A survey of physiological races of *Puccinia helianthi* in Argentina

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ABSTRACT

- Sunflower rust, caused by *Puccinia helianthi*, is common and widespread in Argentina and occurs annually on cultivated sunflower (*Helianthus annuus* L.) and naturalized wild annual species. Even though the sunflower rust fungus is not endemic to Argentina there has been a great deal of pathogenic change in *P. helianthi* during the six decades of sunflower cultivation in this country. Novel pathotypes appear regularly that can rapidly overcome new sources of host resistance and, for this reason, jeopardize the principal tool for controlling this disease. A survey of rust physiological races was conducted to gather information on the diversity of this pathogen and to determine the gene/s that are still appropriate for introgressing into the sunflower breeding programs to obtain genetic control of this disease.

- Seventeen rust isolates were collected on cultivated, wild, ornamental, and adventitious sunflowers from the Chaco Province in Northern Argentina to Pedro Luro in the South of Buenos Aires Province during the period 2004-2011. These isolates were assessed for virulence on the international set of nine differential lines and identified using the triplet coding system.

- Physiological race 700 was the predominant race collected, since 7 out of the 17 isolates belongs to it. Also, rust physiological races 704, 720, 740, 744, 760 were identified. Interestingly, we found evidence of further diversity in the group of isolates belonging to race 700. When seven isolates of this race were inoculated on the USDA line HA-R6, which is resistant to race 777, we found that one of them –collected in Pergamino, Buenos Aires, Argentina- was virulent on this line. This new physiological race, for which we propose the code "7001", indicates that it would be useful to increase the set of sunflower differentials in order to describe more appropriately the diversity of the fungus.

- These results indicate that there was a significative change in the composition of the physiological races of *P. helianthi* in Argentina since the publication of the last survey in 1985. As a matter of fact, no isolate belonging to the group races 100, 300 or 500 was collected in the last years.

- The obtained results suggest that the genes present in inbred lines P386, HAR3 and HAR4 are still effective to control sunflower rust in Argentina. Nevertheless, they should be pyramidized in the final hybrid varieties in order to achieve a sustainable strategy of disease control. Continuous surveys of rust races throughout the entire sunflower cultivated region in Argentina are necessary to assist breeding programs in defining resistant sources and introgression strategies.

Key words: genetic resistance, molecular breeding, rust, sunflower.

INTRODUCTION

Sunflower rust caused by *Puccinia helianthi* (Schw.) is an autoecious and macrocyclic rust that occurs on wild perennial, wild annual, and cultivated sunflower species. Rust is considered an important disease of sunflower in Australia, South Africa, Russia, Canada, Argentina and India (Siddiqui and Brown, 1977; Los et al., 1995; Mayee 1995). Severe epidemics of sunflower rust can significantly reduce yield and quality of cultivated sunflower (Middleton and Obst, 1972). Although fungicides are available for disease management, host plant resistance is the most effective way to control the disease.

Even though the sunflower rust fungus is not endemic to Argentina there has been a great deal of pathogenic change in *P. helianthi* during the six decades of sunflower cultivation in this country (Antonelli, 1969, 1985). Novel pathotypes appear regularly that can rapidly overcome new sources of host resistance as it is the case in other production areas of the world (Sendall et al., 2006) This new pathotypes can jeopardize the main tool for controlling this disease. In Argentina, during the 2006/2007 growing season early and severe rust outbreaks occurred in many sunflower production areas (Huget, 2008). The information obtained during continuous monitoring of the sunflower production area in pursuit of existing rust pathotypes is critical to define breeding strategies. For this reason, a survey of rust physiological races was conducted to gather information on the diversity of this pathogen and to determine the gene/s that are still appropriate for introgressing into the sunflower breeding programs to obtain genetic control of this disease.

MATERIALS AND METHODS

Race identification. Identification of physiological races was done by using a set of nine differentials and the associated coded triplet nomenclature (Gulya and Masirevic, 1988). The differentials used in this system include susceptible inbred line S37-388, Canadian lines MC 90 and MC 29, the Argentine line P-386, and inbred lines HAR1 through HAR5. S37-388 is universally susceptible to all races. In addition HAR6, a line resistance to race 777 was used.

Collection of rust samples. Seventeen samples of rust urediospores were collected from cultivated, wild, ornamental, and adventitious sunflowers during the period 2004-2011. Each urediospore sample was initially increased by inoculating a susceptible sunflower line, and the freshly produced spores were used to inoculate a set of nine differentials (Gulya and Masirevic, 1996).

Inoculation: Plants of the differential lines were grown under greenhouse conditions and were inoculated by the method described by Gulya and Masirevic (1996). The inoculated plants were incubated in a dew chamber, and held 16 to 20 h at 20ºC in the dark. These plants were then grown in a greenhouse maintained at 22ºC ± 2ºC with a photoperiod of 16 h, using sodium vapor lamps for supplemental illumination. The rust symptoms were scored 12-14 days after inoculation. Plants were scored as resistant if they did not present uredia or they showed hypersensitive flecks, otherwise they were considered susceptible.

Comparison with previous surveys: to compare the older race classification system (i.e.: four North American (NA) races (1, 2, 3, and 4), Sackston, 1962) and the triplet coding system, the previous NA race 1 corresponds to race 100 of the coded triplet system, NA race 2 to race 500, NA race 3 to race 300, and NA race 4 to race 700 (Qi et al., 2011).

RESULTS

Sunflower rust was observed over all the sunflower production area in Argentina, and also in wild sunflower populations. Samples of sunflower rust collected in this work came from four Argentinean provinces, including Chaco, Cordoba, Santa Fe and Buenos Aires. Collected isolates and their geographical origin are detailed in Figure 1.

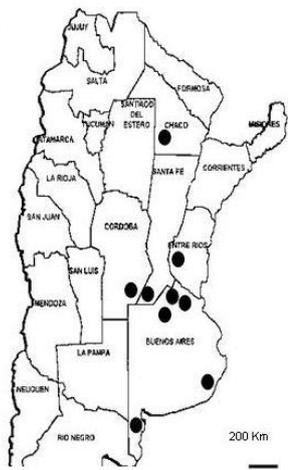| Isolate	Collection Place	Year	Collector
BR401	Las Breñas, Chaco	2004	A. B. de Romano
H201	Venado Tuerto, Santa Fe	2005	N. Huguet
H202	Venado Tuerto, Santa Fe	2005	N. Huguet
MB101	Balcarce, Buenos Aires	2005	M. E. Bazzalo
MB102	Balcarce, Buenos Aires	2005	M. E. Bazzalo
MB103	Balcarce, Buenos Aires	2005	M. E. Bazzalo
MB104	Balcarce, Buenos Aires	2005	M. E. Bazzalo
B302	Pergamino, Buenos aires	2008	M. Bulos
BR506	Baigorrita, Buenos Aires	2008	A. B. de Romano
SC401	Venado Tuerto, Santa Fe	2009	C. Sala
SP101	Junin, Buenos Aires	2009	S. Piubello
SP102	Junin, Buenos Aires	2009	S. Piubello
DA102	Parana, Entre Rios	2010	D. Alvarez
MC102	Pedro Luro, Buenos Aires	2010	M. Cantamutto
A201	Venado Tuerto, Santa Fe	2011	E. Altieri
B301	Arias, Cordoba	2011	M. Bulos
A202	S. A. de Areco, Buenos Aires	2011	E. Altieri

Fig. 1. Isolate code, geographical origin, year of collection, and name of the collector of the rust isolates used in this study. A map of Central and Northern Argentina shows the collection sites by a black dot (●).

As could be seen in Table 1, physiological race 700 was the predominant race collected, since 7 out of the 17 isolates belongs to it. The second predominant race was 740, which was identified in five out of the 17 isolates. Also, rust physiological races 704, 720, 744, and 760 were identified. No clear pattern of geographical distribution of the two predominant races could be observed.

Table 1. Determination of the physiological race for 17 isolates of *P. helianthi*.

Isolate	Reaction over a set of differential lines									Race
	S37-788	CM90	CM29	P386	HAR1	HAR2	HAR3	HAR4	HAR5	
BR401	S	S	S	R	R	S	R	R	R	**740**
H201	S	S	S	R	R	S	R	R	R	**740**
H202	S	S	S	R	R	S	R	R	S	**744**
MB101	S	S	S	R	R	R	R	R	R	**700**
MB102	S	S	S	R	R	R	R	R	S	**704**
MB103	S	S	S	R	R	S	R	R	R	**740**
MB104	S	S	S	R	R	S	R	R	S	**744**
B302	S	S	S	R	R	R	R	R	R	**700**
BR506	S	S	S	R	R	S	R	R	R	**740**
SC401	S	S	S	R	S	S	R	R	R	**760**
SP101	S	S	S	R	R	S	R	R	R	**740**
SP102	S	S	S	R	S	R	R	R	R	**720**
DA102	S	S	S	R	R	R	R	R	R	**700**
MC102	S	S	S	R	R	R	R	R	R	**700**
A201	S	S	S	R	R	R	R	R	R	**700**
B301	S	S	S	R	R	R	R	R	R	**700**
A202	S	S	S	R	R	R	R	R	R	**700**

DISCUSSION

The occurrence of different physiological races of sunflower rust in Argentina was first reported in 1957. Since then, the reports of the variability for this pathosystem were scarce. The last report was published in 1985 and included the physiological races determined over the period 1965-1983 (Antonelli, 1985). Some of the inbred lines and hybrids used as rust differentials in this report were the same as the international set used in the present study, and, for this reason, some conclusions about the changes in the pathogen over time can be drawn. Physiological races 100, 300 and 500 were discovered during the 1960 decade, and the race 700 during the 70s. In the present study, no isolate belonging to group races 100, 300 or 500 was collected. In fact, all the collected isolates belong to group race 700. These indicate that the

deployment of several rust resistance genes (v.g. *R1* and *R2*) in sunflower commercial hybrids during the last 30 years determined a selection pressure over sunflower rust populations and an associate drift in the frequency of virulence genes.

The obtained results suggest that the genes present in inbred lines P386, HAR3 and HAR4 are still effective to control sunflower rust in Argentina. Nevertheless, they should be pyramidized in the final hybrid varieties in order to achieve a sustainable strategy of disease control. Continuous surveys of rust races throughout the entire sunflower cultivated region in Argentina are necessary to assist breeding programs in defining resistant sources and introgression strategies

Observed diversity in the group of isolates belonging to race 700 given by the polymorphism for virulence over the inbred resistant line HAR6 suggest that it would be possible to describe more appropriately the diversity of *Puccinia helianthi* by increasing the number of differential lines. For example, if HAR6 is included in the set of differential lines, the isolate belonging to race 700 and which is virulent on HAR6, should be coded as "7001", and the rest of isolates belonging to race 700 and avirulent on HAR6 should be coded as "7000". Appart from HAR6, several inbred lines show a high level of resistance to almost all the current physiological races. These lines should be evaluated by their discriminative efficiency of the sunflower rust diversity in order to complete a new triplet of public differential lines. This new set of differential lines will be highly useful to describe more precisely the current diversity of the fungus and its changes over time.

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