

Genetic characterization of *Plasmopara halstedii* populations in Argentina using simple sequence repeats (SSR) and effector-based markers

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Introduction

Sunflower downy mildew, caused by the pathogen *Plasmopara halstedii* (Farl.) Berl. et de Toni is an economically important disease of sunflower. Since 2012, *P. halstedii* epiphytotics were identified in several production regions of Argentina. However, the genetic diversity of the pathogen in Argentina remains largely unknown.

Materials and Methods

a) Race determination of *P. halstedii* isolates

42 isolates were collected in years 1991, and 2013 to 2018 in fields located in Central (Buenos Aires) and Northern (Chaco y Santa Fe) Argentina. The races of the isolates were determined by pathogenicity assays (Figure 1).

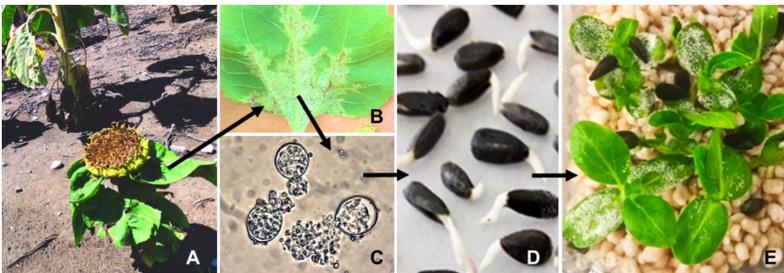


Fig. 1. A= recovery of inoculum at field B= detail of leaf sporulation C= spore solution D= reinfection of differential set of sunflower inbred lines E= phenotyping and race determination.

b) SSR analysis

The isolates were analyzed by eight loci SSR. The number of alleles per locus (APL), the expected (HE) and observed heterozygosity (HO) were determined. SSR data was analyzed with regions (2), collection years (4) and races (5) as variation sources (AMOVA) and the population structure was defined (Bayesian method).

c) Polymorphisms analysis on sequence data

DNA sequences of two effector genes type CRN and RXLR were obtained from two isolates per race and were analyzed along with the polymorphisms reported by Gascuel et al, 2016 on 14 French races. The polymorphisms were analyzed by multiple alignment (ClustalW).

Conclusions

- The race spectrum in Argentina is increasing in the last years. A novel race was detected for the first time (711).
- The heterozygotes deficit found is coincident with the homothallic reproduction of *P. halstedii*.
- The increased genetic variability observed in recent years seems to parallel with the emergence of novel races reducing fungicides and/or R genes efficacy.
- The examined sites from the effector sequences resulted homogeneous among the Argentinean races.
- Race determinations and effector CRN/RXLR polymorphisms were concordant in the pathotypes shared by Argentina and France, except for 330, that showed alternative RXLR haplotypes in each country.
- The study based on two effector genes suggests that the races evolution depends on country specific factors.

Results

- The races of the isolates were 330, 710, 711, 730 and 770.
- Based on AMOVA, genetic variation was observed among regions (7%) and years (32%), but not between races.
- Average number of alleles= 2.625; Average Observed Heterocigosity= 0; He Average expected heterocigosity= 0.369; Average Inbreeding coefficient F=1

Table 1. Preexistent and novel alleles in different years of isolates collection. N= number of analyzed isolates . Nd= non determined.

Year	N	Number of alleles detected	Novel alleles detected	Races determined
1991	1	8	---	770
2013	13	13	5	nd
2016	8	14	3	710
2017	16	17	5	330, 710, 711, 730
2018	4	10	0	710, 770

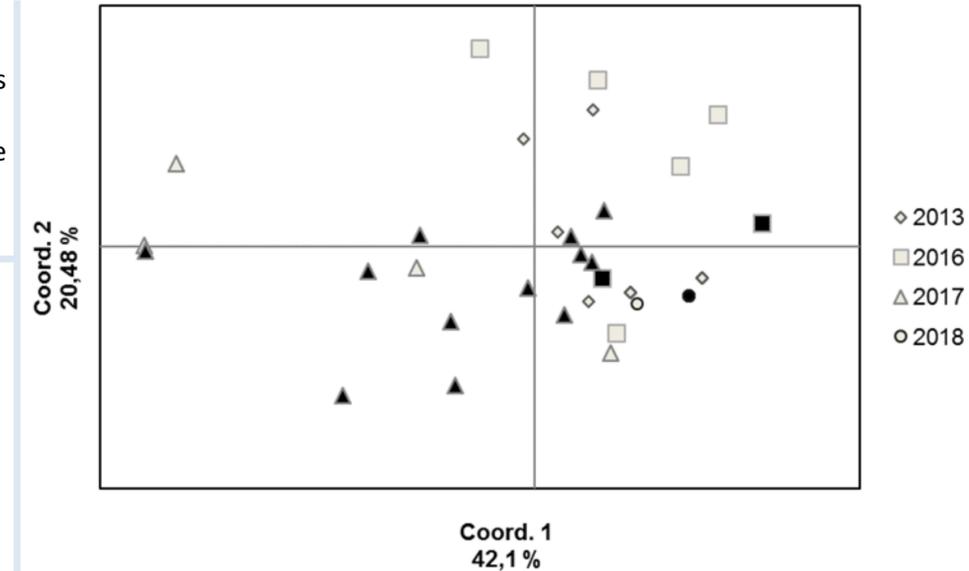


Fig. 2. PCO analysis based on the SSR matrix of genetic distance. Shapes indicate isolates collection years, and black or grey colors indicate Northern and Central regions, respectively.

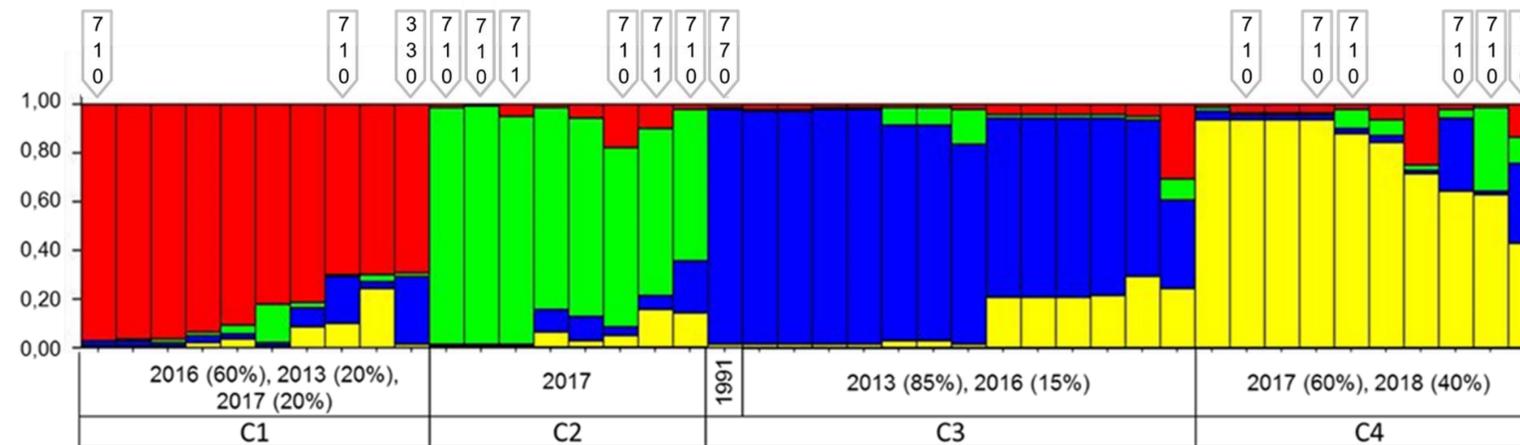


Fig. 3. Histogram of Bayesian clustering. Each individual is represented by a bar proportional to the inferred clusters. In the upper part of the figure are indicated the races of isolates.

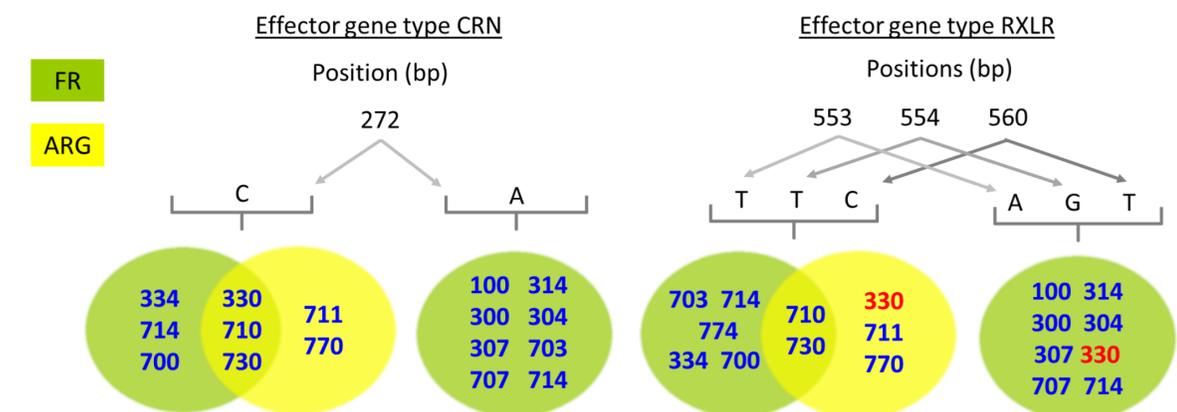


Fig. 4. Polymorphisms in the nucleotide sequences of the effector CRN and RXLR. The races of *P. halstedii* from Argentina (ARG) and France (FR) are shown in color circles