BAG | Journal of Basic & Applied Genetics

Vol XXXV (1): 53-64; Jan.-July 2024

Journal of the Argentine Society of Genetics

DEVELOPMENT OF GENETIC STOCKS OF SUNFLOWER WITH RESISTANCE TO SUNFLOWER CHLOROTIC MOTTLE VIRUS

CONFORMACIÓN DE RECURSOS GENÉTICOS DE GIRASOL CON RESISTENCIA AL VIRUS DEL MOTEADO CLORÓTICO DEL GIRASOL

Fernández Moroni I.[1], Lenardon S.[2,3,4], Giolitti F.[2,3], Álvarez D.[5], Poverene M.[1,6], Presotto A.[1,6], Cantamutto M.[7]

[1] Dpto. Agronomía, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina.

[2] Instituto Nacional de Tecnología Agropecuaria (INTA), Córdoba, Argentina.

[3] Instituto de Patología Vegetal (IPAVE), UFYMA, Córdoba, Argentina.

[4] Universidad Nacional de Río Cuarto (UNRC), Facultad de Agronomía y Veterinaria (FAV), Río Cuarto, Argentina.

[5] Estación Experimental Agropecuaria Manfredi (E.E.A. Manfredi), INTA, Manfredi, Córdoba, Argentina.

[6] Centro de Recursos Naturales Renovables de la Zona Semiárida-Consejo Nacional de Investigaciones Científicas y Técnicas (CERZOS-CONICET), Bahía Blanca, Argentina.

[7] E.E.A. Hilario Ascasubi, INTA, Hilario Ascasubi, Argentina.

Corresponding author:
Ivana Fernández Moroni
ivana.fernandez@uns.edu.ar

ORCID 0000-0001-9779-1184

Cite this article as:
Fernández Moroni I., Lenardon S., Giolitti F., Álvarez D., Poverene M., Presotto A., Cantamutto M. 2024. DEVELOPMENT OF GENETIC STOCKS OF SUNFLOWER WITH RESISTANCE TO SUNFLOWER CHLOROTIC MOTTLE VIRUS. BAG. Journal of Basic and Applied Genetics XXXV (1): 53-64.

Received: 04/12/2023
Revised version received: 05/06/2024
Accepted: 05/05/2024

General Editor: Elsa Camadro

DOI: 10.35407/bag.2024.35.01.04

ISSN online version: 1852-6233

Available online at
www.sag.org.ar/jbag

ABSTRACT

The common race of sunflower chlorotic mottle virus (SCMoV-C) can cause severe yield losses in susceptible genotypes of sunflowers if infection occurs at early plant stages. In Argentina, SCMoV-C is widespread in sunflower production fields and even if its incidence is generally low, in some cases it can reach up to 95%. To date, no complete resistance to SCMoV-C has been detected in commercial cultivars. In the search for resistant germplasm, wild sunflower (*Helianthus annuus* L.) populations from Argentina were tested, as they were exposed to natural selective pressure during their naturalization. After artificial inoculation with SCMoV-C, symptom-free plants were selected and grown for controlled self-pollination, sibling crosses and crosses with inbred lines. Recurrent selection for non-symptomatic plants and self-fertility significantly increased the frequency of asymptomatic individuals after SCMoV-C inoculation in the development germplasm. After eight generations of recurrent selection and controlled crosses, four genetic stocks with complete SCMoV-C resistance were developed. These genetic stocks could be used for breeding programs and genetic studies. The genetic stocks were registered in the Active Sunflower Germplasm Bank of the National Institute of Agricultural Technology (INTA, EEA-Manfredi), for maintenance and public distribution.

Key words: germplasm, *Helianthus annuus*, potyvirus, pre-breeding.

RESUMEN

En los genotipos susceptibles de girasol, la cepa común del *Virus del moteado clorótico del girasol* (SCMoV-C) puede causar graves pérdidas de rendimiento si la infección ocurre en las primeras etapas del desarrollo de la planta. En Argentina, el SCMoV-C está muy extendido en los campos de producción de girasol y aunque su incidencia es generalmente baja, en algunos casos puede llegar hasta el 95%. Hasta ahora, no se ha detectado resistencia completa a SCMoV-C en cultivares comerciales. En la búsqueda de germoplasma resistente, poblaciones de girasol silvestre (*Helianthus annuus* L.) de Argentina fueron testeadas, ya que durante su naturalización estuvieron expuestas a presión selectiva natural. Después de la inoculación artificial con SCMoV-C, se seleccionaron y cultivaron plantas libres de síntomas y se realizaron cruzamientos controlados, entre hermanos, con líneas endogámicas y autofecundaciones. La selección recurrente de plantas asintomáticas y autofértiles aumentaron considerablemente la frecuencia de individuos asintomáticos después de la inoculación con SCMoV-C en el germoplasma en desarrollo. Después de ocho generaciones de selección recurrente y cruces controlados, se desarrollaron cuatro stocks genéticos con resistencia completa a SCMoV-C. Este germoplasma podría utilizarse para programas de mejoramiento y estudios genéticos. Los stocks genéticos fueron registrados en el Banco de Germoplasma Activo de Girasol del Instituto Nacional de Tecnología Agropecuaria (INTA, EEA-Manfredi), para su mantenimiento y distribución pública.

Palabras clave: germoplasma, *Helianthus annuus*, potyvirus, premejoramiento.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) yield is threatened by several diseases that cause economic losses. Generally, the disease problems are caused by specific or generalist fungal pathogens, but viruses are a potential hazard due to their degree of symptomatology (Vasquez and de Romano, 2006; Gontcharov, 2014; Gulya *et al.*, 2019). In Argentina, sunflower chlorotic mottle virus (SCMoV) (Dujovny *et al.*, 1998; 2000) is the most widespread virus infecting cultivated and wild sunflower (Cabrera Mederos *et al.*, 2020). Although its incidence is generally low, i.e. less than 3% in some cases, it can reach up to 95% (Lenardon, 1994). In susceptible genotypes, SCMoV infection at the early plant stages can cause generalized chlorosis, reduced growth, and yield losses exceeding 50% (Lenardon *et al.*, 2001).

SCMoV (*Potyvirus helichloromaculae*) belongs to family *Potyviridae* (ICTV, 2024). Viruses of this genus are transmitted from plant to plant mainly during the feeding action of infected aphid vectors. Five natural hosts of SCMoV were identified: *H. annuus*, *H. petiolaris* L., *Eryngium* sp., *Dipsacus fullonum* L. and *Ibicella lutea* L. In the epidemiology of the virus, the most important are *D. fullonum* and *Eryngium* sp., which are biennial and perennial weeds, respectively, that allow the virus to pass from one growing season to the next (Cabrera Mederos *et al.*, 2020). Preventive measures are needed to manage plant virus diseases since there are no curative treatments when crops are established. Resistant or tolerant genotypes are a simple, economical, and sustainable way to manage viral diseases. In combination with cultural practices (including chemical applications against vectors) and biological control, integrated disease management maximizes the likelihood of reducing yield losses (Jones, 2004; Tatineni and Hein, 2023).

Currently, two strains of SCMoV virus affect sunflower, the chlorotic ringspot strain (Giolitti *et al.*, 2010) and the common (C) strain (Dujovny *et al.*, 1998; 2000). The latter is the most widely distributed in Argentina (Cabrera Mederos *et al.*, 2020).

Lenardon *et al.* (2005) explored the susceptibility to SCMoV-C of more than 200 public and private sunflower inbred lines. Of these, only three lines showed partial resistance to this pathogen. The best response was observed in line L33 (Advanta Semillas S.A.I.C), which was linked to limited systemic infection due to scarce and isolated symptoms of chlorotic mottling and moderate reduction of yield components.

In plants, disease resistance can be genetically controlled by one, a few, or many genes, and it can be partial or total (Agrios, 2005). Total resistance implies that the virus cannot colonize its host, while in partial resistance there is colonization by the pathogen, although it is suppressed. Generally, the severity of symptoms reflects the level of virus replication and accumulation in the host (Revers *et al.*, 1999; de Ronde *et al.*, 2014).

Many crops have undeniably benefited from the useful traits of their wild relatives (Hajjar and Hodgkin, 2007). In sunflower, cytoplasmic male sterility, herbicide tolerance, modified fatty acid profile, disease resistance, among other traits have been successfully introgressed into the cultivated gene pool with very important economic consequences (Seiler *et al.*, 2017).

Naturalized wild sunflower, *H. annuus var. annuus* L., is distributed across the central region of Argentina (Poverene *et al.*, 2002). The invasive process has been associated with high phenotypic (Cantamutto *et al.*, 2010a; Presotto *et al.*, 2009) and genetic diversity (Garayalde *et al.*, 2011; Hernández *et al.*, 2019), probably exacerbated by an intense gene flow from cultivated sunflower (Ureta *et al.*, 2008).

Wild Argentine sunflowers could have developed resistance genes under natural selective pressure, due to the wide diffusion of the virus. To date, no specific research has been carried out on the reaction of wild sunflower germplasm to SCMoV-C. If wild Argentine sunflowers are virus resistant, there is a challenge of introgressing this trait into domestic sunflower. This paper reports the results of 8-years testing and selection for resistance to SCMoV-C in wild Argentine sunflowers, aimed at forming a useful source of germplasm for sunflower breeding.

MATERIALS AND METHODS

Screening for resistance to SCMoV-C in wild sunflower naturalized in Argentina

Nine wild *H. annuus* populations collected from representative geographical habitats (Cantamutto *et al.*, 2008), expressing different phenotypes in a common garden experiment (Cantamutto *et al.*, 2010b), were selected to initiate the evaluation and selection procedure (Table 1). The evaluations were conducted in November 2004, August 2005, and January 2006 on a minimum of 43 plants of each population. Every wild population was evaluated at least twice completing 575 screened plants. The commercial hybrid Contiflor 7 was used as a susceptible control.

Virus maintenance and artificial inoculation

The artificial inoculation and selection of plants without disease symptoms were carried out in greenhouses at the Instituto de Patología Vegetal, Instituto Nacional de Tecnología Agropecuaria (IPAVE-INTA), Córdoba, Argentina. SCMoV-C was maintained on susceptible sunflower cultivars in the greenhouse. Infected leaves

Table 1. Wild *Helianthus annuus* populations from Argentina

Population code	Location	Province	Eco-region
AAL	Adolfo Alsina	Buenos Aires	Pampa
BAR	Colonia Barón	La Pampa	Spinal
DIA	Diamante	Entre Ríos	Spinal
LMA	Las Malvinas	Mendoza	Plains and tablelands forest
CAR	Carhué	Buenos Aires	Pampa
RAN	Rancul	La Pampa	Spinal
MAG	Media Agua	San Juan	Plains and tablelands forest
JUM	La Carlota	Córdoba	Pampa
RCU	Río Cuarto	Córdoba	Spinal

were collected and stored at -80 °C until used as a source of inoculum. Infected leaves were ground in a buffer solution, pH 7, containing silicon carbide added as abrasive. The inoculum was applied to expanding sunflower leaves at V2-6 (Schneiter and Miller, 1981), using a high-pressure airbrush apparatus. Further details about virus maintenance and inoculation protocol were described by Lenardon *et al.* (2005).

Reproduction and crosses of select plants

Selected plants were transplanted in the experimental field of Universidad Nacional del Sur (UNS) Agronomy Department, Bahía Blanca, Argentina, with a spacing of 30 cm between plants and 100 cm between plots. Drip irrigation was provided to supply the water demand of the plants.

The fertile heads used for the controlled crosses were covered with paper or polyamide bags at the R4 stage, for insect and pollen exclusion. The male fertile plants used as females were emasculated manually in the morning and pollinated in the late afternoon. Pollination was carried out with fresh pollen collected from covered heads. Crosses involving the wild resource as the maternal parent were not emasculated because of its high degree of self-incompatibility (Gutierrez *et al.*, 2014).

Generation of the SCMoV-C resistant genetic stocks

Two cycles of selection were performed to conform the SCMoV-C resistant genetics stocks.

First cycle of selection: introgression of the SCMoV-C resistant trait into a domestic strain background

The first cycle of selection comprised five round of SCMoV-C inoculation, selection of asymptomatic plants and its controlled reproduction. Started with the selection of non-symptomatic plants of wild sunflower accessions, BAR and CAR. SCMoV-C free symptoms plants of wild sunflower, as well as new plant selected on next generations, were self-pollinated, sibling mated or interbreeding with the male sterile inbred lines (IL): A10, HA89 and A09, susceptible to SCMoV-C.

Second cycle of selection: fixing the SCMoV-C resistant trait

In 2009, the S10 family, a segregant germplasm developed in the first cycle of selection (Figure S1), was chosen to be the donor of the resistant trait on the second cycle of selection. Four asymptomatic plants after SCMoV-C infection of S10 (S10aRR, S10bRR, S10cRR, and S10dRR) were crossed with A09 and B09 inbred lines. Both lines are susceptible to SCMoV-C. A09 is a male-sterile inbred line with PET1 cytoplasm; while B09 is the male-fertile maintainer line of A09, with normal *H. annuus* cytoplasm (Garayalde *et al.*, 2015; González *et al.*, 2015). Due to the branching condition of S10RR plants, several heads were used in reciprocal controlled crosses between B09 on manual emasculated flowers, and as a pollen donor in crosses with A09. The progenies of those crosses were cultivated in the experimental field during the following season. At R4, heads were bagged until maturity to produce self-pollinated seeds.

In G5 (fifth generation), the progeny of self-fertile plants of the crosses (A09xS10RR; S10RRxB09 and B09xS10RR), were selected to generate the next generation. In G6 (sixth generation), SCMoV-C asymptomatic and self-fertile plants of F_2 families were chosen to ongoing selection. In G7 (seventh generation), F_3 families were submitted to another round of SCMoV-C inoculation. Selected F_3 plants, belonging to different families, were crossed between them or self-pollinated. The offspring of these crosses constituted four genetic stocks named GS-1, GS-2, GS-3, and GS-4. A detailed description of the the successive crosses performed on the second cycle of selection was described in Figure S2, in the supplementary material.

Phenotypic reaction to virus infection and serological virus analysis of resistant plants

The evaluated plants were grown in three-liter plastic pots; in each pot, 1-2 seeds were sown. To break dormancy, seeds had previously undergone a stratification treatment in plastic trays on moistened paper for one week at 4-7 °C (ISTA, 2004). The

susceptible cultivars Contiflor 17 or Contiflor 7 were used as a susceptible control.

SCMoV-C inoculation and selection of plants without the disease symptoms were performed artificially and individually on each plant. The reaction of the plants to SCMoV-C infection was assessed 15 days after inoculation and, on selected asymptomatic plants, after transplanting to the experimental field in the UNS at the reproductive stage. Inoculated plants were classified visually according to the leaf symptoms expression as: 1) SS, when chlorotic mottling was confluent over the entire lamina; 2) MR, when chlorotic mottling was mild; and 3) RR, when they had no visible disease symptoms (Figure 1). Some plants that had very few isolated, chlorotic pinpoints compatible with SCMoV-C after transplant, at the reproductive stage, were recorded as RR*.

On G8 (eighth generation), the visual diagnosis of inoculated plants was confirmed by the DAS-ELISA test. DAS-ELISA was performed as described by Lenardon *et al.* (2005) using an antiserum obtained by Dujovny *et al.* (1998) at least 15 days after SCMoV-C inoculation.

Field SCMoV-C inoculation of the genetic stocks

The selected genetic stocks were cultivated and artificially inoculated with SCMoV-C at V2-V4 in the experimental field of INTA in Manfredi, Córdoba, Argentina, during the 2013-14 growing season. Plant reaction was observed 15 days after inoculation and classified as previously described.

Phenotypic characterization of the SCMoV-C genetic stocks

Seeds of the genetic stocks, preconditioned as previously indicated, were arranged in multi-cell trays (70cc) filled with organic substrate (Terrafertil MULTIPRO®) in October 2014. They were grown in greenhouses until being transplanted in the experimental field of the UNS Agronomy Department, approximately one month later, at V2-4. Between 8 and 31 plants were spaced every 30 cm and grouped in plots according to the germplasm origin. Water demand was supplied by drip irrigation.

Morphological and physiological traits were used to describe the genetic stocks. The following characteristics were determined on mature plants: height (PH), number of leaves on the main stem (LP), leaf area (LA) of a leaf located halfway up the main stem (Aguirrezábal *et al.*, 1996), presence of a main head (MH), anthocyanins (ANT), branches (BRA) on the main stem, and head number per plant (HP). Branching was classified into apical, basal, or fully branching. The number of self-fertilized seeds per head (SAF) was counted in one or two heads per plant. Cypsela length (CL) and width (CW) were determined for 20 to 40 cypselas, and the fresh biomass of 1000 cypselas (B1000) was estimated, based on the fresh weight of at least four samples of 30 to 150 cypselas of each genetic stock, according to Equation 1.

B1000 (g) =(1000*PFcip)/Ncip [Equation 1]

where B1000: fresh biomass of 1000 cypselas, PFcip (g): fresh weight of cypselas, Ncip: number of cypselas.

The duration of the ontogenetic cycle was characterized as the duration in days elapsed from germination until 50% of the plants reached the R5 stage (G-R5) and until 50% of the plants were at the R9 stage (G-R9).

RESULTS AND DISCUSSION

Screening for resistance to SCMoV-C in wild sunflower naturalized in Argentina

No local or systemic symptoms of SCMoV-C disease were observed in at least 13% of the plants of all wild Argentine *H. annuus* populations after artificial inoculation (Table 2). This discovery was of interest because many public and private sunflower varieties from Argentina appear to be susceptible to SCMoV-C (Lenardon *et al.*, 2005), and once infected the virus can cause severe yield losses (Lenardon *et al.*, 2001). Until recently, Argentina was the only country with the presence of this virus, but Bello *et al.* (2023) recorded the first case in Brazil in 2021. This virus will continue to expand to other countries because it is transmitted by aphids, some weeds could act as natural reservoirs (Dujovny *et al.* 1998; Cabrera Mederos *et al.*, 2020) and many sunflower cultivars seem to be susceptible (Lenardon *et al.*, 2001).

Among the wild Argentine accessions, BAR showed the highest frequency, with almost 60% of the plants expressing no disease symptoms after inoculation. The BAR accession might have introgressed with *H. petiolaris* because it was collected in an area where both wild species coexist and it shows morphological evidence of gene flow (Gutierrez *et al.*, 2009). This agroecological situation could have made the emergence of novel variability possible through interspecific crosses.

Generation of the SCMoV-C resistant genetic stocks

The first cycle of virus resistance introgression into the domestic strain was started from a few plants selected from the BAR and CAR accessions, which reached the reproductive stage without symptoms after being inoculated with SCMoV-C and transplanted. Despite the high level of self-incompatibility of wild sunflowers (Fick and Miller, 1997; Gutierrez *et al.*, 2014), some seeds were obtained from self-fertilized and sibling-mated BAR plants. However, all its scarce progeny were susceptible to SCMoV-C. Instead, the crosses BAR-RRxCAR-RR and A10xBAR-RR produced abundant

Table 2. Mean percentage of asymptomatic plants of the wild *H. annuus* populations from Argentina after artificial inoculation with SCMoV-C between 2004 and 2006. Contiflor 7 (CF7) was used as susceptible control.

Population code	Artificial inoculations rounds (No.)	Inoculated plants (No.)	Asymptomatic plants (%)
RCU	3	126	56
RAN	2	43	35
DIA	3	130	49
LMA	2	48	30
MAG	3	105	19
JUM	3	109	35
BAR	3	113	61
AAL	3	115	13
CAR	2	52	35
CF7	3	33	0

seeds and SCMoV-C symptoms-free plants to continue the selection process (Table 3). Plants without disease symptoms after infection were self-pollinated, sibling-mated, or acted as a pollen donor to pollinate inbred sterile plants.

Three new rounds of SCMoV-C inoculation and controlled crosses between asymptomatic plants were performed in 2007-08, 2008-09, and 2009-10. However, after four generations of recurrent selection, the resultant germplasm lacked complete fixation of the SCMoV-C resistance trait, which denoted the absence of homozygosity of the character.

Reduced selection efficacy probably was because some plants without disease symptoms may have been erroneously diagnosed for further selection. Specifically, this situation may have happened in 2008-09, since 40% of plants of the susceptible control had no visual symptoms of SCMoV-C disease after inoculation (Table 3). Additionally, the high level of self-incompatibility of the genetics resources difficult the increment of homozygosity of the resistant trait, which was probably due to the self-incompatibility inherited from wild populations (Gutierrez *et al.*, 2014). In both wild and old sunflower cultivars, self-fertility is prevented mainly by a sporophytic self-incompatibility mechanism (Fick and Miller, 1997). However, sunflower breeding has broken down this reproductive mechanism and encouraging self-pollination (Gandhi *et al.*, 2005; Sun *et al.*, 2012) and now, cultivars can produce up to 100% of seed set under self-pollination (Astiz *et al.*, 2011). Segregation or co-segregation of the male-sterile trait, a product of crossing with male sterile inbred lines, contributes as a barrier to prevent self-fertilization of the resistant individuals, too.

To enhance the chance of fixing the resistant trait, in 2009-10 began the second cycle of crosses and selection. Disease symptom free plants of G4 families derived from crosses that produce abundant seeds, more than 50, by sibling crosses or self-pollination were selected. But, at maturity, only one of the selected crosses produced male fertile plants with a profuse production of pollen. The selected resource was named S10 family (Family identifier code: 25, Table 3). S10 was the result of the first cycle of selection, a segregating cross with wild cytoplasm and about half a percent of resistance.

S10 plants were crossed with the maintainer B09 and the male sterile A09 line. The objective of these crosses was to increase the level of self-fertilization to facilitate the subsequent introgression process of the SCMoV-C resistant trait into the elite germplasm of sunflower (Seiler *et al.*, 2017; Warburton *et al.*, 2017).

Only F_1 self-pollinated seed families were chosen to continue the second cycle of SCMoV-C selection. From that moment on, the chosen individuals were SCMoV-C symptom-free plants selected from progenies obtained by self-fertilization of families with abundant seed production. A higher degree of self-fertility can facilitate breeding since it allows a rapid increase in the homozygosity of the trait under selection (Cubero, 2003).

Once the offspring of highly self-fertilized seed production plants were used to continue the selection process, the proportion of progenies without SCMoV-C symptoms increased quickly as selection progressed. After another two generations submitted to selection, the proportion of SCMoV-C symptoms-free progenies increased up to 100% (Table 4). Similar results were obtained by Jan and Gulya (2006a; 2006b) during the development of genetic stocks with resistance to sunflower mosaic virus (SuMV) using wild-resistant material of American wild *H. annuus*.

Lenardon *et al.* (2005), in reference to SCMoV, as well as Jan and Gulya (2006b) in SuMV, stated that the resistance of their germplasms to the viruses of the *Potyviridae* family was controlled by a dominant gene. Although in many cases plant resistance to virus appears to be under simple dominant or recessive genetic control (Maule *et al.*, 2007), some reports show that resistance may also be under a few genes or polygenic inheritance (Gómez *et al.*, 2009; de Ronde *et al.*, 2014, Rossi *et al.*, 2015).

The segregation pattern of resistance suggests that more than one gene could participate in complete resistance against SCMoV-C. As in this study, research by Melchinger *et al.* (1998) in maize, Gore *et al.* (2002) in soybean, and Lee *et al.* (2017) in pepper, revealed a segregation pattern that did not fit a single-gene model for resistant and susceptible plants. Although it seems

Table 3. Seed production and offspring (O) reaction after SCMoV-C inoculation (RAI), of the controlled crosses performed during the first cycle of selection. Intercrosses with selected wild *Helianthus annuus* conformed the first generation (G1), and the successive generations (G2, G3, and G4) were obtained from new selections (highlighted in bold) and controlled crosses. ORAI: RR, plant without symptoms; MR, plant with mild chlorotic symptoms; SS, susceptible to SCMoV-C. SIB: sibling cross. ▽: self-pollination. Contiflor 17 (CF17) were used as susceptible control. A09, A10, and HA89 are inbred lines. The number at the beginning of an inoculated family indicates the family identifier code (e.g., 3RR SIB= a sibbing cross of resistant plants of BAR-RR x CAR-RR).

Family identifier code	Inoculated family	Produced seeds (No.)	Inoculated plants (No.)	ORAI		
				SS (%)	MR (%)	RR (%)
	G1	**First generation**	**2006-07**			
1	BAR-RR ▽	< 5	No plants were obtained			
2	BAR-RR SIB	< 10	3	0	100	0
3	**BAR-RR x CAR-RR**	**> 100**	**33**	**39**	**6**	**55**
4	**A10 x BAR-RR**	**> 100**	**53**	**63**	**24**	**13**
	CF17		18	100	0	0
	G2	**Second generation**	**2007-08**			
5	**A10 x 3RR**	**> 50**	**18**	**72**	**0**	**28**
6	A09 x 4RR	> 50	21	76	0	24
7	A10 x 4RR	> 50	22	83	0	17
8	**A09 x 3RR**	**> 50**	**18**	**67**	**17**	**16**
9	**HA89 x 3RR**	**> 50**	**24**	**54**	**42**	**4**
10	HA89 x 3RR	> 50	24	46	54	0
11	**3RR SIB**	> 25	9	56	22	22
12	4RR SIB	> 25	24	55	12	33
	CF17		6	100	0	0
	G3	**Third generation**	**2008-09**			
13	**5 RR SIB**	**> 40**	**5**	**0**	**0**	**100**
14	8 RR SIB	> 50	28	0	18	82
15	8 RR SIB	> 50	30	10	7	83
16	**8 RR SIB**	**> 50**	**18**	**0**	**0**	**100**
17	8 RR SIB	> 50	22	19	0	81
18	9 RR ▽	> 20	15	7	0	93
19	9 RR x 7 RR	> 50	30	10	3	87
20	**11 RR x 5 RR**	**> 50**	**13**	**0**	**0**	**100**
	CF17		5	60	0	40
	G4	**Fourth generation**	**2009-10**			
21	13 RR ▽	< 20	8	13	0	87
22	16 RR SIB	> 50	23	52	0	48
23	16 RR ▽	< 5	1	0	0	100
24	**20 RR SIB**	**> 50**	**42**	**52**	**0**	**48**
25	20 RR ▽	> 50	40	52	0	48
	CF17		19	100	0	0

plausible that resistance to SCMoV-C is governed by more than one gene, genetic analysis was outside the scope of the present study. As resistance evaluations on F_2 and F_3 progeny considered only two to ten inoculated individuals, a segregation model was not statistically determined. Future studies, focused on the inheritance of resistance could elucidate the genetic mechanism involved.

In G8, none of the progenies of the selected crosses had any visual symptoms of SCMoV-C after inoculation (Table 4). Negative DAS-ELISA results individually obtained from each inoculated plant confirmed that virus accumulation was strongly inhibited in these germplasms. This is the first time that germplasms completely resistant to SCMoV-C have been produced without segregation of the resistance trait. These genetic stocks represent an important source of genetic variability of SCMoV-C resistance, since no resistant sunflower cultivars are available, and no cases of complete resistance have been reported up to the present time. These germplasms will provide sunflower breeders with a source of resistance against SCMoV-C, should it become an economic problem.

Phenotypic characterization of the resistant genetic stocks

The use of wild relatives in breeding programs can be a great challenge because their useful traits could be masked by agronomically inferior background characteristics (Dempewolf *et al.*, 2017). Even beyond this, there is strong agreement on the benefits of conserving and expanding genetic breeding resources to address production constraints (Seiler *et al.*, 2017; Warburton *et al.*, 2017; Khoury *et al.*, 2022).

Table 4. Parent (P) and offspring (O) reaction after SCMoV-C inoculation (RAI) of the crosses performed during the second cycle of selection. F_2 progeny of crosses performed with selected S10, segregating resistant SCMoV-C population (family code 24, Table 3), with two inbred lines, A09 and B09, conformed the sixth generation (G6), and the successive generations (G7 and G8) were obtained from new selections (highlighted in bold) and controlled crosses. RAI: RR, plant without symptoms; MR, plant with mild chlorotic symptoms; SS, susceptible to SCMoV-C. B09, B10 and Contiflor 17 (CF17) were used as susceptible controls. SIB: sibling cross. ▽: self-pollination. ND: no data available. GS: Genetic Stock. The number at the beginning of an inoculated family indicates the family identifier code (e.g., 30 ▽= a self-pollination of resistant plants of F2(A09xS10cRR)). The letter RR followed by the superscript characters MR or * indicates that a selected RR plant showed mild chlorotic mottling or very few isolated chlorotic spots compatible with SCMoV-C infection after transplanting, respectively.

Family identifier code	Inoculated family	PRAI	ORAI (No.)		
			SS	MR	RR
	G6	**Sixth Generation**	**2011-12**		
26	F_2(A09xS10cRR)	ND	0	3	2
27	F_2(A09xS10cRR)	ND	0	3	1
28	F_2(A09xS10cRR)	ND	3	0	1
29	**F_2(A09xS10cRR)**	**ND**	**1**	**0**	**4**
30	**F_2(A09xS10cRR)**	**ND**	**0**	**0**	**4**
31	F_2(B09xS10bRR)	ND	0	2	1
32	F_2(B09xS10bRR)	ND	1	1	2
33	F_2(B09xS10bRR)	ND	0	1	0
34	F_2(B09xS10bRR)	ND	0	1	0
35	F_2(B09xS10bRR)	ND	1	0	0
36	**F_2(B09xS10dRR)**	**ND**	**0**	**1**	**3**
37	F_2(B09xS10dRR)	ND	1	0	1
38	**F_2(S10cRRxB09)**	**ND**	**0**	**0**	**5**
39	F_2(S10cRRxB09)	ND	0	2	2
40	F_2(S10cRRxB09)	ND	0	1	1
41	F_2(S10cRRxB09)	ND	3	0	2
42	F_2(S10cRRxB09)	ND	0	1	3
43	F_2(S10cRRxB09)	ND	1	0	3
44	F_2(S10cRRxB09)	ND	0	2	3
45	F_2(S10cRRxB09)	ND	1	0	3
	CF17		19	0	0
	G7	**Seventh Generation**	**2012-13**		
46	**29 ▽**	**RR**	**0**	**0**	**7**
47	29 ▽	RR	7	0	2
48	30 ▽	RR	2	0	5
49	30 ▽	RR	4	0	4
50	30 ▽	RR	0	0	2
51	36 ▽	RR	2	0	8
52	**36 ▽**	**RR**	**0**	**0**	**5**
53	**38 ▽**	**RR**	**0**	**0**	**10**
54	38 ▽	RR	1	0	9
55	38 ▽	RR	0	0	3
56	38 ▽	RR	0	0	6
	CF17		20	0	0
	G8	**Eighth Generation**	**2013-14**		
GS-1	53 ▽	RR^{MR}	0	0	68
GS-2	46 x 52	RR x RR^{MR}	0	0	34
GS-3	46 x 52	RR x RR*	0	0	34
GS-4	52 ▽	RR*	0	0	68
	B09		34	0	0
	B10		34	0	0

Table 5. Morphological and physiological traits (mean ± standard error) of SCMoV-C resistant genetic stocks (GS). PH: plant height; LP: leaves per plant; LA: foliar surface; HP: heads per plant; SAF: self-fertilized cypselas per head; B1000: thousand cypselas biomass; CL: cypsela length; CW: cypsela width; G-R5: days between germination and flowering; G-R9: days between germination and plant senescence.

Genetic stock	PH (cm)	LP (No.)	LA (cm²)	HP (No.)	SAF (No.)	B1000 (g)	CL (mm)	CW (mm)	G-R5 (days)	G-R9 (days)
GS-1	144,2 ± 5,5	27 ± 1	235,9 ± 21,9	17 ± 3	101 ± 41	33,3 ± 0,2	8,6 ± 0,2	4,7 ± 0,2	104	154
GS-2	176,4 ± 6,8	26 ± 1	295,6 ± 21,4	22 ± 3	83 ± 15	28,8 ± 2,3	7,3 ± 0,2	4,2 ± 0,1	104	147
GS-3	126,5 ± 10,3	27 ± 1	171,1 ± 28,5	18 ± 6	62 ± 14	23,8 ± 2,5	7,2 ± 0,2	4,2 ± 0,1	107	140
GS-4	95,5 ± 5,2	25 ± 1	96,1 ± 13,6	6 ± 1	54 ± 15	12,4 ± 0,7	6,2 ± 0,1	3,4 ± 0,1	107	133

As SCMoV-C resistant genetic stocks were the result of crosses between cultivated and wild biotype parents, they showed intermediate morphological, reproductive, and phenological characteristics between them, a fact commonly observed in this type of crossing (Gandhi *et al.*, 2005; Baack *et al.*, 2008; Presotto *et al.*, 2011).

The height of SCMoV-C resistant germplasms ranged from 95 to 177 cm. In GS-3 and GS-4 the height was less than 130 cm (Table 5) resembling the height of the parental inbred lines. The height of the lines involved in the development of a hybrid should be 40 cm shorter than the desired height of the hybrid, generally between 150-180 cm (Škorić, 2012).

Due to their wild origin, plants of the four resistant genetic stocks showed branches with several heads. In addition, they presented anthocyanins on the stem or leaf petioles, except for GS-4 (Table 6). These traits resembled the wild Argentine sunflower biotypes (Presotto *et al.*, 2011). However, all the SCMoV-C genetic stocks exhibited a main larger inflorescence (Table 6), 22 to 27 leaves on the main stem, with a leaf area exceeding 90 cm² (Table 5). Apical dominance is a characteristic of domesticated sunflowers, which are generally monoecious with large heads that produce large seeds (Škorić, 2012; Radanović *et al.*, 2018). The intermediate condition, larger main heads, and branching are the product of hybridization between wild and cultivated sunflowers (Presotto *et al.*, 2011).

The level of seed set under self-pollination of the resistant genetic stocks was higher than that observed in the wild populations from which they originated. The developed genetic stocks produced more than 50 seeds per head and the GS-1 genetic stock produced the highest number of seeds by self-fertilization, about 100 per head.

Full flowering (R5) took place between 99 to 107 days after seed germination. The flowering period was long, more than two weeks, because of the pluri-head plant morphology. The phenological stage R9 was reached between 26 and 58 days after flowering (Table 5). The length of the ontogenetic cycle was more than 130 days. The SCMoV-C-resistant germplasms could be considered to have a long growth cycle (Fick, 1978; Škorić, 2012).

The cypsela biomass of resistant genetic stocks was greater than 20 mg, except in GS-4, and the fruit length and width were equal to or greater than 6 and 3 mm, respectively (Table 5). Seed size and biomass were intermediate between the sunflower inbred lines (Pekcan *et al.*, 2015) and wild sunflower (Presotto *et al.*, 2009; 2011).

Male sterile plants were observed in GS-2 and GS-3. These germplasms have PET1-type male-sterile cytoplasm donated by A09 male-sterile line (Figure S2); presumably the nuclear fertility restoration genes are in a heterozygous state (Fick and Miller, 1997; Acquaah, 2012). The GS-2, GS-2, and GS-3 showed *Verticillium* wilt symptoms in the common garden. *V. dahliae* produces early leaf drying (Gulya *et al.*, 1997) and a reduction in crop production in susceptible genotypes (Wang *et al.*, 2021). Therefore, it is possible that evaluated quantitative traits had been undervalued in those genetic stocks. GS-1 did not exhibit any *Verticillium* wilt symptoms

Self-fertilized seeds of the resistant genetic stocks, GS-1, GS-2, GS-3 and GS-4, were deposited in the Active Germplasm Bank of the INTA Manfredi, registered with codes CGGI1351, CGGI1352, CGGI1353 and CGGI1354, respectively.

CONCLUSIONS

The wild Argentine *H. annuus* showed abundant plants without any SCMoV-C symptoms after inoculation. BAR showed the highest proportion among the nine accessions tested.

Crossing of the symptoms-free SCMoV-C BAR plants with inbred lines, followed by a recurrent selection for SCMoV-C resistance and self-fertility, led to the generation of four SCMoV-C resistant genetic stocks, GS-1, GS-2, GS-3 and GS-4, without phenotypic segregation for the resistance trait.

Among the resistant germplasms, GS-1 presented the most suitable phenotype for use as a SCMoV-C resistance donor to the cultivated sunflower. The traits in which it stood out were the absence of segregation for male fertility, high production of self-fertilized seeds,

Table 6. Morphological traits of SCMoV-C resistant genetic stocks (GS). BRA: plants with branches; A: absent; AB: apical branching; BB: basal branching; or FB: full branching. MH: plants with main head. ANT: plants with stem anthocyanin. (*) Plants with signs of Verticillium.

Genetic stock	BRA (%)				MH (%)	ANT (%)	Other traits
	A	AB	BB	FB			
GS-1	0	0	0	100	100	100	Presence of plants with only tubular flowers, larger on the margins than at the center of the head.
GS-2	0	16	0	84	100	68	Presence of male-sterile plants. Presence of plants with only tubular flowers, larger on the margins than at the center of the head. (*)
GS-3	5	28	0	67	100	67	Presence of male-sterile plants. Presence of plants with only tubular flowers, larger on the margins than at the center of the head. (*)
GS-4	10	20	13	57	100	0	(*)

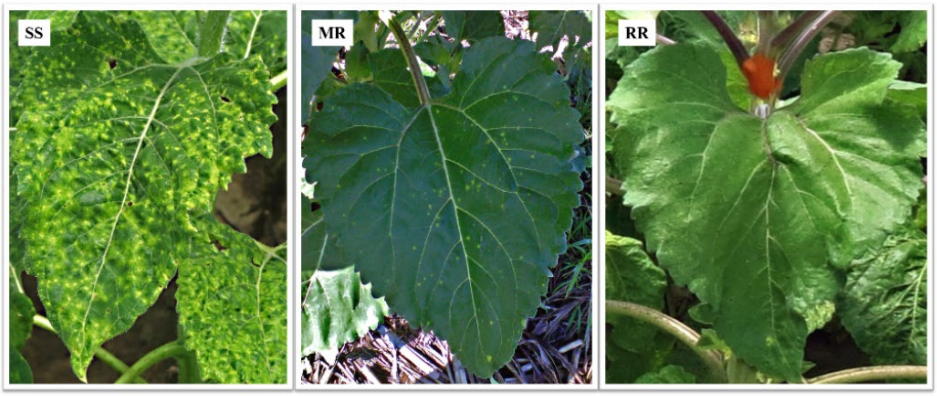

Figure 1. Classification of sunflower plant reaction after artificial SCMoV-C inoculation by lamina symptoms: SS: confluent chlorotic mottling over the entire lamina; MR: mild chlorotic mottling; RR: no visible disease symptoms of SCMoV-C.

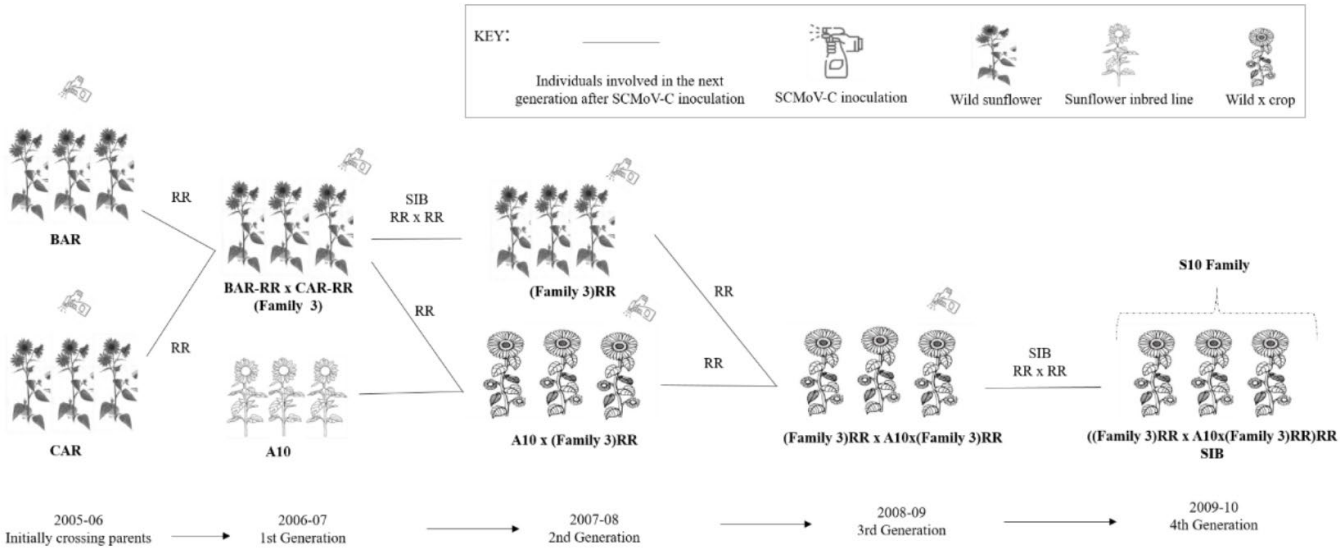

Figure S1. Overview of the crosses performed and inoculation rounds on the first cycle of selection against SCMoV-C infection to conform the S10 family. Plants without symptoms (RR) of SCMoV-C disease after artificial inoculation were chose to continuous selection. A10: male sterile inbred line, BAR: wild sunflower from Colonia Barón. CAR: wild sunflower from Carhué. SIB: sibling cross.

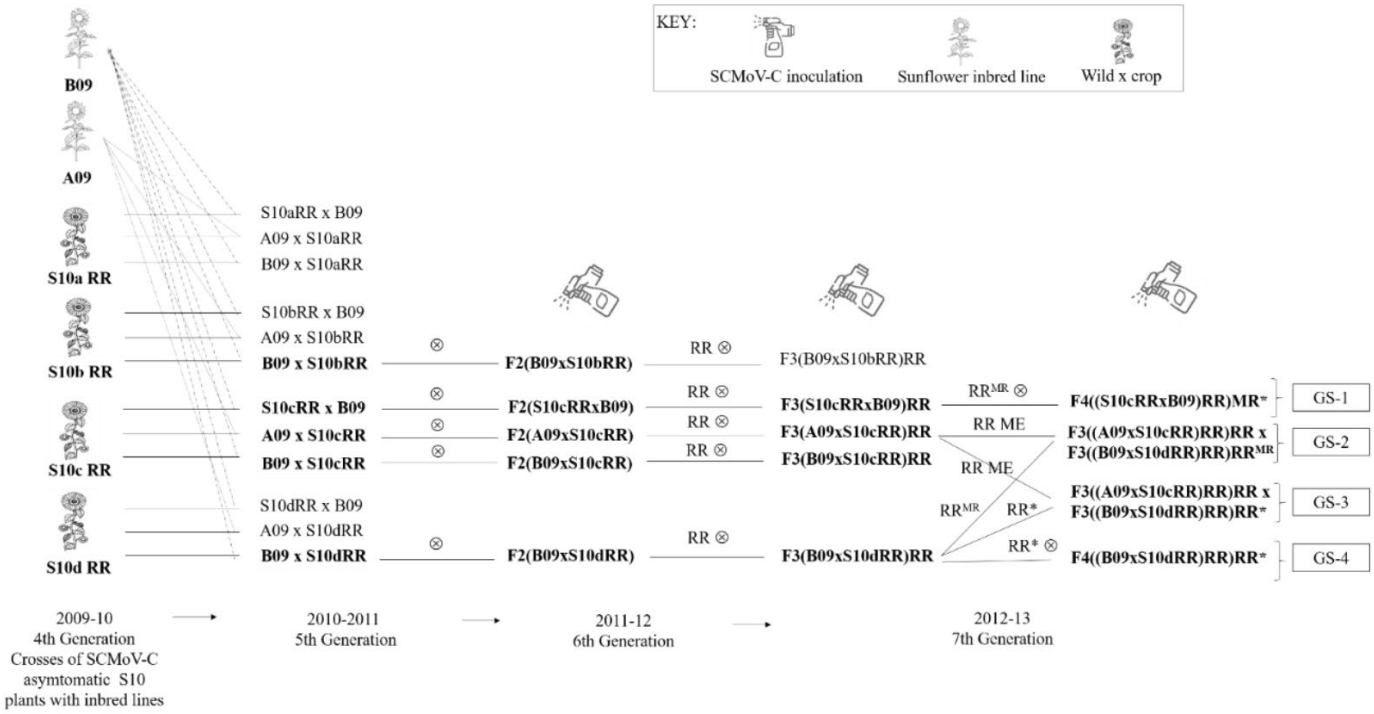

Figure S2. Overview of the successive intercrossings and inoculation rounds carried out in the second cycle of selection to conform the SCMoV-C resistant genetic stocks (GS). RR: plants without symptoms of SCMoV-C disease after artificial inoculation. The letter RR followed by the superscript characters MR or * indicates that a selected RR plant showed mild chlorotic mottling or very few isolated chlorotic spots compatible with SCMoV-C infection after transplanting, respectively. A09: male sterile inbred line. B09: A09 maintainer line. S10: segregant germplasm developed in the first cycle of selection. Lower-case letters added to the S10 to indicate different plants. ⊗: self-pollination.

presence of a main head larger than the secondary ones and seminal biomass greater than 30 mg.

These germplasms are open-access resources for breeders, their contribution to research or the development of new breeding lines or cultivars implies a commitment to appropriate recognition.

ACKNOWLEDGMENTS

We thank the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT) and Universidad Nacional del Sur (UNS) for the fellowship to Ivana Fernández Moroni. This work was supported by grants from ANPCYT (PAE 37100 PICT 020; PICT-2012-2854), INTA and UNS (PGI 24A204) of Argentina.

REFERENCES

Acquaah G. (2012) Principles of plant genetics and breeding. Wiley-Blackwell Editorial, Oxford, UK.

Agrios G. (2005) Plant Pathology. Elsevier Academic Press Editorial, Amsterdam, Netherlands.

Aguirrezábal L., Orioli G., Hernández L., Pereyra V., Miravé J. (1996) Girasol. Aspectos fisiológicos que determinan el rendimiento. Unidad Integrada Balcarce Editorial. Buenos Aires. Argentina.

Astiz V., Iriarte L.A., Flemmer A., Hernández L.F. (2011) Self-compatibility in modern hybrids of sunflower (*Helianthus annuus* L.). Fruit set in open and self-pollinated (bag isolated) plants grown in two different locations. Helia 34 (54):129-138.

Baack E., Sapir Y., Chapman M., Burke J., Rieseberg L. (2008) Selection on domestication traits and quantitative trait loci in crop–wild sunflower hybrids. Mol. Ecol. 17: 666–677.

Bello V.H., Madoglio Favara G., Verruma Bernardi G., Marques Rezende J.A., Barbosa Salaroli R., Watanabe Kitajima E. (2023) First report of sunflower chlorotic mottle virus infecting sunflower plants in Brazil. Sci. agric. 80. https://doi.org/10.1590/1678-992X-2022-0035

Cabrera Mederos D., Torres C., Bejerman N., Trucco V., Lenardon S., Leiva Mora M., Giolitti F. (2020) Phylodynamics of sunflower chlorotic mottle virus, an emerging pathosystem. Virolog. 545: 33-39.

Cantamutto M., Poverene M., Peinemann N. (2008) Multi-scale analysis of two annual Helianthus species naturalization in Argentina. Agric. Ecosyst. & Environ. 123 (1–3): 69-74.

Cantamutto M., Poverene M., Presotto A., Alvarez D., Lenardon S., Rodríguez R., Martín Sánchez J., Fernández Moroni I., Giolitti F., Garayalde A., Haucke A., Bellido A., Fraysse M. (2010a) The argentine wild *Helianthus annuus* L. genetic resource. Helia 33 (52): 47-62.

Cantamutto M., Presotto A., Fernández Moroni I., Alvarez D., Poverene M., Seiler G. (2010b) High infraspecific diversity of wild sunflowers (*Helianthus annuus* L.) naturally developed in central Argentina. Flora 205 (5): 306–312.

Cubero J. (2003) Introducción a la mejora genética vegetal. Wiley-Blackwell Editorial, Oxford, UK.

de Ronde D., Butterbach P., Kormelink R. (2014) Dominant resistance against plant viruses. Front. Plant Sci. 5. https://doi.org/10.3389/fpls.2014.00307

Dempewolf H., Baute G., Anderson J., Kilian B., Smith C., Guarino L. (2017) Past and future use of wild relatives in crop breeding. Crop Sci. 57: 1070-1082.

Dujovny G., Sasaya T., Koganesawa H., Usugi T., Shoara K, Lenardon S. (2000) Molecular characterization of a new potyvirus infecting sunflower. Arch. Virol. 145: 2249-2258.

Dujovny G., Usugi T., Shoara K, Lenardon S. (1998) Characterization of a potyvirus infecting sunflower in Argentina. Plant Disease 82 (5): 470-474.

Fick G. (1978) Breeding and genetics. In: American Society of Agronomy (Ed.) Sunflower science and technology. Madison, US, pp. 279-338.

Fick G., Miller J. (1997) Sunflower breeding. In: American Society of Agronomy (Ed.) Sunflower technology and production. Madison, US, pp. 395-440.

Gandhi S., Heesacker A., Freeman C., Argyris J., Bradford K., Knapp S. (2005) The self-incompatibility locus (S) and quantitative trait loci for self-pollination and seed dormancy in sunflower. Theor. Appl. Genet. 111: 619-629.

Garayalde A.F., Poverene M., Cantamutto M., Carrera A.D. (2011) Wild sunflower diversity in Argentina revealed by ISSR and SSR markers: an approach for conservation and breeding programs. Ann. Appl. Biol. 158 (3): 305-317.

Garayalde A.F., Presotto A., Carrera A., Poverene M., Cantamutto M. (2015) Characterization of a new male sterility source identified in an invasive biotype of *Helianthus annuus* (L.). Euphytica 206 (3): 579-595.

Giolitti F., Bejerman N., de Breuil S., Lenardon S. (2010) Identification and characterization of a new strain of Sunflower chlorotic mottle virus, a Potyvirus infecting Asteraceae in Argentina. J. Phytopathol. 158 (7-8): 536-541.

Gómez P., Rodríguez Hernández A., Moury B., Aranda M. (2009) Genetic resistance for the sustainable control of plant virus diseases: breeding, mechanisms and durability. Eur. J. Plant. Pathol. 125:1-22.

Gontcharov S.V. (2014) Dynamics of hybrid sunflower disease. Helia 37 (60): 99-104.

González J., Mancuso N., Alvarez D., Cordes D., Vázquez A. 2015. Contribution of the Argentine germplasm to the improvement of sunflower. Helia 38 (62): 121-140.

Gore M.A., Hayes A.J., Jeong S.C., Yue Y.G., Buss G.R., Saghai Maroof M.A. (2002) Mapping tightly linked genes controlling potyvirus infection at the Rsv1 and Rpv1 region in soybean. Genome 45: 592–599.

Gulya T., Harveson R., Mathew F., Block C., Thompson S., Kandel H., Berglund D., Sandbakken J., Kleingartner L., Markell S. (2019) Comprehensive Disease Survey of U.S. Sunflower: Disease Trends, Research Priorities and Unanticipated Impacts. Plant Disease 103: 601-618.

Gulya T., Rashid K., Masirevic S. (1997) Sunflower diseases. In: American Society of Agronomy (Ed.) Sunflower technology and production. Madison, US, pp. 263-380.

Gutierrez A., Carrera A., Basualdo J., Rodriguez R., Cantamutto M., Poverene M. (2009) Gene flow between cultivated sunflower and *Helianthus petiolaris* (Asteraceae). Euphytica 172 (1): 67-76.

Gutierrez A., Rueda F., Cantamutto M., Poverene M. (2014) Self-pollination and its implication in invasiveness of *Helianthus annuus* ssp. *annuus* and *H. petiolaris*. BAG 25: 5-15.

Hajjar R., Hodgkin T. (2007) The use of wild relatives in crop improvement: a survey of development over the last 20 years. Euphytica 156: 1-13.

Hernández F., Presotto A., Poverene M., Mandel J. (2019) Genetic diversity and population structure of wild sunflower (*Helianthus annuus* L.) in Argentina: reconstructing its invasion history. J. Hered. 110 (6): 746–759.

ICTV-International Committee on Taxonomy of Viruses (2024) https://ictv.global/report/chapter/potyviridae/potyviridae/potyvirus (accessed June 2024).

ISTA- International Seed Testing Association (2004) International rules for seed testing. International Seed Testing Association Editorial, Zürich, Switzerland.

Jan C.C., Gulya T.J. (2006a) Registration of a sunflower germplasm resistant to rust, downy mildew, and virus. Crop Sci. 46 (4): 1829.

Jan C.C., Gulya T.J. (2006b) Registration of three virus resistant sunflower genetic stocks. Crop Sci. 46 (4):1834-1835.

Jones R.A.C. (2004) Using epidemiological information to develop effective integrated virus disease management strategies. Virus Res. 100: 5–30.

Khoury C., Brush S., Costich D., Curry H., Haan S., Engels J.M.M., Guarino L., Hoban S., Mercer C.L., Miller A.J., Nabhan G.P., Perales H.R., Richards C., Riggins C., Thormann I. (2022) Crop genetic erosion: understanding and responding to loss of crop diversity. New Phytol. 233: 84-118.

Lee J.H., An J.T., Siddique M.I., Han M., Choi S., Know J.K., Kang B.C (2017) Identification and molecular genetic mapping of Chili venial mottle virus (ChiVMV) resistance genes in pepper (*Capsicum annuum*). Mol. Breeding 37 (121). https://doi.org/10.1007/s11032-017-0717-6.

Lenardon S. (1994) Síntomas de etiología viral en cultivos de girasol. In: INTA (Ed.) Enfermedades del girasol en la Argentina. Balcarce, Argentina, pp. 99-103.

Lenardon S., Bazzalo M., Abratti G., Cimmino C., Galella M., Grondona M., Giolitti F., León A. (2005) Screening sunflower for resistance to Sunflower chlorotic mottle virus and mapping the Rcmo-1 resistance gene. Crop Sci. 45: 735-739.

Lenardon S., Giolitti F., León A., Bazzalo M., Grondona M. (2001) Effect of Sunflower chlorotic mottle virus infection on sunflower yield parameters. Helia 24 (35): 55-66.

Maule A., Caranta C., Boulton M. (2007) Sources of natural resistance to plant viruses: status and prospects. Mol. Plant Pathol. 8 (2): 223-231.

Melchinger A., Kuntze L., Gumber R., Lübberstedt T., Fuchs E. (1998) Genetics basis of resistance to sugarcane mosaic virus in European maize germplasm. Theor. Appl. Genet. 96: 1151-1161.

Pekcan V., Evci G., Yilmaz M.I., Nalcaiyi A.S.B., Erdal S.Ç., Cicek N., Ekmekci Y., Kaya Y. (2015) Drought effects on yield traits of some sunflower inbred lines. J. Agric. For. 61 (4): 101-107.

Poverene M., Cantamutto M., Carrera A., Ureta M., Salaberry M., Echeverria M., Rodriguez R. (2002) El girasol silvestre (*Helianthus* spp.) en la Argentina: caracterización para la liberación de cultivares transgénicos. RIA 31 (2): 97-116.

Presotto A., Cantamutto M., Poverene M., Seiler G. (2009) Phenotypic diversity in wild *Helianthus annuus* from Argentina. Helia 32 (50): 37-50.

Presotto A., Fernández Moroni I., Poverene M., Cantamutto M. (2011) Sunflower crop-wild hybrids: Identification and risks. Crop Prot. 30: 611-616.

Radanović A., Miladinović D., Cvejić S., Jocković M., Siniša J. (2018) Sunflower genetics from ancestors to modern hybrids- A review. Genes 9 (11): 528. https://doi.org/10.3390/genes9110528.

Revers F., Le Gall O., Candresse T., Maule A. (1999) New advances in understanding the molecular biology of plant/potyvirus interactions. MPMI 12 (5): 367–376.

Rossi E., Borghi M., Di Renzo M., Bonamico M. (2015) Quantitative trait loci (QTL) identification for resistance to Mal de Río Cuarto virus (MRCV) in maize based in segregate population. Open Agric. J. 9: 48-55.

Schneiter A., Miller J. (1981) Description of sunflower growth stages. Crop Sci. 21: 901-903.

Seiler G., Qi L.L., Marek F. (2017) Utilization of sunflower crop wild relatives for cultivated sunflower improvement. Crop Sci. 57 (3): 1083-1101.

Škorić D. (2012) Sunflower genetics and breeding. Int. Monography. Editorial Serbian Acad. Sci. Arts, Novi Sad, Serbia.

Sun Y., Godwin I.D., Arief V.N., Delacy I.H., Jackway P.T., Lambrides C. J. (2012) Factors controlling self-fertility in sunflower: the role of GCA/SCA effects, alleles, and floret characteristics. Crop Sci. 52:128-135.

Tatineni S., Hein G.L. (2023) Plant viruses of agricultural importance: current and future perspective of virus disease management strategies. Phytopathology® 113 (2):117-141.

Ureta S., Carrera A., Cantamutto M., Poverene M. (2008) Gene flow among wild and cultivated sunflower, *Helianthus annuus* in Argentina. Agric. Ecosyst. Environ. 123: 343–349.

Vasquez A., de Romano A. (2006) Sunflower crop in Argentina to date. Helia 29:159-164.

Wang D., Su Z., Ning D., Zhao Y., Meng H., Dong B., Zhao J., Zhou H. (2021) Different appearance period of *Verticillium* wilt symptoms affect sunflower growth and production. J. Plant Pathol. 103: 513-517.

Warburton M.L., Rauf S., Marek L., Hussain M., Ogunola O., Sanchez Gonzalez J.J. (2017) The use of crop wild relatives in maize and sunflower breeding. Crop Sci. 57: 1227-1240.