

## EVALUATION OF GENETIC VARIABILITY FOR *Sclerotinia sclerotiorum* (Lib.) de Bary RESISTANCE IN SUNFLOWER AND UTILIZATION OF ASSOCIATED MOLECULAR MARKERS

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### SUMMARY

In the framework of a bilateral project Italy – Romania (University of Udine and IC), an experiment was established at the University of Udine Experiment Station in order to: i) evaluate the genetic variability of the tested genotypes for *Sclerotinia* basal stem and head infection; ii) to test two new alternative screening methods against white head rot; iii) to investigate possible relationships among the different tests; iii) to find resistance-associated fragments by AFLP analysis.

The following tests were used: 1) basal stem infection, 2) ascospore head infection, 3) mycelium culture filtrate injection and 4) oxalic acid injection into the back face of the head.

The segregation of responses between resistant and susceptible controls displayed the suitability of mycelium and ascospore tests adopted and the independence of the two tolerance mechanisms.

Conversely, tests 3 and 4 showed a poor discrimination of the controls and a complete lack of relationships with mycelium and ascospore infections. Sunflower reaction against *S. sclerotiorum* may not be restricted to resistance to oxalic acid, but may involve other physiological mechanisms.

The inbred line 28R (coming from *H. argophyllus* wild species) was not only most tolerant against both, basal stem and white head rot infections, but also it gave the best performance in oxalate and culture filtrate tests highlighting a specific resistance to oxalate.

**Key words:** *Sclerotinia sclerotiorum*, sunflower, infection modalities, genetic variability, AFLP, molecular markers

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## INTRODUCTION

*Sclerotinia sclerotiorum* (Lib.) de Bary significantly reduces the yield of sunflower, especially in temperate and humid environments. Although it affects all parts of the plant, the infection on the head (known as "white head rot") causes the greatest yield losses in the main sunflower-growing areas in Italy and abroad (e.g. Argentina, Europe, China). The basal stem attack is not infrequent in some areas of Central Italy with early spring sowing times (Zazzerini *et al.*, 1987).

Resistance to *Sclerotinia sclerotiorum* is polygenic and under additive control (Vear and Tourvielle, 1984; Castano *et al.*, 1992) so that breeding programs have to combine favorable genes from different sunflower genotypes. Different parts of the sunflower plant exhibit in general independent types of resistance (Tourvielle and Vear, 1990), but Castano *et al.* (1989) found a positive correlation between the root and leaf levels of tolerance. These results suggested that simple resistance tests may be practically applied to the capitulum in substitution of the more complicated tests which are presently used.

Chemicals with curative effects available at present are not practical and economical. Although the biotechnological approach by genetic transformation has produced promising results for *Sclerotinia* head resistance (Bazzalo *et al.*, 2000), individuation of tolerant genotypes still remains the main aim of breeders. The field test under natural and/or artificial infection is extensively practiced but it is laborious, time consuming and not always reliable for disease development due to high variability in inoculum pressure and environmental conditions (Hahn, 2000). Moreover, high temperature, often recorded in the summer under greenhouse conditions, can limit the screening ability of the fungus (Boland and Hall, 1987). For these reasons it is necessary to develop other easy and rapid methods as fungal culture filtrate and oxalate tests (Tahmasebi-Enferadi *et al.*, 1998), which are not adversely affected by temperatures up to 40°C.

The aim of this work was: i) to evaluate the reaction of different inbred lines to the two main *Sclerotinia sclerotiorum* infection attacks (basal stem and head); ii) to investigate two new screening tests against white head rot by direct injection into the head of the mycelium culture filtrate and oxalic acid; iii) to find possible relationships among different infection test modalities; iv) to carry out a preliminary molecular analysis by AFLP markers in order to find resistance-associated fragments.

## MATERIALS AND METHODS

### Genetic material

Eight inbred lines, coded 28R, R978, 9304CH, 51333RO, 5134RO, 5141RO, HA89, and 303CH, were tested. The following inbred lines were obtained at University of Udine: 28R, a *Helianthus annuus* x *Helianthus argophyllus*-derived B line

selected for physiological parameters related to drought tolerance; R978, an experimental high oleic restorer line; 9304CH, an experimental restorer line with good GCA; 303CH, an experimental B line, used as control, obtained from a Chinese population after selection for high performance against the natural infection of white head rot. These inbred lines came from the breeding program of Udine University. The experimental inbred A lines 5133RO, 5134RO, 5141RO were obtained by ICCPT, Fundulea (Romania). HA 89, a public inbred line, was used as the susceptible control. All of the above genotypes showed a very similar flowering time (the range was +3 and - 2 days from HA89).

### **Fungal isolates**

- a. ***Sclerotinia mycelium***. The inoculum was obtained according to Tahmasebi-Enferadi *et al.* (1998). After three/four weeks, the infected oat seeds were removed, air dried and directly used as inoculum or stored in sealed Petri dishes.
- b. ***Sclerotinia culture filtrate***. The filtrate was obtained following the protocol of Tahmasebi-Enferadi *et al.*, (1998) with a 2 g/l oxalic acid concentration and pH 4.0. Oxalic acid concentration was determined with an Endpoint enzymatic-colorimetric kit (591C, Sigma Chemical Co. St. Louis, MO, USA).
- c. **Aqueous solution of oxalic acid**. The solution was adjusted at pH 4.0 to the final concentration of 2 g/l.
- d. ***Sclerotinia ascospores***. The ascospores were obtained following the procedure described by Castano and Rodriguez (1997). The production of apothecia begun 38 days after sowing of frozen sclerotia and continued for about 40 days. Periodically, apothecia were harvested, dried under artificial light (40 - 60 W) for 4 h, and ascospores were collected and stored in Petri dishes. The ascospores were counted and diluted with distilled water to a concentration of 2500/ml for artificial infection.

### **Field trials**

Field trials were carried out in 2001, at the Experiment Farm of the University of Udine, at S. Osvaldo (UD) in Northern Italy, (latitude 45° 2' N, altitude 60 m) on a loamy-sandy and shallow soil (about 60 cm), without water table and with 5% of gravel.

The mechanical operations applied were the plowing at 30 cm followed by two light harrowings. The preceding crop was sunflower. At the end of the winter, before sowing, 150 kg N ha<sup>-1</sup>, 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 100 kg K<sub>2</sub>O ha<sup>-1</sup> were applied. Temperature and rainfall records are reported in Figure 1. Hand sowing was performed on 15 May and the emergence occurred 7 days later. Plots were hand thinned to a single plant spacing of 25 cm in the row. The experimental scheme was a rand-

omized block design with three replications. The experimental unit was composed of two 7 m rows spaced 0.50 m apart, with a plant density of 8 p/m<sup>2</sup>.

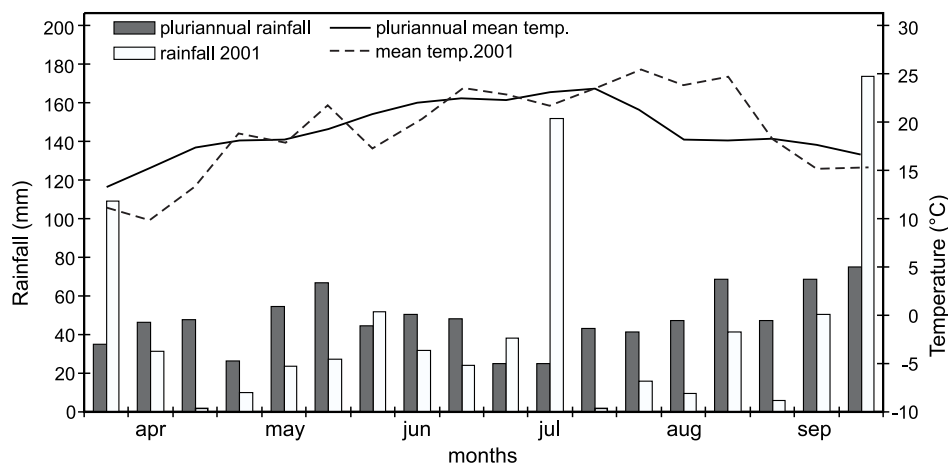


Figure 1: Rainfall and air mean temperature during the year 2001 and the previous ten years

Two sprinkler irrigations (10 mm each) were applied weekly during the entire crop cycle to maintain the soil at non-limiting water condition. Weed control was performed by hand after plant emergence without any pesticide treatment.

#### Resistance tests

- a. **Basal stem infection by mycelium.** Ten plants per plot were inoculated by hand on 7 July, 45 days after emergence, at R2 plant growth stage (Schneider and Miller, 1981). Two oat seeds, infected by mycelium, were positioned over the basal stem and covered with moist cotton wool and a transparent plastic film in order to maintain humidity (Tahamasebi-Enferadi *et al.*, 2000). The percentage (%) of dead plants was recorded fifty days after infection.
- b. **Mycelium culture filtrate and (c) oxalic acid solution tests.** At flower bud (R4) stage (Schneider and Miller, 1981), ten plant samples from each plot were injected on the back of the head with 10 ml of *Sclerotinia* culture filtrate (toxin) (Raducanu, 2000) and 10 ml of oxalic acid solution. Both solutions had the same oxalic acid concentration (2 g/l), corresponding to 20 mg of oxalate per plant. After two weeks, the inoculated capitula started to be observed twice a week for symptoms of white head rot-like. Plant reaction was measured as percentage (%) of brown rotted area per total head area.
- c. **Head infection with ascospore inoculum.** At flowering stage (R5.3), with approximately three external rows of hermaphrodite disk in the pistillate stage (Schneider and Miller, 1981), the floral surface of ten capitula per plot was sprayed with 10 ml (5 ml twice a week) of an aqueous 2500 ascospore/

ml suspension. The inoculated plants were immediately covered with bags in order to avoid drying. After two weeks, the capitula started to be observed twice a week for first symptoms of white head rot. Plant reaction was measured as previously described.

The disease susceptibility index (DSI) was calculated for mycelium, culture filtrate, oxalate and ascospore tests. Plots were rated according to the following severity classes: 0 = no disease symptoms and 1, 2, 3, 4, 5, 6 = 1-10%, 11-20%, 21-40%, 41-60%, 61-80%, 81-100% of the capitulum area with white head rot symptoms, respectively.

The DSI was calculated using the following formula:

$$DSI = \frac{(\text{number of infected plants}) \times (\text{severity class})}{(\text{number of inoculated plants})}$$

The incubation index (I) was also calculated as a ratio between incubation period (in days) of inbred lines and controls inoculated at the same time. This index, calculated for each genotype, is proportional to *S. sclerotiorum* (Alvarez *et al.*, 1999).

Data were arc-sin square root transformed. The four experiments were analyzed separately, within each observation, as Randomized Completely Block Design (RCBD) with three replications, using MSTATC software.

Table 1: Sequences of adapters and primers used for AFLP analysis

<i>EcoRI</i> adapter		5'-CTCGTAGACTGCGTACC-3' 3'-CTGACGCATGGTTAA-5'
<i>MseI</i> adapter		5'-GACGATGAGTCCTGAG-3' 3'-TACTCAGGACTCAT-5'
<i>EcoRI</i> primer+1	E01	5'-GACTGCGTACCAATTCA-3'
	E03	5'-GACTGCGTACCAATTCG-3'
<i>EcoRI</i> primer+3	E40	5'-GACTGCGTACCAATTCAGC-3'
	E41	5'-GACTGCGTACCAATTCAGG-3'
	E65	5'-GACTGCGTACCAATTCGAG-3'
	E69	5'-GACTGCGTACCAATTCGCG-3'
<i>MseI</i> primer+1	M02	5'-GATGAGTCCTGAGTAAC-3'
<i>MseI</i> primer+3	M47	5'-GATGAGTCCTGAGTAACAA-3'
	M48	5'-GATGAGTCCTGAGTAACAC-3'
	M51	5'-GATGAGTCCTGAGTAACCA-3'
	M52	5'-GATGAGTCCTGAGTAACCC-3'
	M53	5'-GATGAGTCCTGAGTAACCG-3'
	M58	5'-GATGAGTCCTGAGTAACGT-3'
	M59	5'-GATGAGTCCTGAGTAACCTA-3'
	M60	5'-GATGAGTCCTGAGTAACCTC-3'
	M62	5'-GATGAGTCCTGAGTAACCTT-3'

### AFLP analysis

The genomic DNA of 8 R28 and R978 plants was isolated from young leaves and stored at -80°C according to Doyle and Doyle (1990) protocol. DNA concentration was determined by the fluorimetric assay and the final concentration was adjusted to 50 ng/μl. High-quality genomic DNA (500 ng) was digested with a pair of restriction enzymes (*EcoRI/MseI*) then ligated to double-stranded *EcoRI* and *MseI* adapters. The ligate was pre-amplified with 1 base selective primers and selective amplification carried out using pairs of 2 bp selective primers. Sequences of adapter and primers are reported in Table 1, and the primer pairs tested are reported in Table 2.

Table 2: Combination of selective primers tested (bold)

Primers		M02								
		M47 CAA	M48 CAC	M51 CCA	M52 CCC	M53 CCG	M58 CGT	M59 CTA	M62 CTC	M62 CTT
E01	E40	<b>E40</b>	<b>E40</b>	<b>E40</b>	<b>E40</b>	<b>E40</b>	<b>E40</b>	<b>E40</b>	<b>E40</b>	<b>E40</b>
	AGC	<b>M47</b>	<b>M48</b>	<b>M51</b>	<b>M52</b>	<b>M53</b>	<b>M58</b>	<b>M59</b>	<b>M62</b>	<b>M62</b>
	E41	<b>E41</b>	<b>E41</b>	<b>E41</b>	<b>E41</b>	<b>E41</b>	<b>E41</b>	<b>E41</b>	<b>E41</b>	<b>E41</b>
	AGG	<b>M47</b>	<b>M48</b>	<b>M51</b>	<b>M52</b>	<b>M53</b>	<b>M58</b>	<b>M59</b>	<b>M62</b>	<b>M62</b>
E03	E65	<b>E65</b>	<b>E65</b>	<b>E65</b>	<b>E65</b>	<b>E65</b>	<b>E65</b>	<b>E65</b>	<b>E65</b>	<b>E65</b>
	GAG	<b>M47</b>	<b>M48</b>	<b>M51</b>	<b>M52</b>	<b>M53</b>	<b>M58</b>	<b>M59</b>	<b>M62</b>	<b>M62</b>
	E69	<b>E69</b>	<b>E69</b>	<b>E69</b>	<b>E69</b>	<b>E69</b>	<b>E69</b>	<b>E69</b>	<b>E69</b>	<b>E69</b>
	GCG	<b>M47</b>	<b>M48</b>	<b>M51</b>	<b>M52</b>	<b>M53</b>	<b>M58</b>	<b>M59</b>	<b>M62</b>	<b>M62</b>

## RESULTS AND DISCUSSION

### Resistance tests

The analysis of variance reported in Table 3 showed statistically significant differences among the genotypes at the end of each treatment adopted to evaluate the tolerance against *Sclerotinia*, confirming the possibility to exploit the genetic variability.

Table 3: Mean squares of the utilized tests at the end of each experiment (Udine, 2001)

Source	d.f.	Stem infection	Ascospore infection	Oxalate test	Culture filtrate test
Replications	2	102.8 n.s.	0.30 n.s.	0.35 n.s.	0.36 n.s.
Genotypes	7	866.2**	3.12**	2.21**	2.01**
Error	14	61.2	0.14	0.26	0.22

\*\*, significant for  $P < 0.01$  probability level

### Basal stem infection

First symptoms appeared 20 days after inoculation and at the end of the experiment the plants were either dead or completely healthy. The field percentage of dead plants 50 days after basal stem inoculation was of 67.8%. Analysis of variance detected highly significant ( $p < 0.01$ ) genotypic differences. The line 28R showed the

highest tolerance level (33.3% of dead plants), without significant differences in relation to the tolerant control (Figure 2).

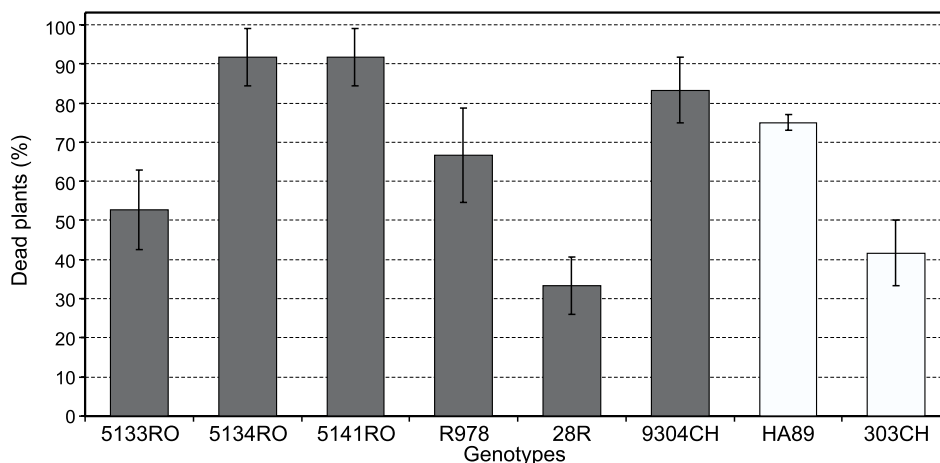


Figure 2: Dead plants (% of the total infected) 50 days after basal stem inoculation; bars represent standard errors

### ***Ascospore infection***

5133RO was the first inbred line to exhibit symptoms, 13 days after inoculation (Figure 3), whereas 29 days after infection the symptoms were present in all genotypes, except in the tolerant control (303CH), which showed the first brown rotted zone on the head after 33 days. The disease symptoms increased in the course of time in all genotypes, reaching the highest level 40 days after the infection. The observations were suspended at that point because of the approach of the physiological maturity. At the end of the experiment the average field DSI was 2.9 (48.3% of disease incidence) and the differences in susceptibility or tolerance to the disease among the genotypes became more evident. The inbred 5133RO was rated as most susceptible (5.25) in the trial, with a significant difference in respect to the susceptible control HA89 (3.66). Conversely, the inbred 28R displayed the highest level of tolerance (1.16), without significant differences from the tolerant control, 303CH (1.65) (Figure 3).

All inbred lines showed the incubation index (I) lower than 1, significantly lower than that in the resistant control (303CH). The average index of the trial was 0.63, with an average latency period of 24 days. This character, strictly depending on the climatic conditions (Figure 1), was in accordance with previous results of Tourvieille and Vear (1984). Statistically significant differences among the genotypes were detected by the analysis of variance, particularly for genotype 5133RO which was most susceptible, with the lowest index (0.39), while genotypes 5141RO and

28R, each with the value of 0.88, showed the highest level of tolerance (data not shown), with similar results to the end of the experiment (Figure 3).

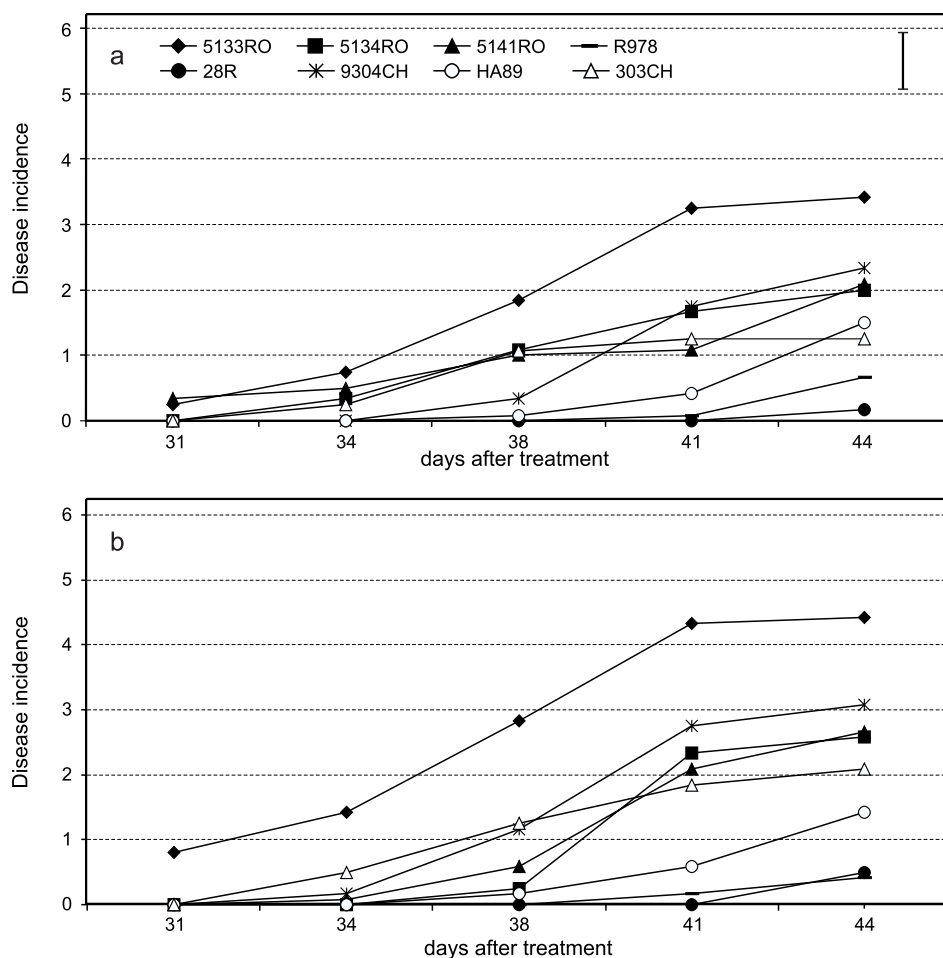


Figure 3: Disease susceptibility index time course of the studied genotypes after mycelium filtrate (a) and oxalic acid tests (b); bars represent the LSD for  $P < 0.01$

#### Culture filtrate and oxalic acid injection

Significant genotypic differences were identified in the response to tests (Figure 4). First deformations and white head rot - like symptoms on the capitulum were observed 31 days after injection with very similar reactions in both treatments. In fact, at the end of the experiment (44 days after treatment), the inbred line 5133RO was most susceptible, whereas 28R and R978 were most tolerant. Both tests were not effective to adequately discriminate the susceptible and tolerant controls since



no significant difference was appreciated between the two genotypes. HA89 showed a lower disease incidence than the tolerant control (303CH), up to the end of the experiment.

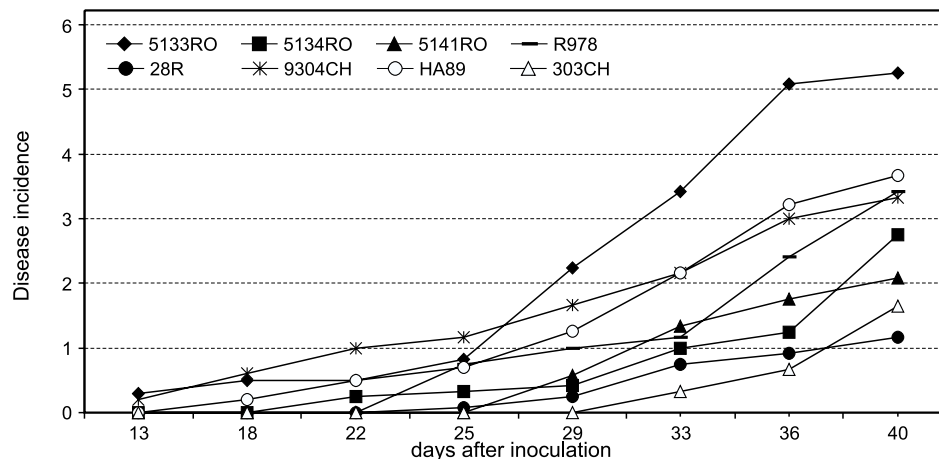


Figure 4: Disease susceptibility index time course of the studied genotypes after ascospore head infection; bars represent the LSD for  $P < 0.01$

Table 4 reports the relationships among the tests adopted. No significant correlations were observed among the analyzed tests except for the positive and highly significant correlation between the culture filtrate and oxalate tests (0.94\*\*).

Table 4: Relationship among the screening tests ( Udine 2001)

Treatment	Ascospore infection	Oxalate test
Culture filtrate test	0.56 n.s.	0.94**
Basal stem infection	0.22 n.s.	0.60 n.s.

Table 5: Number of bands and level of polymorphism for the best primer combinations

Primer combination		Total number of bands	Number of polymorphic bands
E41	M48	75	19
	M51	84	14
	M52	86	13
	M53	75	6
	M59	91	10
	M60	50	1
E65	M58	63	8
	M60	47	9

### AFLP analysis

After autoradiography, the resultant banding patterns for each of the 36 primer pairs tested were manually scored. Table 5 shows the total number of fragments for

the eight primer combinations with the best performance together with the number of polymorphic bands. Each of the eight primer combinations revealed between 1 and 19 polymorphic loci in the analyzed plants. The level of polymorphism detected by the best primer combinations (E41/M48 and E41/M51) was similar to previous results in sunflower (Quagliaro *et al.*, 2001).

## CONCLUSIONS

The mycelium and ascospore tests confirmed that these two attack forms of *Sclerotinia* are not closely related, as already reported by other authors (Tourvieille and Vear, 1984). In fact, the resistant and susceptible lines used as control were not similar in their response to the two different types of infection (Table 4). The two mechanisms studied may be independent, so that the best breeding program should combine the genes controlling each mechanism within the same genotype.

Oxalic acid is well known as responsible for the appearance of *Sclerotinia* symptoms in the plant (wilting) (Huang and Dorrel, 1978; Noyes and Hancock, 1981). Moreover, the results of this experiment showed that it could also cause symptoms very similar to those of white rot on the head. Mycelium culture filtrate caused the same symptoms and, in spite of the presence of many other chemical compounds, oxalic acid was most responsible for the symptoms appearance, as confirmed by the strictly positive relationship between the two methods (Table 4). These methods were insensitive to the environmental conditions and they were easier and faster than the ascospore test, but in this experiment, they displayed a very poor discrimination of the susceptible and tolerant control lines in respect to infections by mycelium and ascospores (Table 4). Physiological reaction against *Sclerotinia sclerotiorum* may not be restricted to oxalic acid resistance. According to some authors (Marciano *et al.*, 1993; Bazzalo *et al.*, 2000), the resistance to oxalate is a specific resistance mechanism that may work singly, but more likely it works in combination with alternative physiological mechanisms at the cellular level, such as biosynthesis of phytoalexins, antimicrobial compounds produced by the infected host plant to inhibit fungal development (Bret *et al.*, 1993), biosynthesis of shikimic acid, a lignin precursor involved in the thickening of the cell wall (Lehninger, 1979), and the biosynthesis of phenolic compounds (Castano *et al.*, 1992). All these compounds provide a consistent level of resistance to *Sclerotinia* in the field.

The inbred line 28R (coming from *H. argophyllus* wild species) appeared to be most tolerant to both basal stem and white head rot infections (Figures 2 and 3). Moreover, it showed the best performance in oxalate and culture filtrate tests highlighting a specific resistance to oxalate (Figure 4) as reported in previous experiments (Tahmasebi-Enferadi *et al.*, 2000).

Although further studies are necessary to obtain additional information, the inbred line 28R provides new breeding perspectives for improving *Sclerotinia* scle-

*rotiorum* resistance in cultivated sunflower and for studying the physiological and genetic mechanisms involved in the resistance.

For the above reasons, with the aim to obtain new segregating populations, crosses between 28R and other susceptible lines (9304CH, HA89) are in progress. The preliminary results with AFLP markers pointed out their suitability for more detailed analysis of segregating populations, to study the inheritance of resistance to *Sclerotinia* and to map QTL that controls this resistance.

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## REFERENCES

- Alvarez, M., Castano, F., Rodriguez, R., 1999. Evaluation of *Sclerotinia sclerotiorum* infection of sunflower hybrids. Ann. Appl. Biol., 134 (supplement): 46-47.
- Bazzalo, M., Bridges, I., Galella, T., Grondona, M., Leon, A., Scott, A., Bidney, D., Cole, G., D'Hautefeuille, J., Lu, G., Mancl, M., Scelonge, C., Soper, J., Sosa-Domiongue, G., Wang, L., 2000. *Sclerotinia* head rot resistance conferred by wheat oxalate oxidase gene in transgenic sunflower. 15<sup>th</sup> International Sunflower Conference, Toulouse, France, June 2000, Tome II: K60-K65.
- Boland, G.F., Hall, R., 1987. Epidemiology of white mold of white bean in Ontario. Can. J. Plant Pathol., 9: 300-309.
- Bret, E., Vear F. and Tourvieille, D., 1993. Utilisation des marqueurs moleculaires pour la resistance au *Sclerotinia*. Les Rencontres Annuelles du CETIOM, INRA, Centre Recherches Agronomiques Clermont-Ferrand, France, pp. 4.
- Castano, F., Hemery-Tardin, D., Tourvieille, D., Vear, F., 1992. The inheritance and biochemistry of resistance to *Sclerotinia sclerotiorum* leaf infections in sunflower (*Helianthus annuus* L.). Euphytica, 58: 209-219.
- Castano, F., Rodriguez, R., 1997. A simple technique to produce ascospores of *Sclerotinia sclerotiorum*. International Sunflower Yearbook 1997-1998, pp. 77.
- Castano, F., Vear, F., Tourvieille, D., 1989. L'utilisation de plusieurs tests simultanes dans la selection pour la resistance du tournesol vis-a-vis de *Sclerotinia sclerotiorum*. Inf. Tech. CETIOM, 107: 14-20.
- Doyle, J.J. and Doyle, J.L., 1990. Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.
- Hahn, V., 2000. Resistance to *Sclerotinia* head rot in sunflower after artificial infection with inoculated millet seed. 15<sup>th</sup> International Sunflower Conference Toulouse, France, June 2000, Tome II: K19-K22.
- Huang, H.C. and Dorrel, D.G., 1978. Screening sunflower seedlings for resistance to toxic metabolites produced by *Sclerotinia sclerotiorum*. Can. J. Plant Sci., 58, 1107-1110.
- Quagliaro, G., Vischi, M., Tyrka, M., Olivieri, A. M., 2001. Identification of wild and cultivated sunflower for breeding purposes by AFLP markers. Journal of Heredity, 92: 38-42.
- Raducanu, F., Vranceanu, V., Hagima, I., Pectu, H., 2000. Studies about the influence of *Sclerotinia sclerotiorum* filtrates on some quantitative and qualitative traits in Romanian

- sunflower genotypes *in vitro* and *in vivo* tested. 15<sup>th</sup> International Sunflower Conference Toulouse, France, June 2000, Tome II: K29-K34.
- Tahmasebi-Enferadi, S., Vannozzi, G.P., Gomez-Sanchez, D. and Baldini, M., 1998. Results of screening sunflower for resistance to toxic metabolites produced by *Sclerotinia sclerotiorum* (Lib.) de Bary and prospects for the use of biochemical markers in breeding. *Agr. Med.*, 128, 47-58.
- Tahmasebi-Enferadi, S., Turi, M., Baldini, M., Vannozzi, G.P., 2000. Comparison between artificial inoculation and culture filtrate of *Sclerotinia sclerotiorum* (Lib.) de Bary treatments on nine sunflower genotypes. 15<sup>th</sup> International Sunflower Conference Toulouse, France, June 2000, Tome II: K23-K28.
- Tourvieille De Labrouhe, D. and Vear, F., 1984. Comparaison de méthodes d'estimation de la résistance du tournesol à *Sclerotinia sclerotiorum* (Lib.) de Bary. *Agronomie*, 4, 517-525.
- Tourvieille, D., Vear, F., 1990. Heredity of resistance to *Sclerotinia sclerotiorum* in sunflower. III Study of reactions to artificial infection of roots and cotyledons. *Agronomie*, 10: 323-330.
- Vear, F., Tourvieille, D., 1984. Recurrent selection for resistance to *Sclerotinia sclerotiorum* in sunflowers using artificial infections. *Revue d'Agronomie*, 8: 789-794.
- Zazzerini, A., Monotti, M., Tosi, L., 1987. Results of a two-years investigation on the most important pathogens in Italy. *Helia*, 10: 47-51.
- Marciano, P., Lenna, P.D. and Magro, P., 1983. Oxalic acid, cell wall-degrading enzymes and pH in pathogenesis and their significance in the virulence of two *Sclerotinia sclerotiorum* isolates on sunflower. *Physiol. Plant Pathol.*, 22: 339-345.
- Noyes, R.D. and Hancock, J.G., 1981. Role of oxalic acid in the *Sclerotinia* wilt of sunflower. *Physiol. Plant Pathol.*, 18, 123-132.
- Lehninger, A.L., 1979. Biosintesi degli aminoacidi aromatici fenilalanina e triptofano. In: *Biochimica*. (ed.) Zanichelli, 2<sup>nd</sup> edition, Italy, 639-641.
- Schneider, A.A., Miller, J.F., 1981. Description of sunflower growth stages. *Crop Sci.*, 21, 901-903.

## **EVALUACION DE LA VARIABILIDAD GENETICA PARA LA RESISTENCIA DEL GIRASOL A *Sclerotinia sclerotiorum* (Lib.) de Bary POR LA UTILIZACION DE MARCADORES MOLECULARES**

### **RESUMEN**

Dentro del proyecto bilateral entre Italia y Rumania (Universidad de Udine y Instituto en Fundulea), fue establecido un experimento en la Estacion Experimental con fines siguientes: i) evaluacion de la variabilidad genetica de genotipos testados a la infeccion de *Sclerotinia* na parte baja del tallo y de la cabeza; ii) control de dos nuevos metodos alternativos de pruebas de la resistencia a la podredumbre blanca de la cabeza; iii) determinar las relaciones posibles entre diversas pruebas; iiiii) constatar los fragmentos ligados con la resistencia por medio del analisis de AFLP.

Fueron utilizadas las pruebas siguientes: 1) infeccion de la parte baja del tallo, 2) infeccion de cabezas por ascosporas, 3) inyeccion de filtrados del cultivo de micelio y 4) inyeccion del acido oxalico en la parte posterior de la cabeza.

Las diferencias en las reacciones de las plantas de control resistentes y sensibles indican que los testes con micelio y ascosporas son convenientes para el trabajo y la independencia reciproca de dos mecanismos de tolerancia presentes.

Por lo contrario a eso, los testes 3 y 4 diferenciaban poco las plantas de control, asi como la ausencia total de enlace con las infecciones causadas por micelio y ascosporas. Parece que la reaccion del girasol a *S. sclerotiorum* no

puede ser limitada solo a la resistencia al acido oxalico, pero la reaccion incluye tambien otros mecanismos fisiologicos.

La linea consanguinea 28R (de origen de la especie silvestre *H. argophyllus*) indico no solo la mas grande tolerancia a las dos formas de infeccion, a la infeccion de la parte baja del tallo y a la infeccion de la cabeza, pero esa era la mejor en los testes con oxalado y filtrados del cultivo (de hongo), indicando la resistencia especifica al oxalado.

### **ÉVALUATION DE LA VARIABILITÉ GÉNÉTIQUE DANS LA RÉSISTANCE DU TOURNESOL ENVERS LA *Sclerotinia* *sclerotiorum* (Lib.) de Bary À L'AIDE DE MARQUEURS MOLÉCULAIRES**

#### **RÉSUMÉ**

Dans le cadre d'un projet bilatéral entre l'Italie et la Roumanie (Université d'Udine et Institut de Fundulei), une expérience a été faite à la station expérimentale de l'Université d'Udine. Les buts en étaient les suivants: i) évaluation de la variabilité génétique des génotypes testés envers l'infection *Sclerotinia* de la base de la tige et de la tête; ii) vérification de deux nouvelles méthodes alternatives d'évaluation de la résistance à la moisissure blanche de la tête; iii) établissement de liens possibles entre différents tests; iiiii) constatation de fragments liés à la résistance à l'aide de l'analyse SFLP. Les tests suivants ont été utilisés: 1) infection de la base de la tige, 2) infection de la tête par ascospores, 3) injection de filtrats de culture de mycélium et 4) injection d'acide oxalique dans la partie arrière de la tête.

Les différences de réactions entre les plantes résistantes et les plantes de contrôle sensibles démontrent l'opportunité des tests avec mycélium et avec ascospores et l'indépendance réciproque des deux mécanismes de tolérance présents.

Au contraire, les tests 3 et 4 ont montré une faible différenciation des contrôles ainsi qu'une absence totale de relation avec les infections au mycélium et aux ascospores. Il semble qu'on ne puisse limiter la réaction du tournesol envers la *S. sclerotiorum* à la résistance à l'acide oxalique mais que cette réaction puisse inclure d'autres mécanismes physiologiques.

La ligne inbred 28R (ayant son origine dans l'espèce sauvage *H. argophyllus*) a non seulement montré la plus grande tolérance envers les deux formes d'infections, celles de la base de la tige et de la tête, mais elle a aussi eu la meilleure performance dans les tests avec oxalate et avec filtrats de culture (mycologique) en plus de montrer une résistance spécifique à l'oxalate.

