Combining ability among some Argentine sunflower populations for *Sclerotinia sclerotiorum* resistance

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Introduction

Sclerotinia sclerotiorum Lib.de Bary is one the most dangerous pathogens in the sunflower crop because it can significantly reduce the yield especially in temperate and humid environments. Although it can produces damage in almost all organs of sunflower plant, white rot in capitula is the principal disease in Argentina (Castaño and Rodriguez, 2001) and basal stem attack is frequent in Centre and North Italy, especially with early spring sowing times (Zazzerini *et al*. 1987).

Diverse control procedures have been proposed to limit damage by *S. sclerotiorum* but at present sowing hybrids, obtained by crossing selected inbred lines with a good resistance level to the disease, is the most encouraging method to diminish risk of epidemics (Castaño *et al*., 2001).

Sunflower breeders normally use different populations as sources of genetic variability (Fick and Miller, 1987). These populations must be genetically characterised in order to choose the most efficient breeding method to develop parental inbred lines with a high combining ability for agronomic traits as disease resistance**.**

In breeding programs for *Sclerotinia* resistance, the field test under both natural or artificial infection remains one of the most practised protocols in the detection of moderately resistant genotypes even if laborious and time consuming and often not reliable due to high variability in inoculum pressure and environmental conditions for the disease development.

For this reason it seems necessary to develop others methodologies, as a greenhouse resistance test, that could be rapidly and easily used, and less time consuming for determining the genotype performance for *Sclerotinia* resistance (Tahmasebi-Enferadi *et al*., 2000).

The aim of this work was: 1) to evaluate the performance of four Argentinean populations and their progeny to *S. sclerotiorum* inoculation on basal stem of sunflower seedlings under greenhouse condition, in order to assess their usefulness as sources of resistance, and 2) to estimate the genetic components of complex population variance with a diallel crossing system for a fixed model, using Griffing Method II.

Materials and Methods

A synthetic population (B1) and three Argentinean varieties (Norkinsol 2, Pehuén INTA and Antilco) and their progeny, obtained by a diallel crossing system for a fixed model with Griffing method II (without reciprocals), were used.

A greenhouse test was conducted at the University of Udine, Italy, with the daytime temperature maintained at about 22°C and the night temperature at 18°C, with a relative humidity of 60% and 70% respectively. A constant 16 hours of photoperiod was provided by a timer connected to 10 lamps (Philips SGR 200/400) which assured a minimum photon flux density of about 900μ E m^{-2} at the top of the seedlings.

Seeds of 10 genotypes were sown in a sandy loam soil in aluminium pots (30 cm long, 20 cm wide and 6 cm deep). Twenty seeds per pot and four pots for each genotype, three for inoculated and one for non-inoculated seedlings as control, were used. Experimental scheme was a completely randomised design, with three replications (pots) with 12 inoculated seedlings in each plot. Soil was maintained at approximately field capacity until the end of the experiment.

Inoculation was carried out 28-days after sown, at V8 plant stage of sunflower development (Schneiter and Miller, 1981), placing an infected oat grain with *S. sclerotiorum* mycelium on the basal stem of seedlings. Inoculum was covered with moistened cotton and a plastic film to maintain humidity.

The percentage of dead seedlings at 25 days after inoculation was scored. Data were first analysed to test significance of genotypic differences. Then an analysis of variance of Griffing's combining abilities was made and both general combining ability (GCA) and specific combining ability (SCA) effects were estimated. The software MSTATC was used.

Results and Discussion

Analysis of variance of the dead seedlings, 25 days after *S. sclerotiorum* inoculation on basal stem, detected highly significant ($p<0.001$) genotypic effects and none significant blocks difference (Table 1).

Table 1. Analysis of variance, Method II, of genotypic responses (as dead seedlings in %) to *Sclerotinia sclerotiorum* inoculations on basal stem of sunflower seedlings

Source	SS	Df	MS	F
Genotypes	10709.01	9	1189.89	71.56**
Blocks	29.57	2	14.78	0.89
Error	299.30	18	16.62	

** = $p<0.001$

An experimental mean of 58.3% of dead seedlings, 25 days after inoculation, was scored (Table 2); this value indicates that less than a half of the inoculated seedlings survived the test. The low coefficient of phenotypic variability (7%) evidenced a good precision of the experiment. The least significant differences test (l.s.d. =7, $p<0.05$) showed that the progeny of the cross Norkinsol 2 x B1 (NxB) and Pehuén INTA (P) had the highest resistance level (24.2% and 27.8% of dead seedlings, respectively) and conversely Antilco x Pehuén INTA (AxP) was the most susceptible one (88.8%).

Table 2. Average of percentage of dead seedlings 25 days after *Sclerotinia sclerotiorum* inoculations on basal stem of 10 sunflower genotypes

Genotypes	Mean of percentage of dead seedlings
Antilco x Pehuén INTA (AxP)	88.8
Norkinsol 2 x Pehuén INTA (NxP)	76.1
Pehuén INTA x B1 (PxB)	70.6
Antilco (A)	62.7
Norkinsol 2 (N)	62.5
B1 (B)	60.7
Norkinsol 2 x Antilco (NxA)	56.5
Antilco x B1 (AxB)	52.8
Pehuén INTA (P)	27.8
Norkinsol 2 x B1 (NxB)	24.2
Mean	58.3
CV (%)	7.0
Lsd ($p<0.05$)	7.0

The GCA and SCA effects were statistically significant ($p<0.001$), suggesting that a considerable genetic variability exist among parents and hybrids (Table 3). This result indicates that both additive and non-additive effects were involved in the expression of the dead seedlings percentage, although the non-additive variance was six times higher. This indicates the non-additive genetic control is preponderant, but the additive effect is far to be negligible.

Table 3. Analysis of variance of Griffing's General and Specific Combining Abilities, Method II, Fixed Model.

Source	SS	Df	MS	F
G.C.A.	814.03	3	271.34	16.32**
S.C.A.	9894.98	6	1649.16	99.18**
Error	299.30	18	16.62	

** = $p<0.001$

Table 4 presents both general and specific combining abilities values for the sunflower populations crossed to calculate genetic component of variance. Antilco (A) and B1 (B) populations presented significant ($p<0.05$) GCA effects; given that B1 had the most favourable GCA effect (-3.72%) it may be considered the best combiner in this experiment. All SCA effects were significantly different from zero. The best cross, showing the most favourable estimates of SCA effect (-28.75%) was Norkinsol 2 x B1 (NxB), suggesting that this population-cross had a higher percentage of survived seedlings than it was expected since the GCA effects of each parent.

Table 4. General Combining Ability, in diagonal, and Specific Combining Ability, off diagonal, ± Standard Error, of sunflower genotypes evaluated by the percentage of dead seedlings 25 days after inoculation at basal stem.

Genotypes	N[$]	A	P	B
N	-1.59 ± 1.44	-5.54* ± 2.57	19.46** ± 2.57	-28.75** ± 2.57
A		5.36* ± 1.44	25.20** ± 2.57	-7.10* ± 2.57
P			-0.04 ± 1.44	16.10** ± 2.57
B				-3.72* ± 1.44

[$]= See table 2

* , ** = $p<0.05$ and $p<0.01$, respectively

Although further research is necessary to produce additional information, it is possible to make some important considerations. The experiment occupied little space in the greenhouse and it could be carried out in an off-season environment. The inoculation protocol was easily made and the data were obtained less than two months after sowing date with a relatively high precision. Results allowed to differentiate genotypes according to the percentage of dead seedlings and to detect superior parents, B1 (B), and crosses, Norkinsol 2 x B1 (NxB), which could be used in programs of intra and inter-population selection for *Sclerotinia* resistance.

References

Castaño, F. and R. Rodríguez, 2001. El cultivo de girasol y un enfoque genético sobre la resistencia a la Podredumbre Blanca de los capítulos. In: http://www.fyo.com.ar/granos/produccion/especiales/girasol/ampliar.asp?idinformacion=12412

Castaño, F., F. Vear and D. Tourvieille, 2001. Horizontal resistances in sunflower: a review of a workshop at the 15th International Sunflower Conference. OCL, 8: 211-215.

Fick, G. and J. Miller, 1997. Sunflower breeding. In: Sunflower Technology and Production, Schneiter, A. (Ed.), ASA, CSSA, SSSA, Madison, 395-439 p.

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

Tahmasebi-Enferadi, S., Turi, M., Baldini, M. and G.P. Vannozzi, 2000. Comparison between artificial inoculation and culture filtrate of *Sclerotinia Sclerotiorum* Lib. de Bary treatments on nine sunflower genotypes. 15th International Sunflower Conference Toulouse, France, June 2000.Tome II:K 23-K28.

Zazzerini, A., Monotti, M. and L. Tosi, 1987. Results of a two-years investigation on the most important pathogens in Italy. Helia 10: 47-51.

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