RECENT DEVELOPMENTS IN BREEDING FOR RESISTANCE TO SUNFLOWER BROOMRAPE

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Orobanche-Helianthus co-existence

The genus *Helianthus* is native to North America, where cultivated sunflower was domesticated

***Orobanche cumana* is a holoparasitic plant from the Old World, mainly distributed around the Black Sea and the Caspian Sea. In the wild, it mainly parasitizes on wild Asteraceae, e.g. *Artemisia maritima*.**

Orobanche-Helianthus co-existence

Sunflower was introduced in the Old World in the 16th century.

Parasitization of *O. cumana* (named in many old papers as *O. cernua*) was first detected in the 1890s in the Saratov Oblast of Russia, in the natural distribution area of the parasite.

Orobanche-Helianthus co-existence

As broomrape became a problem for sunflower production in the area, resistance breeding was successfully conducted resulting in the first resistance line developed in 1918.

That initial resistance was surpassed as early as in 1926.

From that time, the history of sunflower breeding has been driven to a large extent by the appearance of new populations of *O. cumana* that overcome all known resistance sources.

Orobanche-Helianthus co-existence

This responds to the singularity of the parasitic system sunflower-*O. cumana*, which in general follows a gene-for-gene interaction in which a resistant *Or* gene interacts with a dominant avirulence gene *Avr* in the parasite.

This was first demonstrated by Antonova et al. (1996) at the physiological level and Rodríguez–Ojeda et al. (2013) at the genetic level.

According to this hypothesis, each dominant *Or* gene identified in sunflower so far (*Or1*-*Or7*, Or_{deb2}) should have its counterpart dominant avirulence gene in *O. cumana*. This was demonstrated by Rodríguez-Ojeda et al. for *Or5*.

Orobanche-Helianthus co-existence

After more than a century of co-existence, the situation is more complex than even before.

Unlike previous situations in which racial situation was more or less clear, the current situation is becoming more complex.

Since the racial situation is becoming more complex, the main questions for the breeder are:

- **For which race am I developing resistance?**
- **Where can I use my resistant materials?**
- **For how long?**

Nowadays, these questions have difficult answers

Causes of broomrape variability

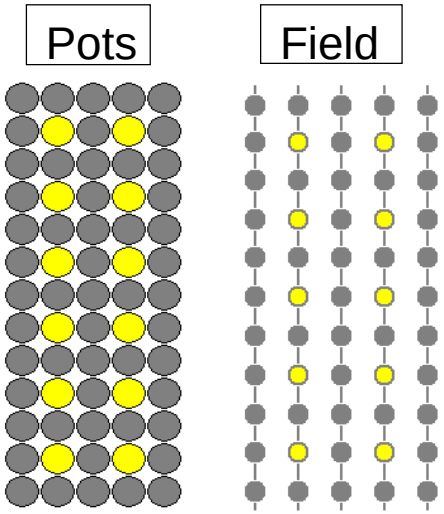
Causes of broomrape variability

Traditionally, broomrape variability has been attributed only to mutation

This was based on the large amount of seeds produced by every single plant and to studies pointing to self-fertilization

However, recent studies have shown the existence of a certain level of cross-fertilization and genetic recombination between individuals of different gene pools.

Causes of broomrape variability



Using the unpigmented plant trait as a visual marker in pot and field experiments

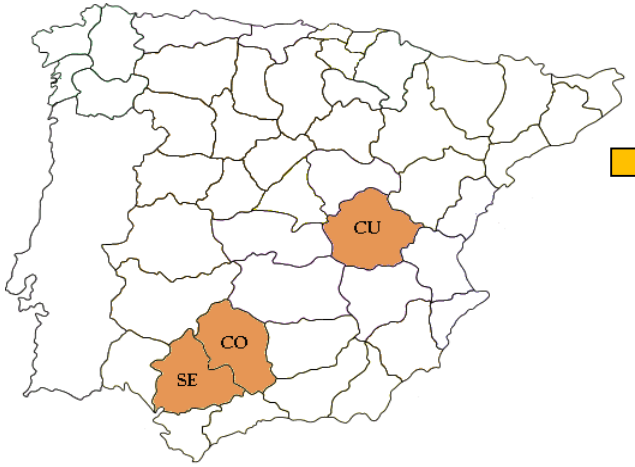
Single unpigmented plants surrounded by a large number of pigmented plants at a certain distance

The occurrence of cross-fertilization resulted in the presence of partially pigmented heterozygotes in the progenies of unpigmented plants

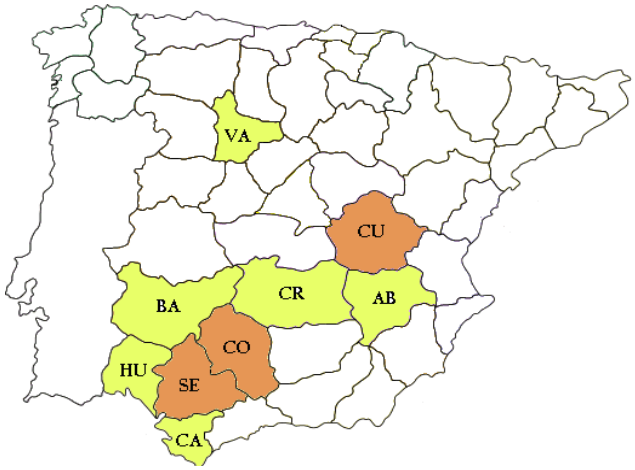
The rate of cross-fertilization: **14.8%** to **34.4%** in the pot experiment and **15.8%** to **40.0%** in the field

Causes of broomrape variability

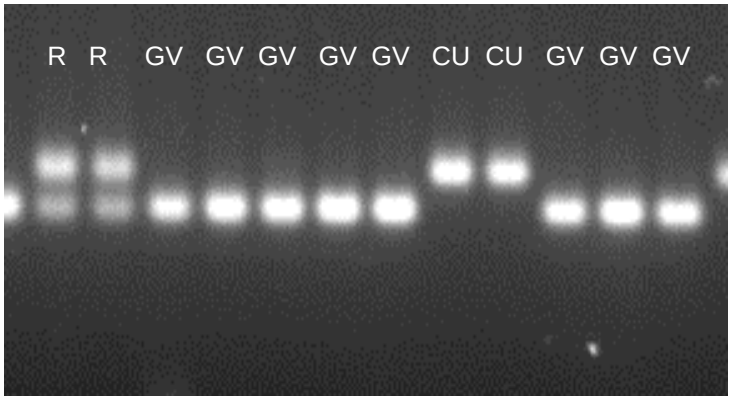
1990´s



2008



First observation of genetic recombination between individuals of the two gene pools



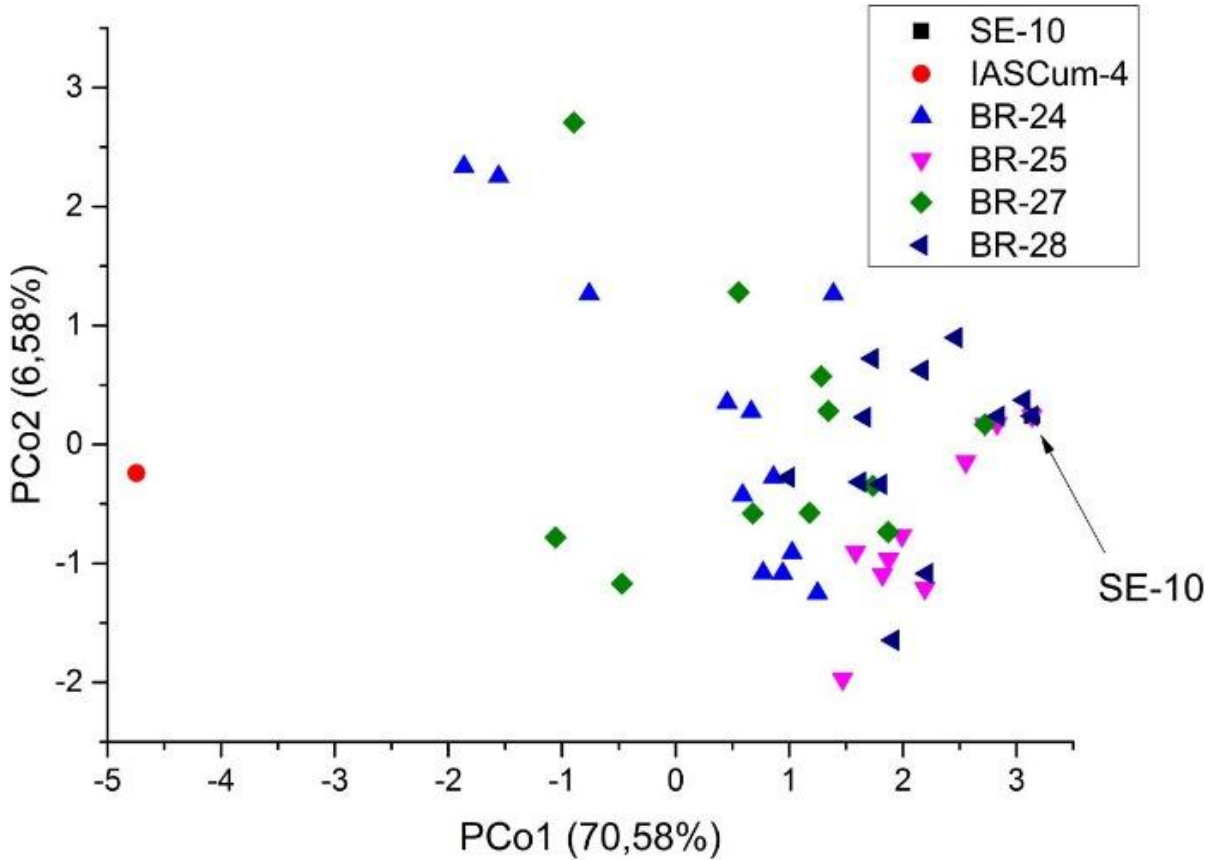
Causes of broomrape variability

Later, in the study of populations with increased virulence in the Guadalquivir Valley, we found that increased virulence was associated with increased variability (Martín-Sanz et al., 2016).

Our hypothesis was that the new virulent populations (G_{GV}) resulted from the recombination of avirulence genes from the gene pool of race F from the Guadalquivir Valley (F_{GV}) and the gene pool of race F from Cuenca (F_{CU})

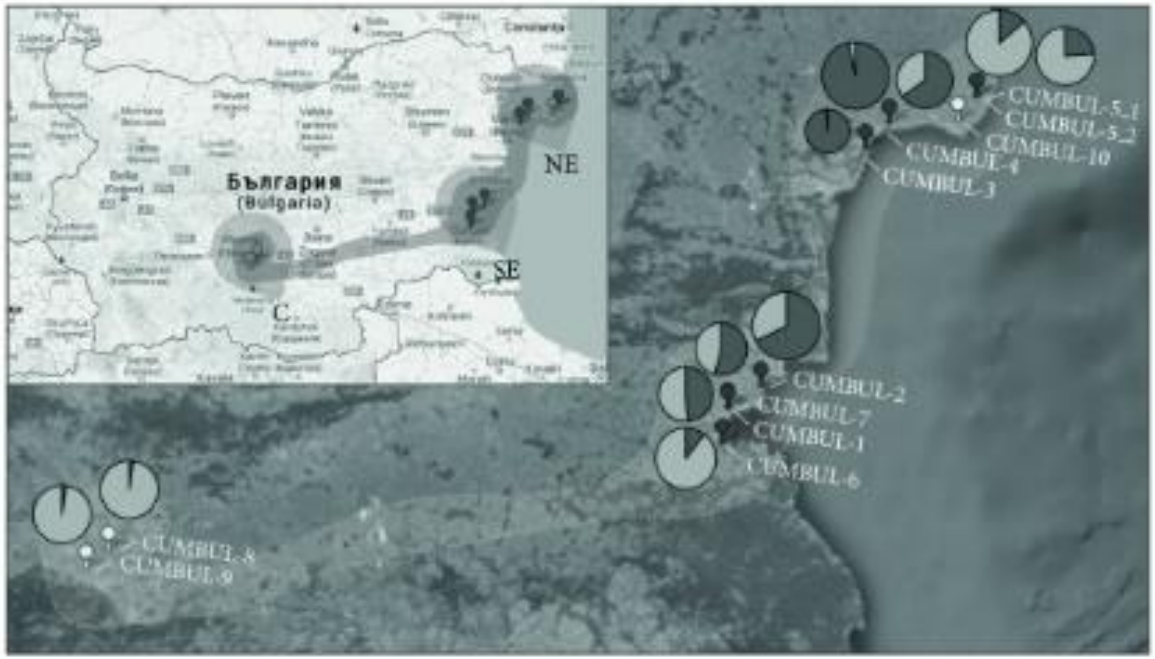
Causes of broomrape variability

This was the first indication that a new race can arise from genetic recombination rather than from a point mutation



Causes of broomrape variability

In a study conducted in the Black Sea area of Bulgaria, it was found that there was gene flow between populations parasitizing wild hosts and populations parasitizing sunflower.



Causes of broomrape variability

Accordingly, genetic recombination can be considered as an important mechanism leading to increased virulence in sunflower broomrape, in addition to mutation.

Key research strategies in a scenario of racial uncertainty

Research strategies

1. Racial characterization of broomrape populations
2. Discovery of new resistance genes
3. Genetic and physiological characterization of resistance genes in sunflower
4. Genetic and physiological characterization of avirulence genes in broomrape
5. Developing disgnostic molecular markers for Resistance and for Avirulence genes

1. Racial characterization of populations

In the mid term, racial characterization of broomrape is expected to be conducted by using diagnostic molecular markers for avirulence genes. This will be discussed later.

In the short term, the only viable alternative is an international collaboration to create a set of differential lines and to evaluate a large set of broomrape populations from all infested áreas

This will be proposed specifically by Stéphane Muños (INRA) in this Symposium, so, I will pass over this in my presentation

2. Racial characterization of populations

There is also a presentation of Alberto Martín-Sanz in the next session about the creation of an universal set of differential sunflower genotypes for race classification of sunflower broomrape populations

2. Discovery of new resistance genes

The two last presentations in this section will deal with the importance of wild sunflower relatives as a source of resistance genes.

New genes are being discovered in recent years in wild *Helianthus* species. For example Or_{deb2}, identified in *H. debilis* subsp. *tardiflorus* has been found to be very effective against the current race G and G+ populations from Spain and Eastern Europe.

2. Discovery of new resistance genes

But breeding for broomrape resistance cannot be based on single genes in a scenario of incresing broomrape dispersion and variability.

It is absolutely necessary a detailed screening of resistance genes in wild *Helianthus* germplasm, particularly in the anual species, from which resistance genes can be easily introgressed into cultivated sunflower.

For example, a new source of posthaustorial resistance from *H. praecox* will be the subject of a presentation in the next sesión, but more resistance genes are required to be discovered and, over all, characterized.

3. Characterization of resistance genes

Characterization of resistance genes should follow two primary objectives:

a. Genetic characterization

b. Physiological characterization

Both are similarly important as I will discuss now

3. Characterization of resistance genes

a. Genetic characterization

Resistance genes are generally arranged in clusters of genes in the sunflower genome, with multiple duplications.

Identification of the linkage group and also the approximate position of the gene is relatively easy, but this provides useless information for pyramiding genes located in the same cluster.

Additionally, gene editing technologies (CRISPR) offer a powerful tool for creating multiple mutations if the sequence of the specific resistance gene is known.

3. Characterization of resistance genes

a. Genetic characterization

Accordingly, the exact identification of the new resistance genes that are being discovered is required to facilitate gene pyramiding strategies and to investigate the function of allelic variants of the gene.

An example of this kind of detailed characterization of resistance genes will be presented in the next session for the resistance gene *Or7*.

3. Characterization of resistance genes

b. Physiological characterization

Gene pyramiding should be also based on a physiology-based strategy.

Pyramiding genes underlying different physiological mechanisms will result in a more durable resistance than a strategy based only on the map position of the genes.

Thus far there is only limited information on resistance mechanisms underlying broomrape reistance genes.

3. Characterization of resistance genes

b. Physiological characterization

More attention to the physiological characterization of resistance sources should be paid in the future

A detailed review of resistance mechanisms was presented yesterday. However, our knowledge about the resistance mechanisms and the molecules involved is still very limited.

Gene expression (next presentation), transcriptomic and metabolomic studies are of paramount importance to advance in our knowledge on how sunflower fights againts broomrape.

4. Characterization of avirulence genes

If our knowledge about resistance genes in sunflower is limited, information on avirulence genes in the parasite is practically nil.

As stated at the begining of the presentation, our current knowledge points to a gene-for-gene interaction in the sunflower-broomrape system, with dominant resistance genes interacting with dominant avirulence genes.

Characterization of avirulence genes is important to understand the function of resistance genes.

4. Characterization of avirulence genes

But the identification and characterization of avirulence genes will also provide fabulous tools for characterization of the racial composition of broomrape populations, beyond the use of differential lines and phenotypic evaluations.

5. Diagnostic markers

The availability of diagnostic molecular markers for identification of resistance genes in sunflower and avirulence genes in broomrape is a kind of science-fiction approach that is becoming a reality in the case of resistance genes and that will also become a reality for avirulence genes, although a lot of research is still required.

With such tools available, our capacity to develop sunflower germplasm with a durable resistance to broomrape will go undoubtedlly beyond our current expectations.

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Thanks for your attention