Genetic control of sunflower partial resistance to black stem (*Phoma macdonaldii*) by the use of Recombinant Inbred Lines (RILs).

A. SARRAFI, L. GENTZBITTEL, A. ROUSTAEE,

Department of Biotechnology and Plant Breeding, BAP, IFR, INP-ENSAT

18 Chemin de Borde Rouge BP 107, 31326 Castanet France

Tel : +33(0)5 62 19 35 80, Fax : +33(0)5 62 19 35 81, E-mail : sarrafi@ensat.fr

Web site: *http://www.ensat.fr/pperso/sarrafi/pages/index.html*

Abstract :

Genetic variability for partial resistance to black stem in sunflower, caused by *Phoma macdonaldii* was investigated in 83 recombinant inbred lines (RILs) of the cross between two genotypes 'PAC-2' and 'RHA-266'. The experiment was undertaken in a randomized complete block design with two replicates, in a controlled growth chamber. Twelve seeds per replicate per genotype were planted in plastic containers (60x40x8cm) containing moistened vermiculite. Twelve-day-old seedlings were inoculated with an agressive French isolate of the pathogen. Genetic variability was observed among the 83 RILs for partial resistance to the disease. Some RILs were significantly more resistant than the more resistant parent, 'PAC-2'. Genetic gain when the best RIL or 10% of selected RILs are compared with 'PAC-2' was significant. A QTL analysis of resistance to black stem showed that seven QTLs were detected on linkage groups 3, 4, 8, 9, 11, 15 and 17. The detected QTLs together explain about 90% of the phenotypic variation of the trait. Crosses between RILs contrasted for their resistance to black stem and exhibiting molecular polymorphism in detected QTLs will be made, in order to focus more precisely on the genomic region of interest.

Key words: *Quantitative trait loci. Sunflower. Recombinant inbred lines. Partial resistance. Black stem.*

Introduction

Black stem caused by *Lepstosphaeria lindquistiii* (*Phoma macdonaldii*) is one of the most important diseases of sunflower. It is present in many European countries including Yugoslavia, Italy, Romania and Bulgaria (Acimovic 1994), China (Hua and Ma 1996), Australia and the USA (Acimovic 1984). Since 1990 the disease has expanded constantly and is now recognized as one of the most serious diseases of sunflower in France (Peres and Lefol 1996).

The symptoms appear principally on the stems and petioles. Disease lesions usually begin at the base of the petiole and spread rapidly to form a large black area on the stem. Premature ripening of diseased plants caused a 10 - 30% reduction in yield (Penaud 1996) and 1000 seed weight (Carson 1991). It can also cause premature death of the plant (Donald et al. 1987).

Genetic variability for partial resistance to black stem in sunflower has been observed in field conditions (Peres et al. 1994). A study of parental genotypes and their F1 hybrids showed that additive genetic effects which are important in predicting the progeny performance of crosses are significant for some inbred lines used in the research work (Roustaii et al. 2000). Additive genetic effects were also reported in sunflower ,by Deglene et al. (1999) for resistance to *Phomopsis Helianthii,* Vear et al. (1997) for resistance to mildew and by Castano et al (1992) for partial resistance to *Sclerotinia sclerotiorum*.

Identification of chromosome regions with effects on resistance to black stem would increase our under standing of genetic control of this character. The development of molecular marker techniques has provided an additional tool to determine quantitative trait loci (QTLs). In sunflower QTLs for *Sclerotinia sclerotiorum* resistance (Mestries et al. 1998) .Candidate genes for downy mildew (Gentzbittel et al. 1998) have been identified..

Some studies have shown the advantages of recombinant inbred lines (RILs) for detecting QTLs (Austin and Lee 1996). RILs are homozygous and can be propagated without further regeneration. The RILs undergo multiple cycles of meiosis before homozygosity is reached; in consequence, linked genes have a great probability of recombination and the pleiotropic effect can be detected (Burr and Burr 1991). This effect increases the power of testing differences between genotypic classes.

The objective of the investigation presented here was to evaluate variability and genetic gain for partial resistance to black stem in 83 recombinant inbred lines (F8) of sunflower. We also carried out a QTL mapping analysis to characterize genomic regions involved in partial resistance to black stem.

Materials and methods

A population of 83 recombinant inbred lines (RILs) developed by the SSD method from the cross between 'PAC-2' and 'RHA-266' were used in the experiment. These recombinant inbred lines (F8) and their parents were produced by INRA-France.

A monopicniospore isolate of phoma produced from naturally infected plants in the south-west of France was used in this study. The pathological characteristics of this isolate, which is one of the most aggressive isolates of the pathogen, were kept constant during the period of the experiment using the method of conservation described by Arabi et al. (1992) for *Drechslera teres*.

The experiment was conducted in a controlled environment chamber at a temperature of 25 ± 1°C (day) / 18 ± 1°C (night). Light intensity was 200 ME m^{-2} S^{-1} with a 14h photoperiod and a relative humidity of 75-80%. The experiment was designed as a randomized complete block with two replications. Each replicate consisted of 12 seedlings of each genotype. Seeds were sterilized for 5 min in a 6% sodium hypochloride solution and washed in sterile distilled water. Two rows of 6 seeds per genotype per replication were planted in plastic containers (40x30x30 cm) filled with moistened vermiculite. Plantlets were irrigated with a nutrient solution (NPK 6-3-6 and micronutrients: Substral, Boulogne Billancourt, France).

Twelve-day-old seedlings were inoculated at the junction of te cotyledon petiole and hypocotyl with 20 µl of a pycniospore suspension (10^6 pycniospores per ml of water, containing 0.25% of gelatine) using a micropipette. After inoculation, each container was enclosed for 72 h using a special transparent cover (pexiglass) to maintain a near-saturated humidity favourable for fungal inoculation.

Small chlorotic lesions appeared on the surface of the cotyledon petiole 1-2 days after inoculation. Three days later, they had elongated and transformed into necrotic lesions depending on the reaction of the lines. In severe infection of susceptible lines, the necrotic lesions elongated and then spread down the hypocotyl. Thus, the percentage of surface area in the upper part of the cotyledon petiole occupied by the fungus varied with the susceptibility of the line. Seven days after inoculation, both cotyledon petioles of the seedling were scored according to the percentage of the petiole area exhibiting disease symptoms (necrosis). A rating scale from 1 to 9, based on the percentage of infected cotyledon petiole area, was used, where: 1=0-5%, 2=6-10%, 3=11-20%, 4=21-30%, 5=31-40%, 6=41-60%, 7=61-80%, 8=81-99%, 9=100% with necrosis spreading down the stem. The measures of severity did not need any transformation to normalize the distribution.

Analysis of variance was performed for resistance to the disease and the means separated using a Newman-Keuls-test (P=0.05). Additive, environmental variances and narrow- sense heritability were calculated according to Kearsey and Pooni (1996), using least-square estimates of the genetic parameters.

A set of 99 RILs and their parents 'PAC-2' and 'RHA-266' were used for DNA extraction and AFLP analysis. Then the same set was screened with 333 AFLP markers and a linkage map was constructed based on 254 linked loci , as previously described (Flores-Berrios et al. 2000).

The chromosomal location of QTLs for resistance to black stem were resolved by composite interval mapping (CIM) using QTL cartographer V1.13 model 6 (Baston et al. 1999). In the QTL carte model 6 integrates two parameters: the number of markers which control the genetic background (*np*) and a window size (*ws*) that will block out a region of the genome on either side of the markers flanking the test site (Basten et al. 1999). Inclusion of the black ground makes the analysis more sensitive to the presence of a QTL in the target interval. At each marker locus, the significance of the association was tested by the likelihood-ratio statistic (Haley and Knott 1992).

Results and discussion

Analysis of variance of the 83 RILs and their parental genotypes 'PAC-2' and 'RHA-266' showed a high significant genotype effect (Data not presented). The genetic variability of the parental genotypes and RILs for partial resistance to black stem are presented in Table1. 'PAC-2' showed a higher level of partial resistance when compared with 'RHA-266', but the difference is not statistically significant. The difference between RILS ($\overline{X}$ RILs) and their parents ($\overline{X}$ P), is not significant showing that the 83 RILs used in this experiment are representative of the entire possible RILs of the cross 'PAC-2' X 'RHA-266'.The comparison between the best parent ('PAC-2') and the best RIL and with the mean of10% of selected RILs showed a significant difference for resistance to black stem (Table 1).This phenomenon, considered as genetic gain, might be due to the polygenic control of partial resistance to the disease and the accumulation of favourable alleles for resistance to black stem in the RILs. Narrow sense heritability was 58.23% indicating that selection for the trait is possible in progenies of crosses. Genetic variability and heritability (60%) for partial resistance to black

stem in F1 hybrids and their parents as well as additive and dominant effects of the genes controlling black stem were also shown by Roustaee et al. (2000).

Table 1 Genetic gain and heretability for black stem resistance in recombinant inbred lines (RILs) of sunflower. The values represent the mean of the host reaction rate, scale range for susceptibility, 1 to 9, from two replications.

Item	Host reaction
PAC-2 (P1)	5.05
RHA266 (P2)	6.25
P1-P2	-1.20^{ns}
$\overline{X}_P$=(P1+P2)/2	5.65
$\overline{X}_{RILs}$[a]	6.58
$\overline{X}_{RILs}$- $\overline{X}_P$	-0.93^{ns}
Best RIL (BRIL)	1.00
GG[c]=BRIL-BP[b]	-4.05*
10% SF8L[d]	2.93
GG[e]=10%SF_8L-BP	-2.12*
h^2	58.23%

* Significant at P = 0.05 ns = not significant at P=0.05

[a] $\overline{X}$ RILs, mean of all recombinant inbred lines.

[b]BP, Best parent ('PAC-2')

[c,e]GG, Genetic gain when the best RIL or 10% of the selected RILs (10% SF8L) are compared with the best parent ('PAC-2')

[d]10%SF_8, 10% of the best recombinant F_8 lines

Significant peak values of LOD scores the position of these peaks, the percentage of phenotypic variance explained and the estimate of QTL effects based on a composite interval mapping analysis for partial resistance to black stem, are shown in Table 2. Seven QTLs were detected and designed according to the trait and linkage group. The effects of each QTL are

Table 2 Map positions and effect of QTLs detected in recombinant inbred lines (RILs) for black stem resistance in sunflower.

QTL	Linkage group	position[a]	LOD	Proportion of variance explained[b]	Additive effect (a)
bsr3.1	III	102	3.8	6.40	0.511
bsr4.1	IV	47	5.0	13.00	0.850
bsr8.1	VIII	133	7.6	17.30	0.912
bsr9.1	IX	89	5.6	8.90	0.581
bsr11.1	XI	73	5.7	13.50	0.901
bsr15.1	XV	179	7.5	16.60	0.986
bsr17.1	XVII	95	8.1	17.40	-0.966

[a]= Value determined by QTL Cartographer, Version 1.13 (Basten et al. 1999)

[b=] Expressed in Kosambi CM, from north of the linkage group (Flores-Berrios et al. 2000).

moderate ranging from 6 to 17% (Table 2). The transgressive phenotypes observed could be explained by the presence of QTLs of opposite sign in each parents. The detected QTLs together explain 92% of the phenotypic variation (Table 2). QTLs for resistance to *Sclerotinia Sclerotiorum* (Mestriees et al. 1998) and candidate genes for downy mildew (Gentzbittel et al. 1998) have been also identified in sunflower. As far as we know genetic markers for resistance to phoma are not presented in the literature. Crosses between RILs contrasted for their resistance to black stem and exhibiting molecular polymorphism in detected QTLs will be made, in order to focus more precisely on the genomic region of interest.

References

Acimovic, M., 1984: Sunflower diseases in Europe, the United States and Australia, 1981-1983. Helia **7,** 45- 54.

Arabi MI, Barrault G, Sarrafi A, Albertini L, 1992. Variation in the resistance of barley cultivars and the pathogenicity of *Drechslera teres* f.sp. *maculata* and *D. teres* f.sp. *teres* isolates from France. *Plant Pathology* **41**, 180-6.

Austin DF, Lee M, 1996. Comparative mapping in $F_{2:3}$ and $F_{6:7}$ generations of quantitative trait loci for grain yield and yield components in maize. *Theorical and Applied Genetics* **92**, 817-826.

Basten CJ, Weir BS, Zeng Z-B, 1999. QTL cartographer version 1.13: program in statistical genetics. Department of statistics, North Carolina State University.

Burr B, Burr F, 1991. Recombinant inbred lines for molecular mapping in maize: theoretical and practical considerations. *Trends Genetics* **7**, 55-60.

Carson ML, 1991. Relationship between *Phoma* black stem severity and yield losses in hybrid sunflower. *Plant Disease* **75**, 1150-3.

Castaño F, Hémery-Tardin MC, Tourvieille de Labrouhe D, Vear F, 1992. The inheritance and biochemestry of resistance to *Sclerotinia sclerotiorum* leaf infections in sunflower (*Helianthus annuus* L.). *Euphytica* **58**, 209-19.

Deglene L, Alibert G, Lesigne P, Tourvieille de Labrouhe D, Sarrafi A, 1999. Inheritance of resistance to stem canker (Phomopsis helianthii) in sunflower. *Plant Pathology* **48**, 559-563.

Donald PA, Venette JR, Gulya TJ, 1987. Relationship between *Phoma macdonaldii* and premature death of sunflower. *Plant Disease* **71**, 466-8.

Flores-Berrios E, Gentzbittel L, Kayyal H, Alibert G, Sarrafi A, 2000. AFLP mapping of QTLs for in vitro organogenesis traits using recombinant inbred lines in sunflower (*Helianthus annuus* L.). *Theorical and Applied Genetics* **101**,

Gentzbittel L, Mouzeyar S, Badaoui S, Mestries E, Vear F, Tourvieille de Labrouhe D, Nicoals P, 1998. Cloning of molecular markeres for disease resistance in sunflower, *Helianthus annuus* L. *Theorical and Applied Genetics* **96**, 519-525.

Haley CS, Knott SA, 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity* **69**, 315-324.

Hua Z, Ma G, 1996. A review of sunflower diseases research of China. In: *Proceedings of the 14th International Sunflower Conference, Beijing, China.* Shenyang, China: Academy of Agricultural Science, **2**, 754-2.

Kearsey MJ, Pooni HS, 1996. The genetical analysis of quantitative traits. Chapman and Hall, London, UK.

Mestriest E, Gentzbittel L, Tourvieille de Labrouhe D, Nicolas P, Vear F, 1998. Analysis of quantitative trait loci associated with resistance to *Sclerotinia sclerotiorum* in sunflowers (*Helianthus annuus* L.) using molecular markers. *Molecular Breeding* **4**, 215-226.

Penaud A, 1996. *Phoma* du tournesol: recherche des époques de contamination et mise au point de la protection fongicide. In: *Proceedings of the 14th International Sunflower Conference, Beijing, China.* Shenyang, China: Academy of Agricultural Science, **2**, 694-9.

Peres A, Allard AM, Deverchere J, Penaud A ,1994. *Phoma* du tournesol: étude de la protection fongicide au champ. In: *4ème Conférence Internationale Sur les Maladies Des Plantes, Bordeaux, France*. Paris, France: Association Nationale de Protection des Plantes, 1179-85.

Peres A, Lefol C, 1996. *Phoma macdonaldii* Boerema: élément de biologie et mise au point d'une méthode de contamination artificielles en conditions controlées. In: *Proceedings of the 14th International Sunflower Conference, Beijing, China.* Shenyang, China: Academy of Agricultural Science, **2**, 687-93.

Roustaee A, Barrault G, Dechamps-Guillaume G, Lesigne P, Sarrafi A, 2000. Inheritance of partial resistance to black stem (*Phoma macdonaldii*) in sunflower. *Plant Pathology* **49**, 396-401.

Vear F, Gentzbittel L, Philippon J, Mouzeyar S, Mestries E, Roeckel-Drenet P,

Tourvieille de Labrouhe D, Nicolas P (1997) The gentics of resistance to five races of

downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus* L.). Theorical

and Applied Genetics 95:584-589.