The relations between The recessive gene for apical branching (*b1*) and Some disease resistance and agronomic characters

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Summary:

In previous studies of QTL for *Sclerotinia* resistance, seed weight per plant and seed oil content in cultivated sunflower, it was found that these characters were partially controlled by QTL near to, or in the same zone as, the recessive apical branching gene *b1*. The F2 progeny of a cross between two sunflower lines, XRQ and PSC8, were used to map the linkage group containing the *b1* gene in detail, using the RFLP technique. Analyses of the F3 families from this cross showed a positive effect of the branched phenotype on oil content and a negative effect on seed weight. The effect of branching was also favourable on *Sclerotinia* resistance as measured by ascospore test. In contrast, the branched families were more susceptible to the mycelium test.

Résumé :

Des études sur la résistance au champignon nécrotrophe *Sclerotinia sclerotiorum* et sur l'expression de caractères agronomiques tels que la teneur en huile et le poids de semences chez le tournesol cultivé ont montré que ces caractères sont partiellement contrôlés par des QTL situés dans la région où a été cartographié le gène de ramification apicale *b1*. La descendance F2 d'un croisement entre deux lignées de tournesol, XRQ et PSC8, a été analysée par la technique RFLP (Restriction Fragment Length Polymorphism) pour établir la carte détaillée du groupe de liaison contenant le gène *b1*. Les analyses des familles F3 de ce croisement ont montré un effet du phénotype ramifié favorable sur la teneur en huile et défavorable sur le poids de semences. L'effet de la ramification était également favorable pour la résistance au *Sclerotinia* mesurée par le test « ascospores ». A l'inverse, les familles ramifiées ont montré plus de sensibilité au test « mycélium ».

Introduction

The necrotrophic fungus *Sclerotinia sclerotiorum* is one of the most important pathogens of sunflower in France, causing white rot and wilt (Purdy, 1979; Boland and Hall, 1994). It can attack each part of the plant and each form of attack can be considered as a different disease since, for a given genotype, the level of resistance developed by plants varies according to plant part (Castaño *et al.*, 1993). Both phenotypic observations and genetical studies have shown that this resistance is polygenic (Mestries *et al.*, 1998).

The available composite map of cultivated sunflower genome (Gentzbittel *et al.*, 1999) which is made up of 17 linkage groups for a total lengh of 1607cM, allows precise analysis of agronomically important quantitative characters, such as disease resistance. It has revealed genomic zones involved in *Sclerotinia* resistance by quantitative trait loci (QTL) identification (Mestries *et al.*, 1998). Some of the agronomic and resistance characters have been localised near the recessive branching gene *b1* (Putt, 1964). In particular, it was found that plants which express the apical branching phenotype show a high level of resistance to *Sclerotinia* (Mestries *et al.*, 1998).

The question remains as to whether the collocation of the recessive gene *b1* and major resistance and agronomic QTL is found in all sunflower genotypes and is related to a pleiotropic effect, due to small capitulum size in branched plants, or whether it is due to a real genetic linkage, which could be broken by recombination. This paper reports a preliminary analysis of some agronomic and *Sclerotinia* resistance characters of branched and unbranched F3 families from a cross between two sunflower lines which show polymorphism for many agronomic characters.

Materials and Methods

Sunflower genotypes:

The two parental inbred lines of the cross were bred by INRA. XRQ was a selection for its downy mildew resistance from a cross between HA89 and the Russian population Progress (Vear *et al.*, 1998). PSC8 was a selection from a population with recurrent selection for capitulum resistance to *Sclerotinia* (Vear *et al.*, 1992). XRQ, unbranched, shows a medium level of resistance to *Sclerotinia,* but a high level of resistance to Phomopsis, high oil content and good seed production. PSC8 exhibits the apical branching gene (*b1*) phenotype, a high level of *Sclerotinia* resistance, high oil content, but low seed weight per capitulum. The male fertile forms of the two lines were crossed in both directions. The F1 plants were selfed by covering the capitula with grease-proof paper bags a few days before flowering to obtain the F2 generation, which was in turn selfed to obtain the F3 families.

Field observations and resistance tests:

Leaves were sampled from the 335 F2 plants to determine their molecular characters. These plants were selfed and 220 F3 families were observed for agronomic characters: 5 plants per family for oil content and seed weight and two replications of 25 plants were tested for the *Sclerotinia* resistance of their capitula, using ascospore (Tourvieille and Vear, 1984) and mycelium tests (Castaño *et al.*, 1993). With the ascospore test, the percentage of attack on the two replications was calculated and for each plant showing symptoms, the time between

ascospore infection and first symptom appearance, compared with the mean delay of two control genotypes infected at the same time, gave a «latency index». The greater the value of this index, the more resistant was the plant. For the mycelium test, the results are lesion areas measured 3 days after infection, compared with the mean lesion size of a control infected at the same time, giving a "mycelium index". The weight of seeds per capitulum (g) was determined and seed oil content was measured on 2g samples from each capitulum by nuclear magnetic resonance (Brucker Minispec 10). Each plant was identified for apical branching phenotype.

Molecular analysis (RFLP):

Sunflower DNA was extracted from green leaves from field grown plants before flowering. Extractions, digestions by restriction enzymes (*Eco*R1 and *Hin*d3) and Southern hybridisations were performed as described by Gentzbittel *et al.* (1994). The RFLP patterns of the 335 F2 plants were established using DNA ^{32}P-labelled probes covering the branching gene linkage group described in previous experiments (Gentzbittel *et al.*, 1995). These probes were tested and chosen for their polymorphism between parental lines XRQ and PSC8.

Data analysis:

Hybridisation bands were identified and linkage phase of each band was determined by comparison with the hybridisation patterns of the parents XRQ and PSC8. The expected F2 segregation proportions were checked for each molecular and genotypic (*b1* gene) marker by Chi-square homogeneity tests (risk α=5%). The normality of distributions and homoscedasticity conditions for the 5 characters studied were tested (Chi-square test, Bartlett's test, α=5%). When necessary, data were converted using Arcsinus-square root (for % attack), Log for mycelium index or square root transformations (for latency index, oil content and seeds weight). The goodness of these distributions and variances were checked again after transformations. Analysis of variance were carried out to test the effect of branched genotype on the four quantitative characters studied. The proportion of phenotypic variance of each character explained by segregation of *b1* gene was determined by the R^2 value.

Results

The RFLP patterns of the 335 F2 plants of the cross were obtained for 17 probes giving polymorphism between the parental lines XRQ and PSC8 and selected about ones giving markers previously mapped on the branching gene linkage group. The proportion of markers showing a codominant segregation was 82%. These molecular data and field observations on F2/F3 plants for branching phenotype, allowed the establishment of the linkage map of the group where the apical branching gene *b1* is located. This map is analogous to those of Mestries *et al.* (1998) and Gentzbittel *et al.* (1999). This map shows 13 markers (compared with 6 for Mestries *et al.* and 21 for the consensus map of Gentzbittel *et al.*) and should thus permit detailed definition of QTL on this linkage group.

For all the characters studied, data followed continuous distributions. One-way analyses of variance were carried on F3 data for both *Sclerotinia* resistance criterions and agronomic traits. Table 1 presents the analyses of variance on the 5 quantitative traits. In all cases there were highly significant differences between F3 families, and for all these

characters the presence of branching affected the mean values of the characters. The percentage of variability explained varies from 7 to 41% (R^2 values). The trait where the effect was greatest was oil content. For *Sclerotinia* resistance, the latency index was less affected than percentage attack, with the mycelium index showing an intermediate value.

Table 1. Analyses of variance on *Sclerotinia* resistance and agronomic traits of F3 (XRQ x PSC8)F3 families.

Quantitative traits	Nbr families	F (genotype)	P>F	R^2 (%) (effect of *b1* gene
Sclerotinia resistance				
% attack	220	18.75	0.000	14.74
Latency index	220	5.97	0.0028	2.66
Mycelium index	220	18.59	0.000	7.92
oil content	217	77.01	0.000	41.85
seed weight	220	17.21	0.000	13.70

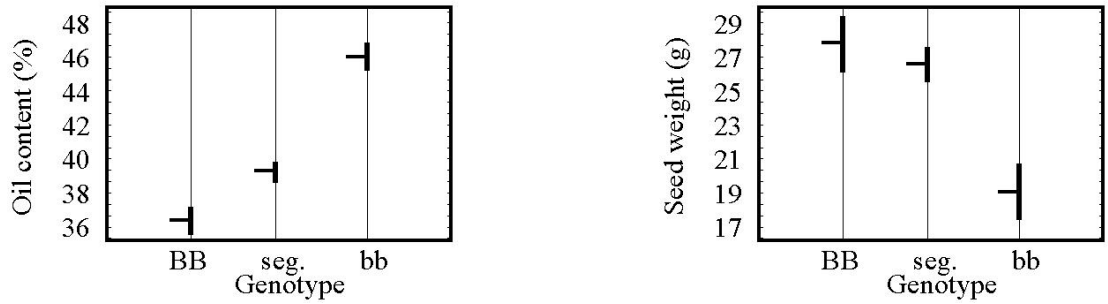
Figure 1 presents graphs of the means for each of the three genotypes of the F3 families (*BB*: homozygous unbranched, *seg*: segregation for branching and *bb*: homozygous branched). Oil content was greater and seed weight smaller in the branched families, with the families showing intermediate values. Similarly, *bb* genotypes showed the best *Sclerotinia* resistance determined both as a low percentage attack and a long latency index. However, for the mycelium index, the branched families much appeared more susceptible than the unbranched genotypes (0.70 and 0.49 respectively).

Discussion

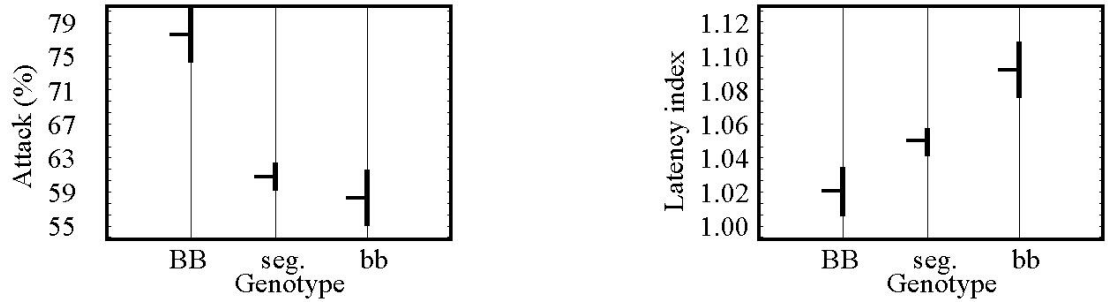
The present results show effects of the branching gene of comparable importance to those reported by Mestries *et al.*, (1998) in a cross between two different sunflower lines (GH x PAC2). The proportion of phenotypic variance explained by the branching gene for seed oil content (33.37%) calculated by these authors was quite similar to the present results (41.85%) and the same was true for seed weight per plant (18.42% and 13.70% respectively). This suggests that the effects of the branching gene on these characters may well be pleiotropic.

For *Sclerotinia* resistance, the conclusion may not be the same. The effects of the branching gene were of a similar level but not all in the same direction. Mestries *et al.* (1998) carried out mycelium tests on capitula and leaves and found significant positive effects of the branching gene on resistance to mycelium extension on capitula (R^2 = 14.96% for F3 and 21.57% on F4) but no effect on extension on leaves. In contrast, in the present cross, the branched plants appeared the more susceptible to the capitulum mycelium test (Figure 1, R^2=7.92). This is evidence that the linkage of capitulum mycelium reaction and branching is not a pleiotropic effect but a true genetic linkage. The measurement of the mycelium lesion

Agronomic characters



Sclerotinia resistance "ascospores test"



Sclerotinia resistance "mycelium test"

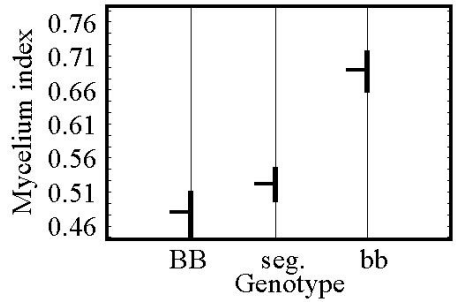


Figure 1: Mean values of the branched (bb) segregating (seg) and unbranched (BB) F3 families for agronomic and *Sclerotinia* resistance characters.

size is made 3 days after infection, and is planned to intervene before the mycelium reaches the edge of the capitulum, so that capitulum size should not directly affect results. It may thus be suggested that this branching gene linkage group carries a region controlling this type of resistance and that a favourable allele had been fixed in the branched line PAC2 but an unfavourable one in PSC8. The results of Castaño *et al.* (1992), who reported that PSC8 had very good resistance to the ascospore test but only moderate resistance to the mycelium test are in agreement with this hypothesis.

The fact that for the cross XRQ x PSC8, the ascospore test showed positive effects of the branching gene on capitulum resistance to *Sclerotinia*, measured both by the percentage attack and by the latency index indicates that these characters are also controlled by regions on this linkage group. The R^2 is much higher for the percentage attack (14.74%) than for the latency index (2.66%). It is possible that part of the effect of branching on percentage attack is pleiotropic, since the smaller capitula dry more quickly than in the case of unbranched plants, and, if symptoms develop too slowly, the capitulum may dry before they appear. However, the small but significant effect of the branching gene on the latency index, calculated for diseased plants and so for which the branched phenotype should have no direct effect, suggests that a QTL for this character also linked to the branching gene may exist. It would appear to be different from that controlling the mycelium index, since, in this case, the branched types present the favourable allele.

In conclusion, the first report by Mestries *et al.* (1998) that the *b1* gene intervenes in many agronomic characters is confirmed with different sunflower genotypes. Oil content and seed weight may be pleiotropic effects but capitulum resistance to *Sclerotinia* appears at least in part, to be determined by different but linked genome regions. Fine mapping of this linkage group should make it possible to position all the QTL compared with the branching gene.

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