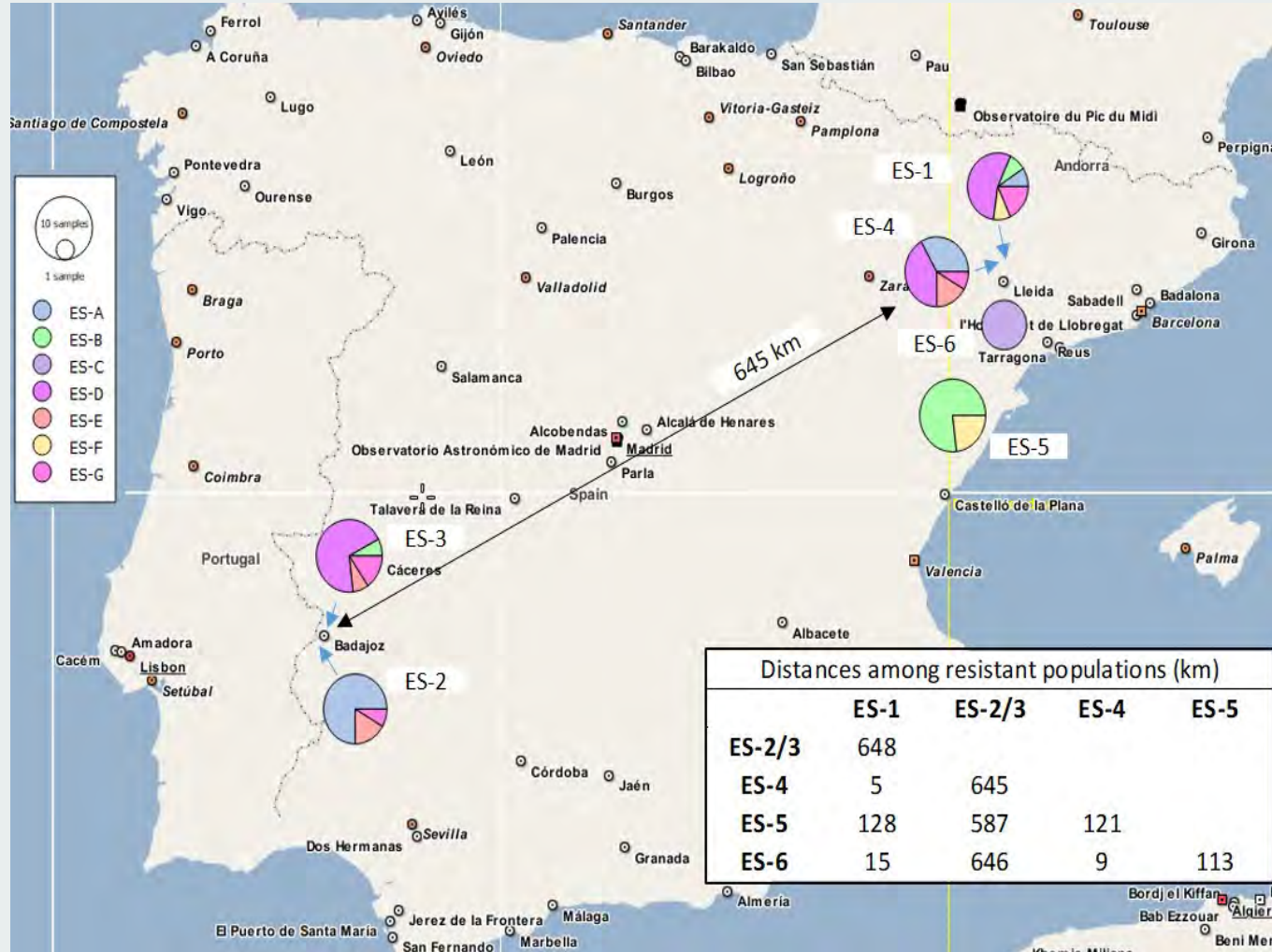
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Abstract

BACKGROUND: The herbicide-resistant invasive weed species *Amaranthus palmeri* threatens agricultural production and native plant ecology in Spain, as well as in other European countries. Understanding whether resistance alleles evolve *in situ* or *ex situ* in resistant populations is crucial for their control. Bioassays were performed on two Spanish *A. palmeri* populations to characterize multiple resistance to ALS- and EPSPS-inhibiting herbicides. Additionally, molecular analysis on extra-chromosomal circular DNA (eccDNA) was performed to determine whether glyphosate resistance evolved *in situ* by selective pressure or was introduced by seed.

RESULTS: Both populations exhibit multiple resistance mechanisms to ALS and EPSPS-inhibiting herbicides, conferring cross-resistance. Eight different ALS allele mutations were identified in resistant plants, including Pro-197-Ile, previously reported only in one species. Glyphosate resistance in the two populations is due to gene duplication mediated by eccDNA. Spanish and North American eccDNAs showed complete identity, with no single nucleotide polymorphisms (SNPs) found between the partial analyzed sequences of non-coding regions.

Evolution of ALS resistance



02

Glyphosate resistance evolution



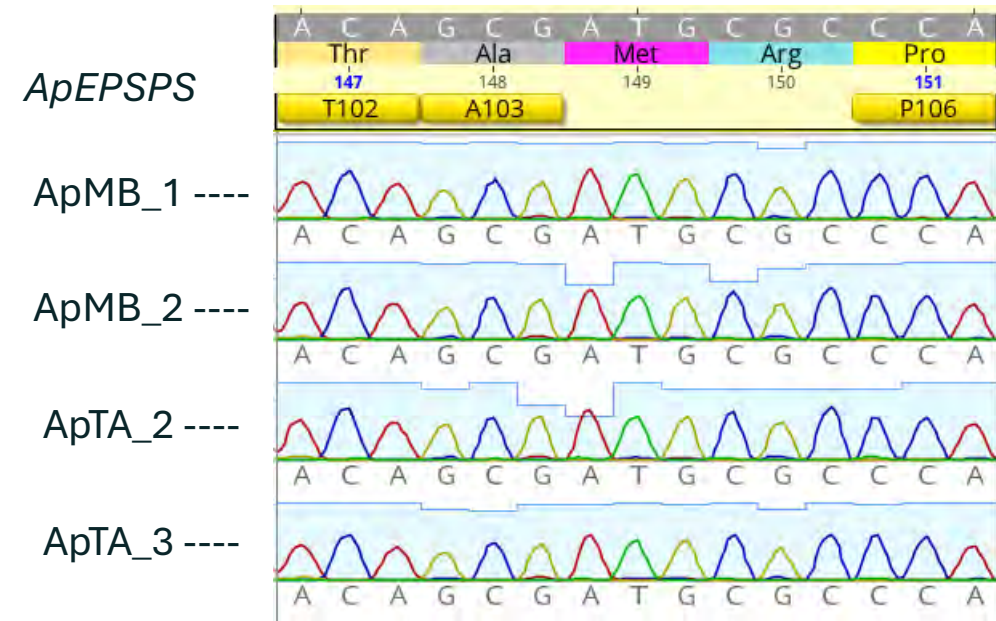


Resistant mechanism

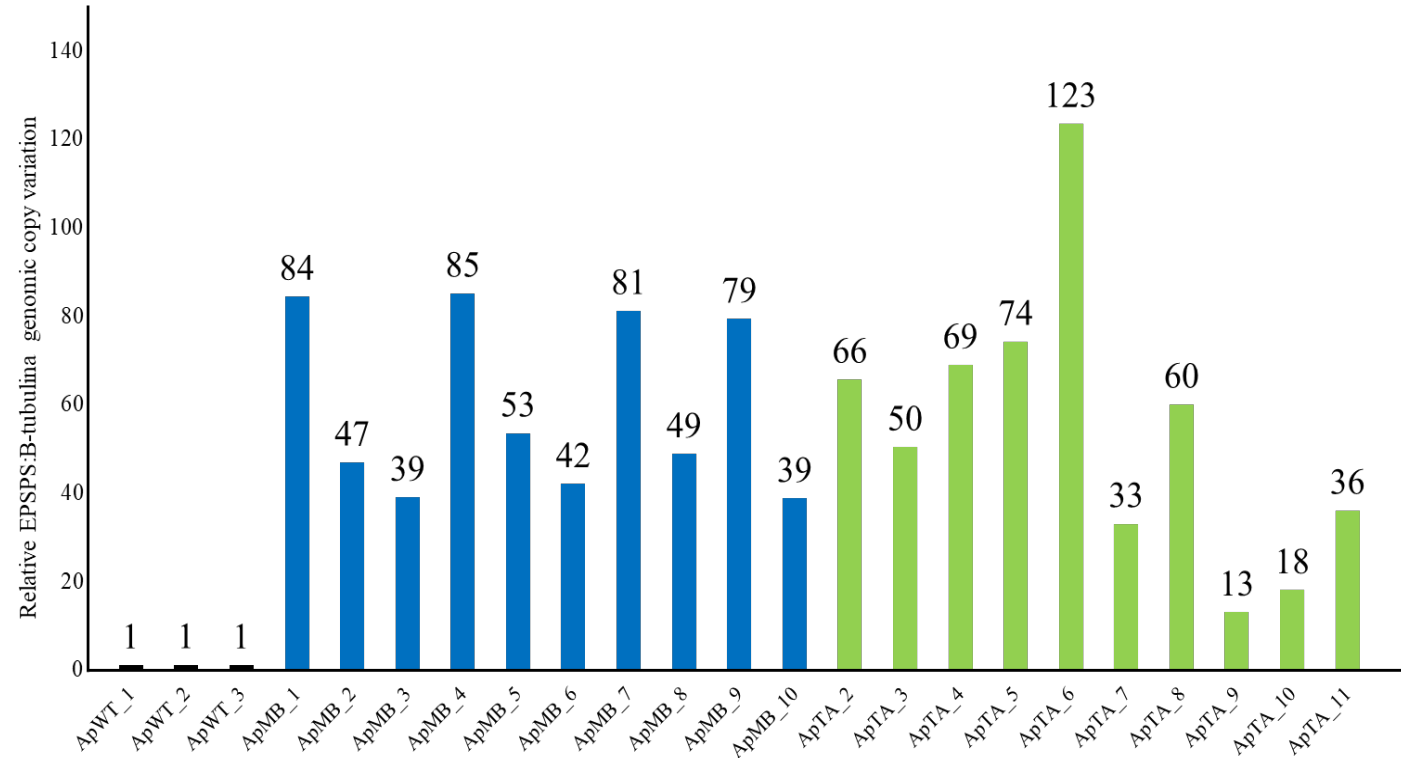
EPSPS inhibitor



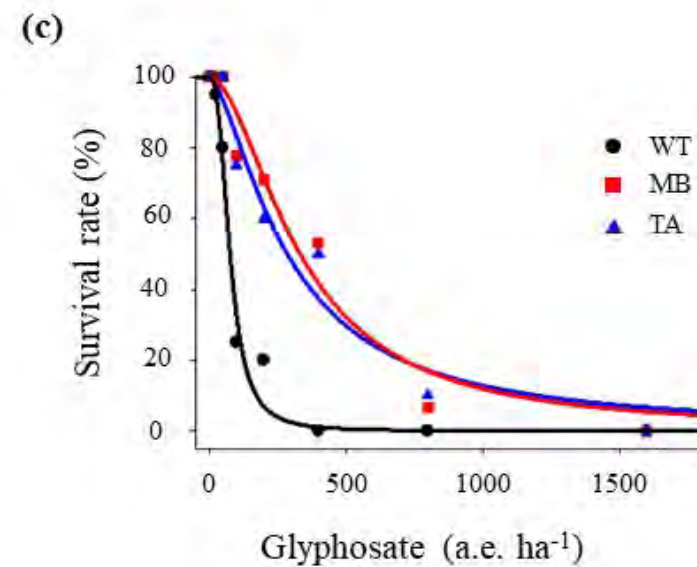
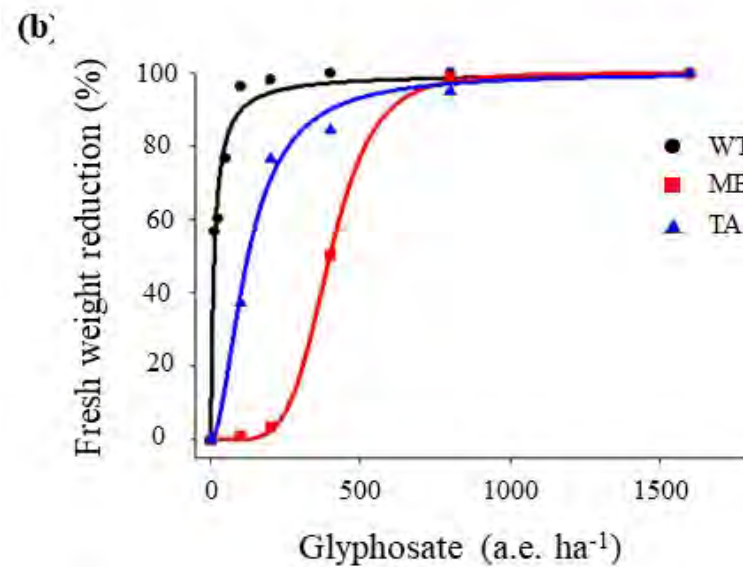
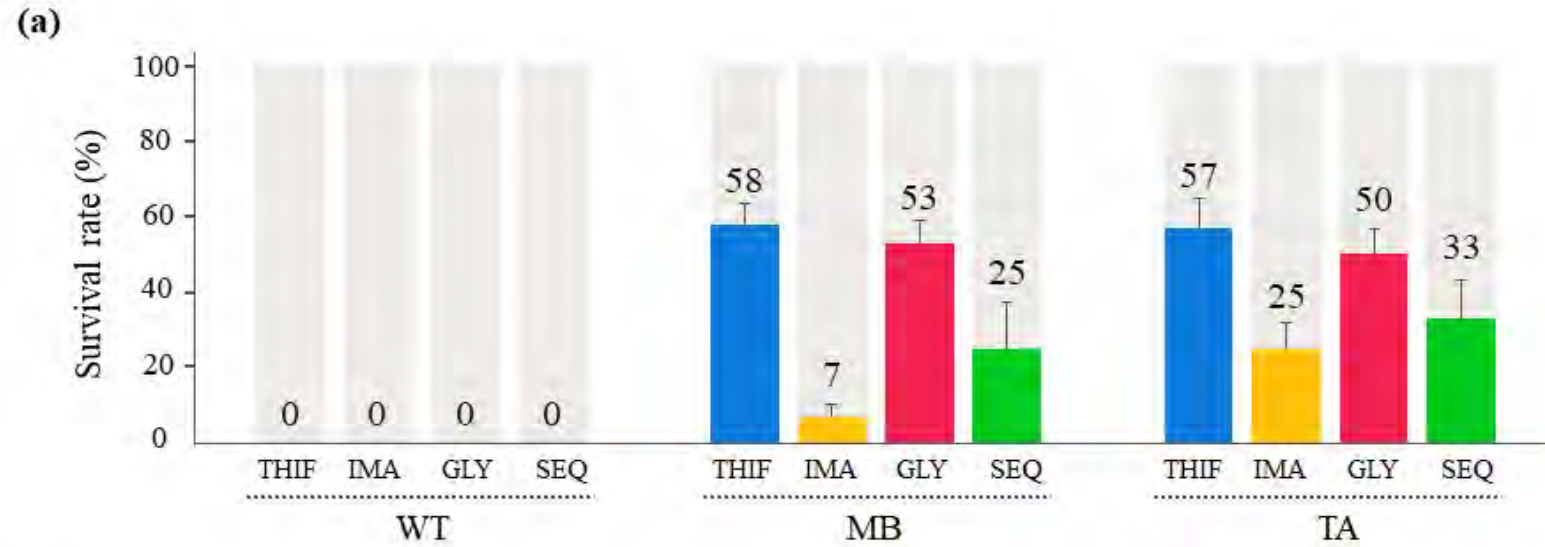
EPSPS partial amplification



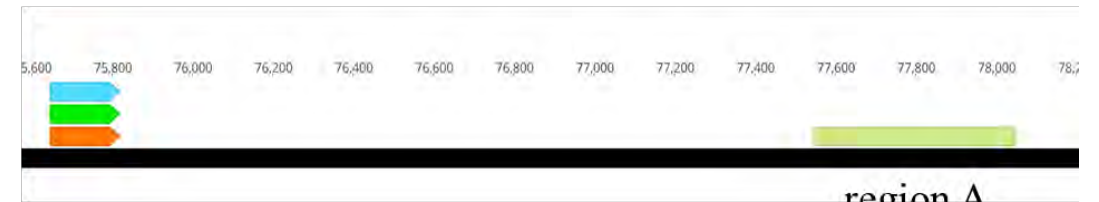
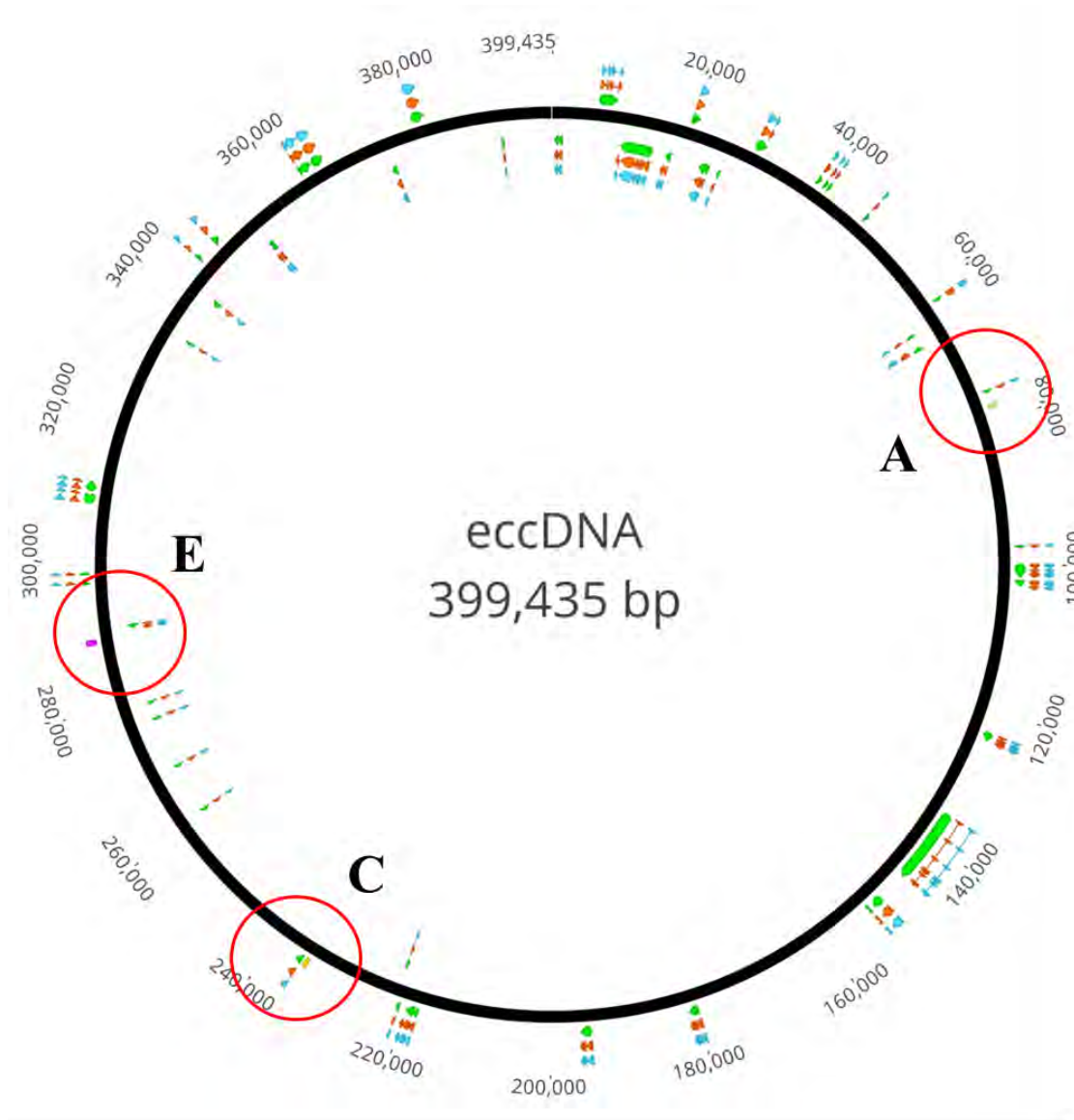
EPSPS gene over expression



Multiple resistance



Glyphosate resistance evolution



region A
1,635 bp



region C
1,513 bp



region E
987 bp



EPSPS sequencing

Consensus identity (%)



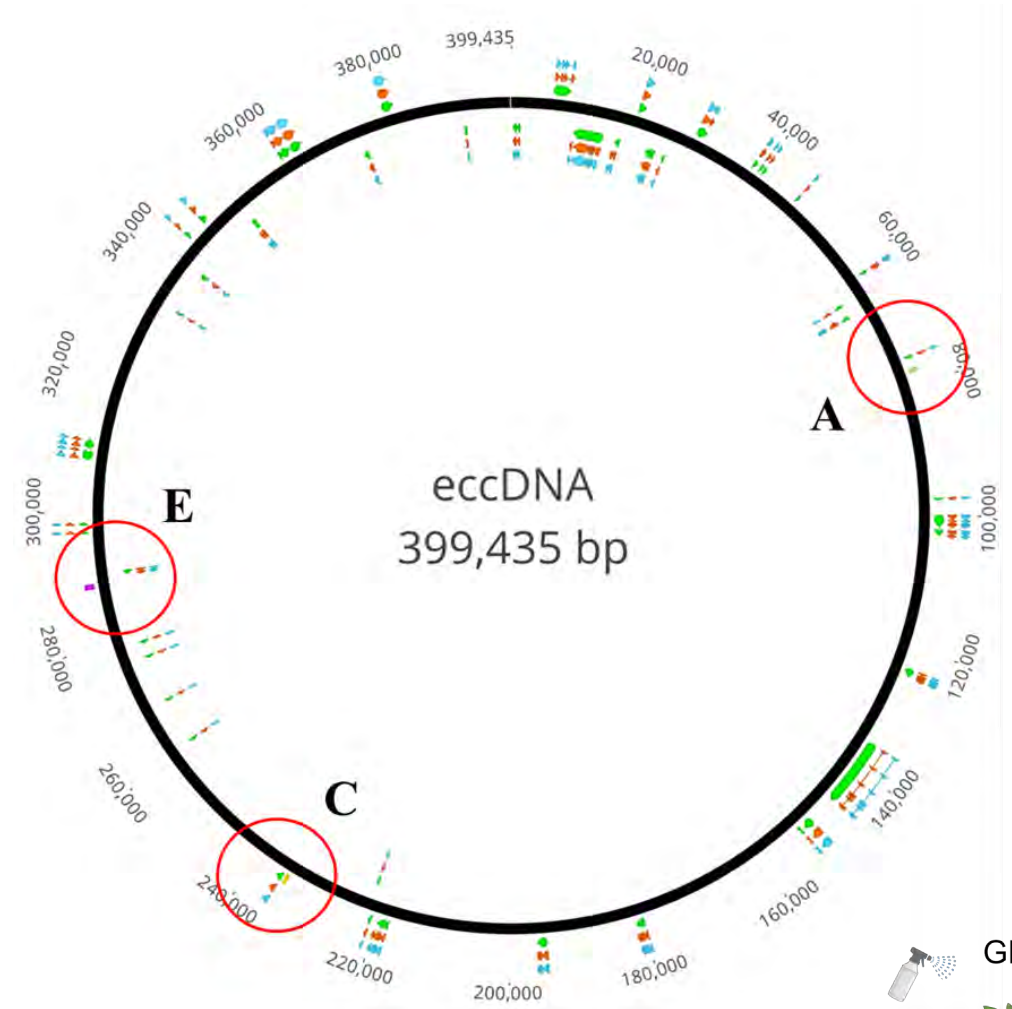
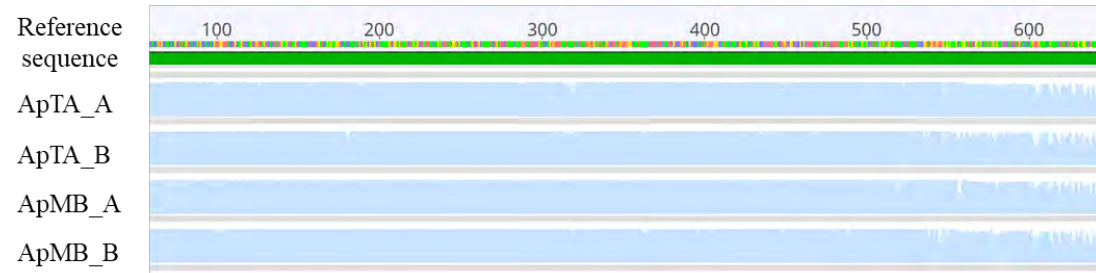
region A



region C



region E





Multiple resistance



MontBlanc (MB)

Herbicide treatment	Sample ID	Pro-197-Ser	Pro-197-Thr	Asp-376-Glu	Trp-574-Leu
GLY	MB_A1				
	MB_A2		✓		
	MB_A3			✓	
	MB_A4		✓	✓	
	MB_A5				✓
	MB_B1	✓			
	MB_B2		✓	✓	
	MB_B3		✓		
	MB_B4		✓	✓	
	MB_B5	✓			
Total		2/10	5/10	4/20	1/10

Tarragona (TA)

Herbicide treatment	Sample ID	Pro-197-Ser	Pro-197-Thr	Pro-197-Ile	Pro-197-Ala	Trp-574-Leu
GLY	TA_A1					✓
	TA_A3	✓				✓
	TA_A4					✓
	TA_A5					
	TA_A6			✓		
	TA_B1				✓	
	TA_B2	✓				
	TA_B3		✓			
	TA_B4			✓		
	TA_B5	✓				
Total		3/10	1/10	2/10	1/10	3/10



03

Whole genome sequencing





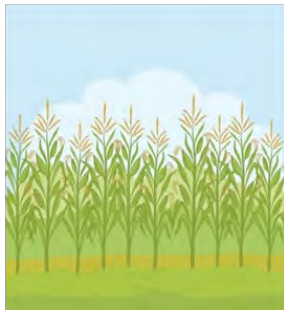
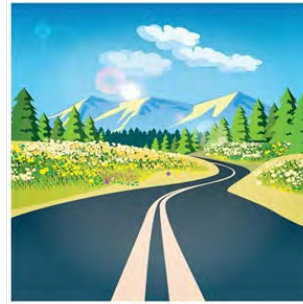
Objectives



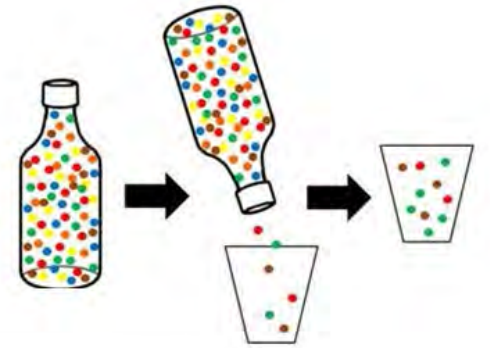
1. Population structure



2. Adaptation and genetic variation

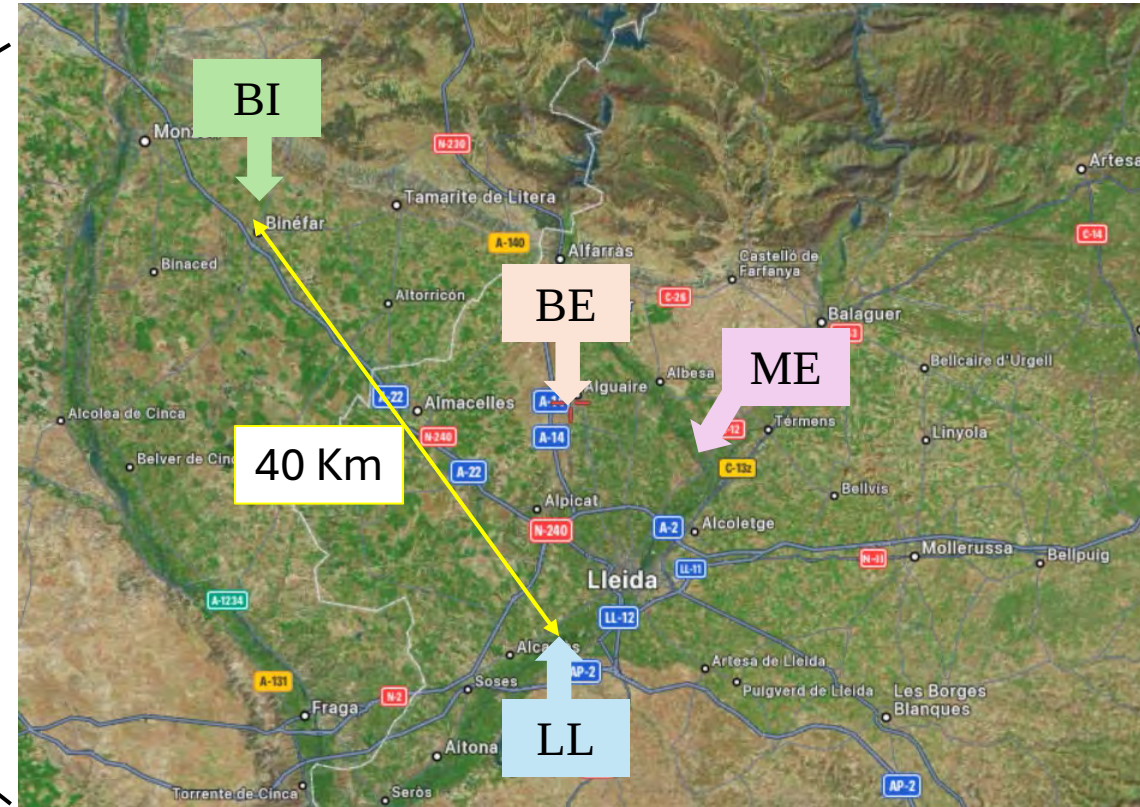
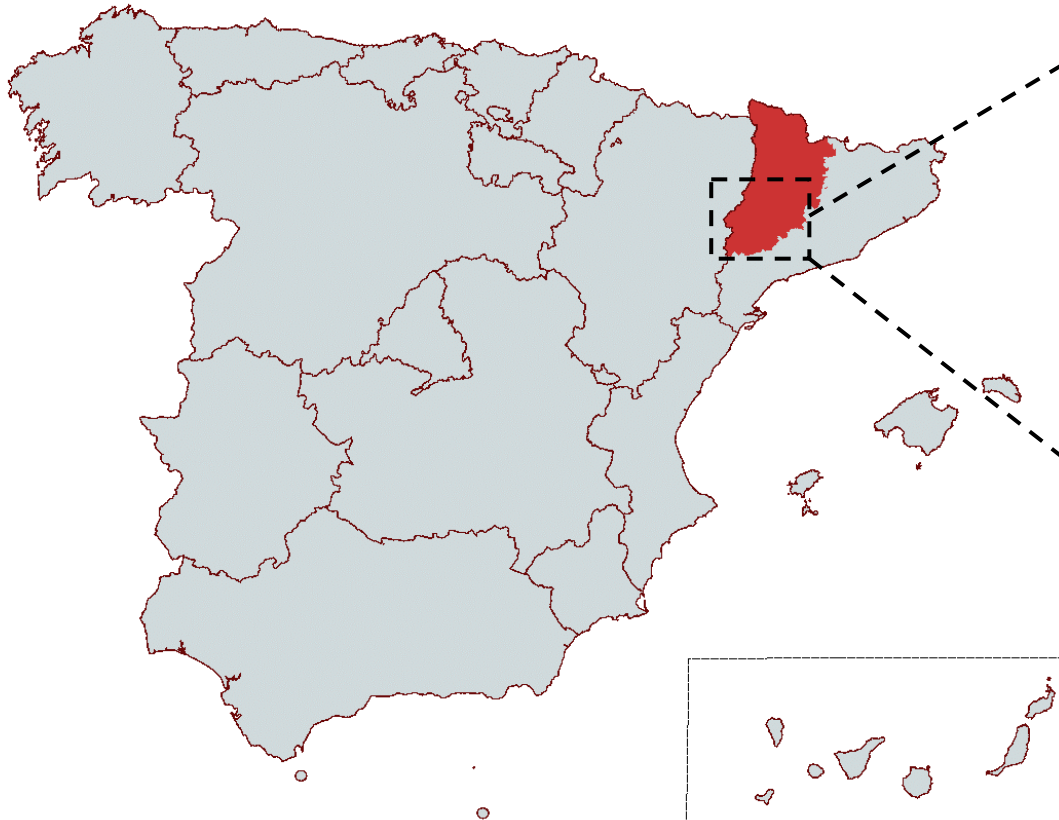


3. Gene Flow (H.R.)



Plant material

Province of Lleida, Catalunya, Spain



- **Collection site:** corn field (BE), roadside (LL and BI) and mais field edge (ME);
- **Herbicide resistance:** BE, BI and LL cross resistance to Thifensulfuron methyl and imazamox, ME resistant to Thifensulfuron methyl (Torra et al., 2020, *Agronomy*; Manicardi et al. 2023, *PMS*)



Plant material

BE



20X

BI



20X

LL



20X

ME



20X

Sow 10-20 sedes/mother plant

DNA extraction from 25 plant/population

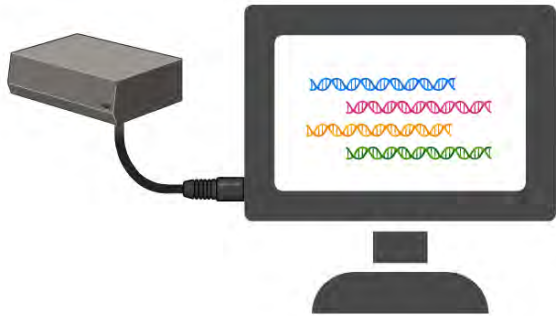
Whole Genome Sequencing, library preparation



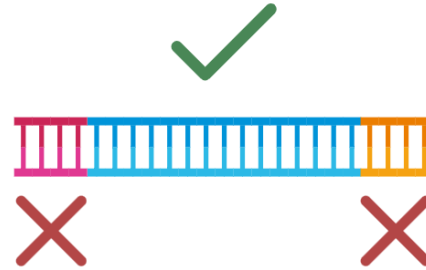
Work steps



1. Download Raw data



2. Select HQ data (trimming)

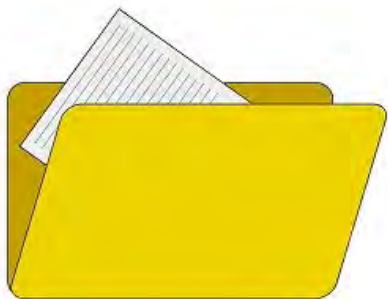


3. Alignment

SeqA: → **C A T - T C A - C**
SeqB: → **C - T C G C A G C**

Vertical pink lines connect the 'C' at position 1, 'A' at position 2, 'T' at position 3, 'C' at position 5, 'A' at position 6, and 'C' at position 7 of SeqA to the corresponding positions in SeqB, illustrating the alignment process.

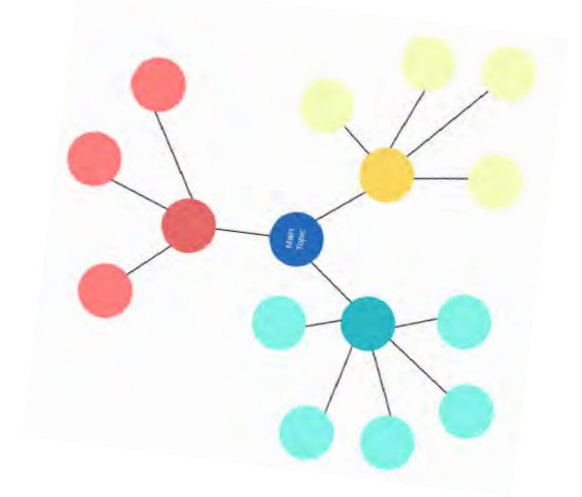
4. Variants call



5. Genomic datastore



6. Static analysis





Variants calling (SNPs)



Reference sequence * * * *

A A A C T G C T A....

Plant A



A A A **T** T G C T A....

Plant B



A A A C T G C T **C**....

Plant C



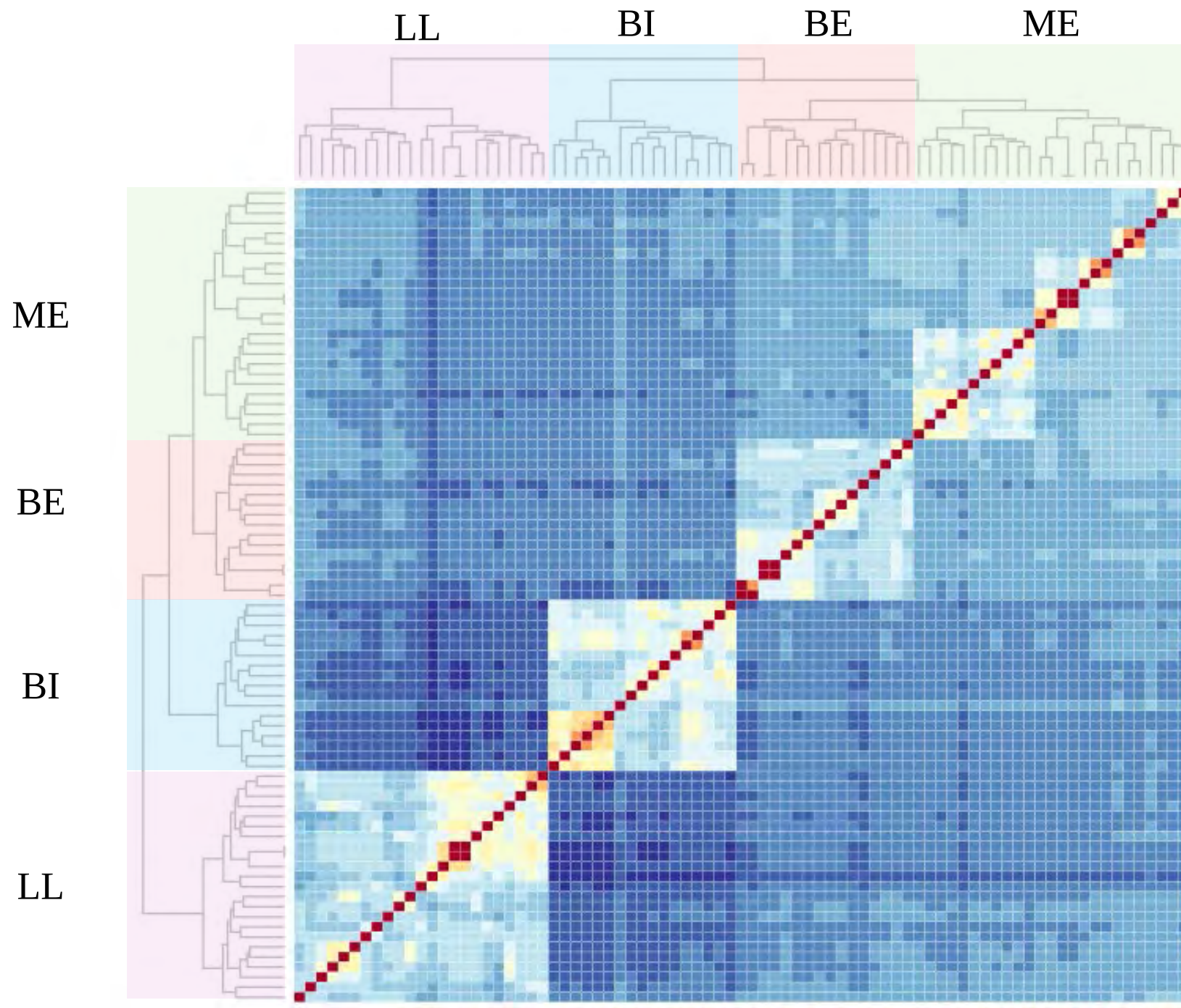
A A A C T G C T **G**....

Plant D



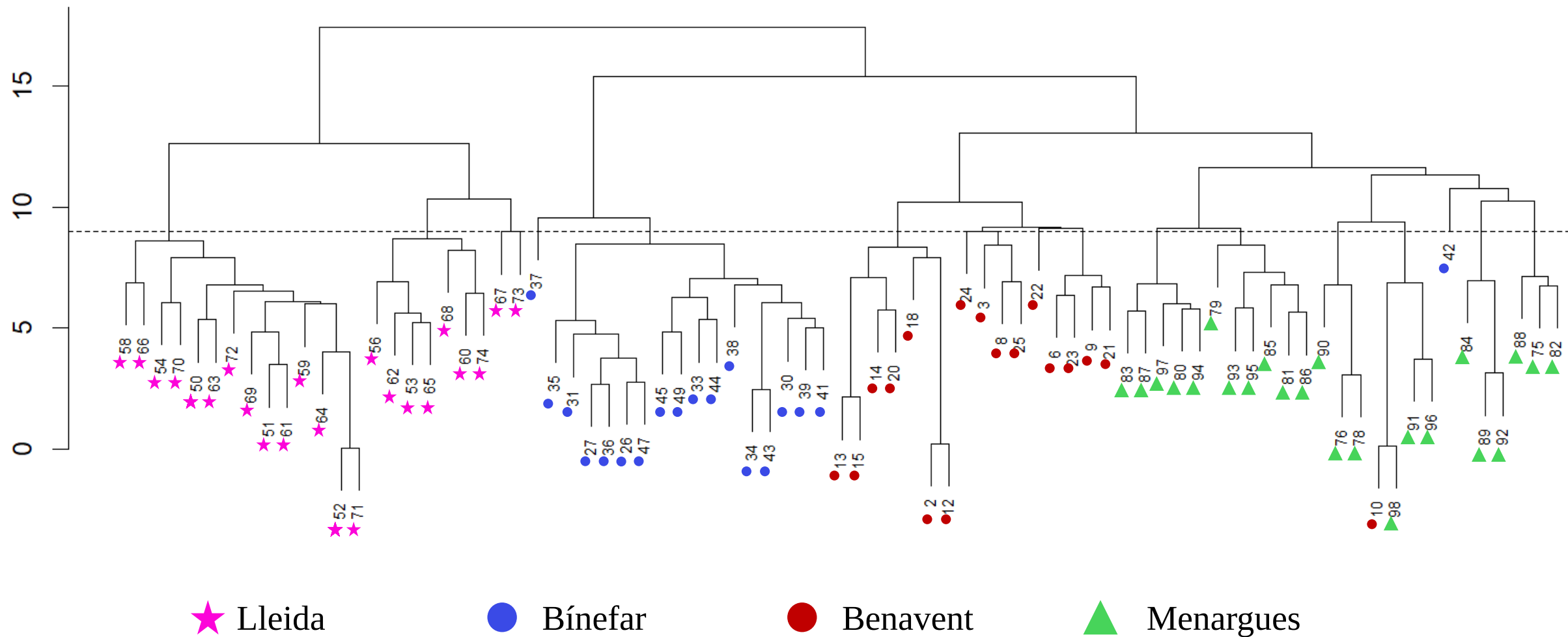
G A **C** C T G C T A....

CHROM	COMMOM	BE	BI	LL	ME
Chr00A	452	0	0	0	0
Chr01A	90269	4	798	1551	55
Chr02A	61757	3	469	1838	19
Chr03A	51660	15	161	1056	32
Chr04A	59257	23	141	1852	59
Chr05A	53679	674	1146	1338	6
Chr06A	39585	65	710	944	36
Chr07A	63510	46	214	255	45
Chr08A	55438	24	104	824	13
Chr09A	52135	21	965	1010	24
Chr10A	43621	3	658	661	85
Chr11A	45151	20	134	198	62
Chr12A	31633	13	371	512	12
Chr13A	43299	4	181	1241	3
Chr14A	39141	44	69	91	12
Chr15A	44184	15	416	474	85
Chr16A	41252	31	29	995	32
Chr17A	39360	4	70	903	85
	855383	1009	6636	15743	665



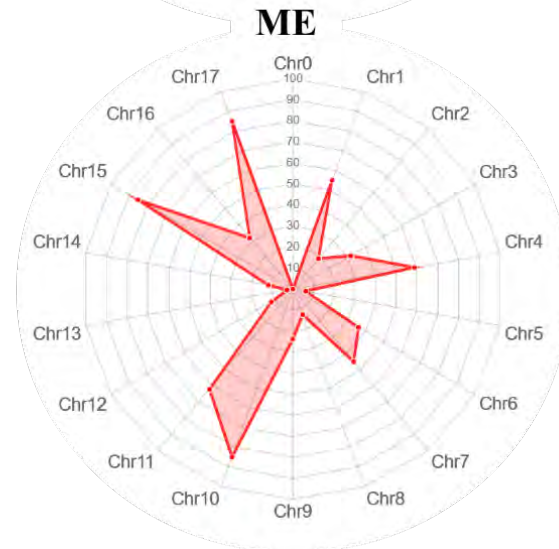
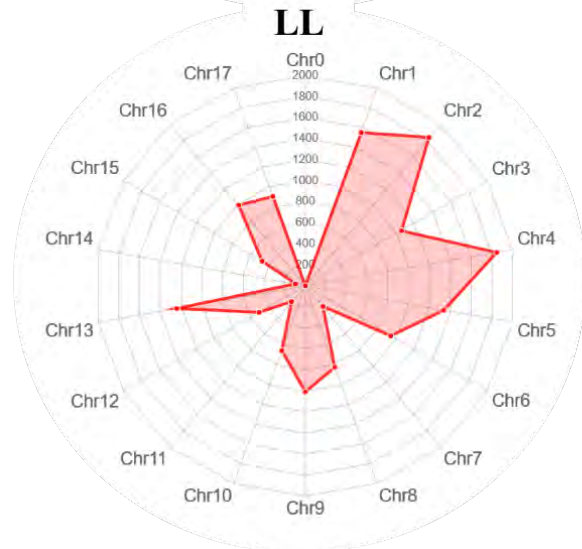
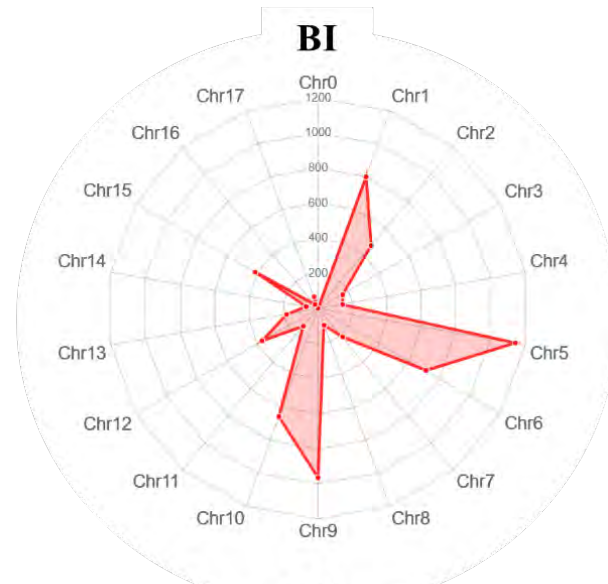
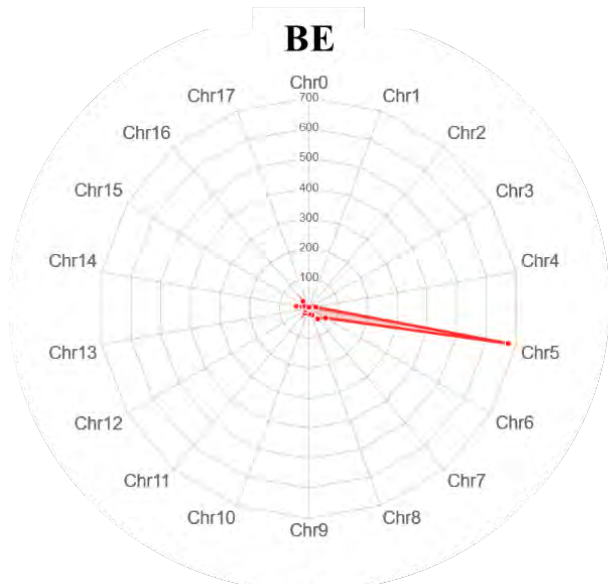


Populations structure

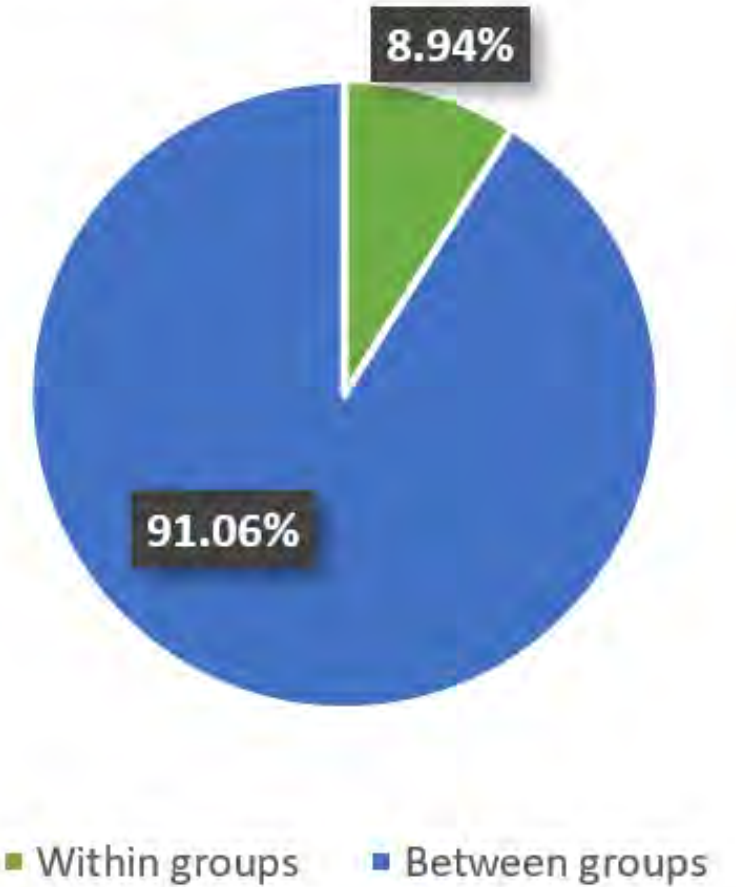




Genetic variation



Total genetic variation



04

Take-home message



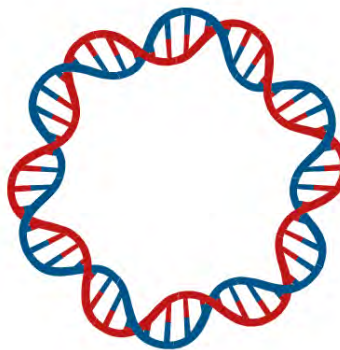
Take-home message

ALS mutations evolution



- Evolved in the country of origin
- Is spread by pollen and seeds
- No sign of independent evolutionary event *in situ*

eccDNA evolution



- Unique evolutionary origin of glyphosate resistance
- Gene flow contribute equal or greater than independent evolutionary event

Population structure



- Genetic differences within populations is greater than between populations
- Suspected multiple origin of introduction
- 9% of difference between Spanish and American genome





EWRS 2025

20th European Weed Research Society Symposium

1-4 July 2025 Lleida (Catalonia, Spain)

Joint approaches for sustainable weed management

