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Studying genetic control fo partial resistance to *Aphanomyces cochlioides* (Drech) in Sugar-Beet (*Beta vulgaris* L.)

Mathilde Liorzou

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SESVANDERHAVE

sugar beet seed

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BELGIUM

Mémoire de Fin d'Études

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**Studying genetic control of partial resistance to *Aphanomyces cochlioides* (Drech) in
Sugar-beet (*Beta vulgaris* L.)**

Mathilde LIORZOU



Volet à renseigner par l'enseignant responsable de l'option/spécialisation*
ou son représentant

Date : .../.../... Signature

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Maître de stage : Stefaan Horemans

Enseignant référent : Maria Manzanares-Dauleux

Autres membres du jury (Nom, Qualité) : Mélanie Jubault et Régine Delourme

"Les analyses et les conclusions de ce travail d'étudiant n'engagent
que la responsabilité de son auteur et non celle d'AGROCAMPUS OUEST".

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Introduction

In the current developing world, there is a need to produce more food to feed the growing population. This will require an increase in the yield of cultivated crops. Today, these yields are limited by several biotic and abiotic factors such as diseases, pests or environmental conditions.

Sugar beet (*Beta vulgaris spp.vulgaris L.*) is the second most important source of sugar after sugarcane, and is grown as a root crop in more than 50 countries in temperate climates. In 2011, about 270 million tons of sugar beet were produced, worldwide. The top 5 producers are France (31 million tons), USA (28 million tons), Germany (25 million tons), Russia (21 million tons) and Ukraine (18 million tons) (FAO, 2012).

SESVanderHave is an international company specialized in developing, producing and commercializing sugar beet seeds. This company exists since 2005, when the company "Maison Florimond Desprez" bought the sugar beet breeding part of Advanta seeds (which was a fusion between Zeneca seeds and VanderHave). They are continuously investing in breeding for resistance to diseases including the fungus *Aphanomyces cochlioides*.

The sugar beet root disease *Aphanomyces cochlioides* is omnipresent and causes significant yield losses mostly in the United States, in eastern and northern Europe (Metzger, et al., 2008) (Moliszewska, et al., 2008) (Piszczek, 2004) (Amein, 2006). Control of *A. cochlioides* has been attempted by biological, chemical, agronomic and breeding methods, but currently no really effective methods have been developed to control this disease. The most effective chemical control is Hymexazol (Tachigarin, 3-hydroxy-5-methyl-isoxazole), but its efficacy remains variable, it is expensive and is ineffective past the seedling stage (Payne, et al., 1990), (Whipps, et al., 1993), (Rush, et al., 1993). The best approach seems to be resistance breeding (Yu, 2004). Until now, only partial resistance to *Aphanomyces cochlioides* has been found (Bockstahler, et al., 1950).

Resistance breeding can be supported by molecular tests that may be more precise than conventional techniques. To use those molecular tests, genetic control of partial resistance to *Aphanomyces cochlioides* has to be found. The aim was to search for quantitative trait loci (QTL) that are potentially involved in partial resistance to *Aphanomyces cochlioides*. In 2012, a study was made by SESVanderHave that placed QTL for resistance on chromosomes 4 and 6 of the SESVanderHave map. The objective of the present study was to locate these more precisely and to search for flanking markers. The major problem was to evaluate and quantify resistance to *A. cochlioides*. Some tests already exist in the literature (Windels, et al., 1989) (Peterson, et al., 1988) (Rao, et al., 1995) (Davis, et al., 1995) (Infantino, et al., 2006) (Panella, et al., 2008). At SESVanderHave, the current screening test was based on the inhibition of plant growth related to the growth of the fungus into the root. The parameter tested was the root wet weight, but there was confusion between the inherent capacity of the plant to grow and the real effect of the disease. Therefore it has been proposed to create a new screening system based on the observation of symptoms. It has been developed on seedlings in order to get results quicker and reduce heterogeneity between plants. Several parameters have been tested such as substrate, date of inoculation or inoculum concentration. A method of scoring has also been developed. Having this method established, the assay from 2012 has been retested with the newly developed protocol to compare the obtained results with both techniques. Another set of recombinant lines has been tested to evaluate their resistance level. With the SNP (Single Nucleotide Polymorphism) marker data available, phenotypic and genotypic data have been analysed by statistical methods in order to study linkage between the data and discover loci potentially involved in the resistance; this aims to define markers linked to resistance.

In this report, some bibliographic information will first be summarized, to provide a better understanding of the subject. Then, material and methods will be explained, including the explanation of the screening system developed. Results will then be shown followed by a discussion.

Genus <i>Beta</i>	
Section <i>Beta</i> (syn. <i>Vulgares</i> Ulbrich)	
2x, 3x, 4x ^a	<i>Beta vulgaris</i> L.
2x, 4x	<i>Beta maritima</i> L. Arcang.
2x	<i>Beta patula</i> Ait.
<div style="display: flex; justify-content: space-between; align-items: center;"> <div style="font-size: 2em;">}</div> <div> ssp. <i>vulgaris</i> ssp. <i>maritima</i> (L.) Arcang. ssp. <i>adansensis</i> (Pamuk.) Ford-Ll. et Will. </div> <div style="font-size: 2em;">{</div> </div>	
<div style="display: flex; justify-content: space-between; align-items: center;"> <div style="font-size: 2em;">{</div> <div> Leaf beet group^b Garden beet group^c Fodder beet group^d Sugar beet group </div> <div style="font-size: 2em;">}</div> </div>	
Section <i>Corollinae</i> Ulbrich	
2x, 4x	<i>Beta lomatogona</i> Fisc. et May.
2x	<i>Beta macrorhiza</i> Stev.
4x	<i>Beta corolliflora</i> Zos. ex Buttler
4x	<i>Beta intermedia</i> Bunge
4x, 6x	<i>Beta trigyna</i> Waldst. et Kit.
Section <i>Nanae</i> Ulbrich	
2x	<i>Beta nana</i> Boiss. et Heldr.
Section <i>Procumbentes</i> Ulbrich (syn. <i>Patellares</i> Tranzschel)	
2x	<i>Beta procumbens</i> Sm.
2x	<i>Beta webbiana</i> Moq.
4x	<i>Beta patellaris</i> Moq.

^aNumber of chromosomes (2x = 18; 3x = 27; 4x = 36; 6x = 72).
^bAlso named Mangold, Spinach beet, Chard, Swiss chard etc.
^cAlso named red beet.
^dAlso named forage beet.

Figure 1 : Sugar beet classification (Bosemark, 2006)

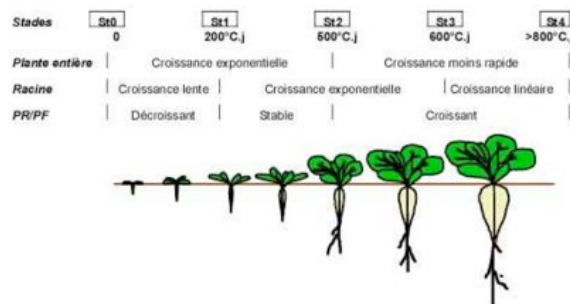


Figure 2 : Phenological stages of sugar beet during the vegetative growth (ITB)

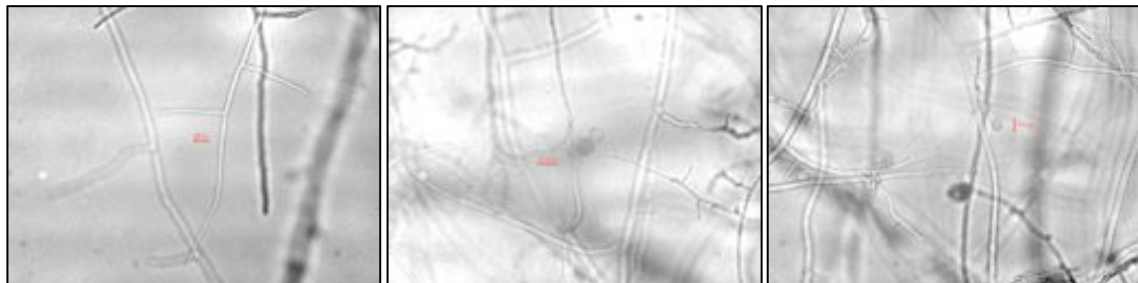


Figure 3 : *Aphanomyces cochlioides* (personal photography)

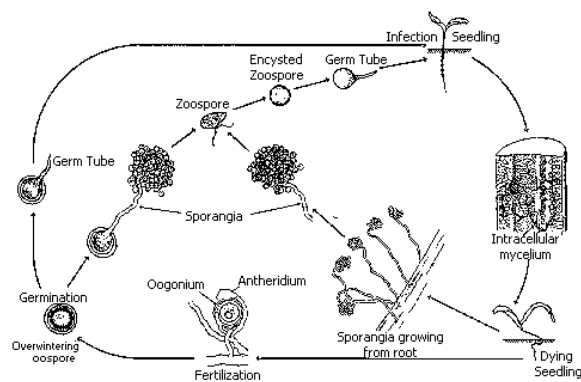


Figure 1. Life cycle of *Aphanomyces cochlioides* on sugarbeet. When root rot occurs later in the season, older plants should be substituted for seedlings in this illustration.

Figure 4: life cycle of *Aphanomyces cochlioides* (Windels & Engelkes, 1995)

Bibliography

A. Taxonomy and biology of sugar beet

Sugar beet (*Beta vulgaris*) is a dicotyledonous plant belonging to the Chenopodiaceous family (Figure 1). It has a biennial cycle (UFS, 2013). It is propagated with seeds (Doré, et al., 2006).

The first year of the reproduction cycle is the vegetative phase. The plant grows (Figure 2) and accumulates reserves in what we call the root, but which is actually 90% root, the upper 10% being derived from the hypocotyl (Cooke, et al., 1993). The plant reaches its final weight around October, depending on the sowing date, region... At this stage, the root can be harvested to produce sugar. This root has a truncated form and has a zone that is particularly rich in sugar named sacchariferous furrow (Doré, et al., 2006).

The optimal growth temperature is between 20 and 28°C, depending on the variety. (AgroParisTech, 2003)

B. Aphanomyces cochlioides in sugar beet

1. Life cycle

Aphanomyces cochlioides Drechsler is an oomycete water mold which causes damping-off and root-rot diseases in Sugar beet. (Figure 3) It has a facultative necrotrophic growth habit (Garrison, 2008).

This disease occurs in all sugar beet-growing regions of the United-States, Canada, Europe and Japan. It can also cause damage on other crops like *Spinacia oleracea* and wild *Beta* spp. This contributes to the survival and increase of inoculum levels in the soil by serving as source of colonization. (Harveson, et al., 2009)

Oospores are stimulated to germinate by root exudate components including Cochliophilin A (5-hydroxy-6,7-methylenedioxyflavone, 1) (Wen, et al., 2006) when the soil is warm (22-28°C optimal) and wet. Those oospores may either form biflagellate zoospores or produce germ tubes that are capable of penetrating the tissues directly (Cerenius, et al., 1985) and infect roots. More typically, the germ tube of an oospore develops into a sporangium that produces motile zoospores (100 to 200 per sporangium (Harveson, et al., 2009)) that move through the soil water and infect roots. These zoospores can swim limited distances (<50cm) in the soil water column (Yu, 2004). In infected tissue, mycelia form sporangia and release zoospores into the soil profile or form oospores within the infected root tissues. (Figure 4)

The disease develops in light soils but development is favored in heavy-textured soils and parts of fields that tend to remain wet (Harveson, et al., 2009). *A. cochlioides* oospores can survive several years in soil and debris of infected weed hosts or sugar beet (Kirk, et al., 2001).

A previous study made by SESVanderHave demonstrated that the optimal temperature of growth for *Aphanomyces* is 28.8°C.

2. Symptoms

The fungus penetrates elongating zones of the plants that are weaker. This induces that the fungus can attack the plant from the germination and at each stage of plant development. At the seedling stage, the hypocotyl and the roots are elongating. When attacked, it causes seed-rot and damping-off. During the rest of the plant life cycle it can attack the growing roots, causing root growth reduction, sprangling (tap root



Figure 5: Damping-off (SESVANDERHAVE)



Figure 6: symptoms on older roots (SESVANDERHAVE)



Figure 7: Above ground symptoms (SESVANDERHAVE)

disappearance) and sometimes plant death. All of this results in yield decreases. This is the reason why breeders are trying to create varieties that are resistant to this disease.

Most of the infection occurs on older roots. Aboveground symptoms initially include undersized, non-vigorous plants with yellow leaves that wilt on hot sunny days, but recover overnight and on cool, cloudy days. Leaves can have a scorched appearance and become brittle. Severely infected plants often die. (Figures 5,6,7)

On the taproot and at the junctions with lateral roots, symptoms begin as water soaked lesions with a tan yellow color and can be superficial, turning light to medium brown as they dry. These lesions can result in stunting of the root as well as rotting of the root tip and sometimes in shriveled vascular tissue. This latter syndrome is known as taproot tip rot. Infection on younger plants may induce the excessive production of lateral roots. This reaction allows the plant to compensate for the loss of a root tip and can be confused with symptoms of rhizomania. In severe cases, the disease can destroy most of the taproot. (Harveson, et al., 2009)

Roots with active or inactive infections show reduced yield and sucrose content and higher levels of impurities (non-sucrose constituents), which make sucrose extraction difficult and expensive. *Aphanomyces cochlioides* also negatively affects the storability of sugar beet roots (Harveson, et al., 2009).

C. Genetics and genomics of sugar beet

1. **Genome facts**

Sugar beet has a genome of nine chromosomes. It is diploid at the natural stage ($n=9$), but it also exist some artificial polyploids that are used in breeding programs (Yu, 2004). The total genome size is 758 million basepairs (Mbp) per haploid genome, varying between different sub-species (Arumuganathan, et al., 1991), (Schmidt, et al., 1998). Herwig et al. (Herwig, et al., 2002) estimated at 25 000 the number of different genes in sugar beet genome. Numerous genetic maps have been made with different types of molecular marker:

- RFLP, restriction fragment length polymorphisms (Barzen, et al., 1992), (Barzen, et al., 1995), (Pillen, et al., 1992)
- RAPD, randomly amplified polymorphic DNA (Barzen, et al., 1995)
- AFLP, amplified fragment length polymorphisms (Schondelmaier, et al., 1996)
- SSR, simple sequence repeats (Rae, et al., 2000)
- SNP, single nucleotide polymorphisms (Möhring, et al., 2004), (Schneider, et al., 2007)

They usually show nine linkage groups

The physical map of sugar beet was published on the 14th February 2012 (Dohm, et al., 2012). This was the result of a sequencing work done by several companies and laboratories. Because these results were not entirely public, SESVanderHave has to make its own sequence with its own markers.

2. **Breeding in sugar beet**

Genetic variation of sugar beet is thought to be limited since it was selected from white fodder beet (Yu, 2004). However, it has a short breeding history because it started to be selected in the 18th century (Achard, 1803) (in comparison to wheat which has been selected since 14 000 years). Moreover, this plant has long cycles of two years thus the process of selection is slowed down. Annual progress in yield is above 1% per year (Zimmermann, et al., 2000).

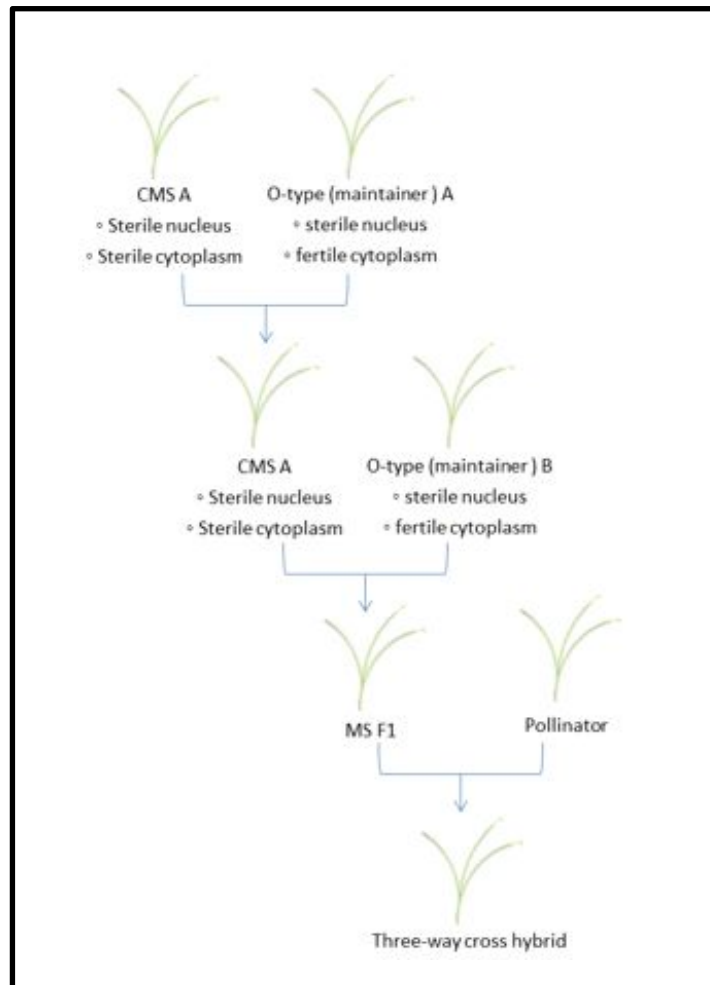


Figure 8 : Making a three-way cross hybrid

Because sugar beet is an allogamous plant, hybrids can be easily made and their vigor can be used. At first, hybrids were made randomly, then their production was more organized. Using tetraploids, breeding companies could make triploid plants that could not be multiplied by farmers. In the 50's, monogerm and male sterility were discovered both in a low yielding female plant (Biancardi, et al., 2004). Initially tetraploid plants could be crossed with this female and form strong hybrids because two chromosomes out of three were coming from the male. Current sugar beet hybrids are mostly, if not all, diploid. Male sterile lines are produced with the help of maintainers plant (known as O-types) which carry the same sterility genes as the male sterile but in normal cytoplasm (they are equivalent). Female plant of an hybrid is usually an hybrid of a CMS with another non-equivalent O-type. The hybrid produced is a three-way cross hybrid. (figure 8) Progress in male plant breeding is faster and easier but progress in the female plant breeding has a larger room for progress because they were less selected until now.

Hybridization with *Beta maritima* or *B. vulgaris* and artificial hybridization with *B. procumbens* or *B. webbiana* can be sources of new traits (Biancardi, et al., 2011).

The objectives of sugar beet breeding programs are to create stable, reliable varieties that have the highest possible yield of sugar per unit area in relation to cost of production, and that meet other requirements of the environment, growers and sugar factories (Bosemark, 2006).

Among the breeding traits, the most important are seed quality (germination rate, monogerm), sugar yield (from 3,5 t/ha in 1946 to about 13 t/ha in 2011 (Desprez, 2013)), industrial quality (sugar content, ratio sugar molasses/sugar content, low impurities content), decrease of soil tare level (soil which sticks to the sacchariferous furrow), decrease of nitrogen needs.

However, because there is almost always a negative correlation between root yield and sugar percentage, maximum expression of both characters is difficult to obtain in the same genotype. Moreover, to produce the high and stable yields required today, sugar beet cultivars must have resistance or tolerance to important pests and diseases such as Rhizomania, Nematodes, Rhizoctonia, Cercospora, and curly top. Similarly, to counter effects of possible climatic changes in the year ahead, breeders may soon have to breed for tolerance to drought or other climatic or edaphic stress factors (Bosemark, 2006).

3. Breeding for disease resistance

a. What is resistance?

Pressured by the presence of a pathogen, plants react differently. They can be susceptible or resistant. Two types of resistance exist, vertical or horizontal. Vertical resistance is generally total and specific to one isolate. It triggers a hypersensitive response like apoptosis. It is based upon one or a few major genes. It is easy and quick to use. However, there is a high risk of collapse of the resistance; this is called the 'vertifolia' effect.

Horizontal resistance is partial and its effect is more to slow down the infection with some barriers rather than to stop it completely. It is a polygenic resistance with several minor genes. The major advantage is that it is a stable resistance. On the other hand, the transfer is slow and difficult and the genetic background can play a key role. (personal communication M. Meulemans, 2013, (Lepoivre, 2003))

b. Resistance to *Aphanomyces cochlioides*

The best approach to control *Aphanomyces cochlioides* seems to be resistance breeding (Yu, 2004). However, the genetic mechanism of resistance is poorly understood

(Campbell, et al., 1993) and *Aphanomyces* is the disease for which least progress has been made, because of the lack of highly resistant sources and the difficulty of creating reproducible *Aphanomyces* infections for lab or field screening (Yu, 2004). A lot of varieties were tested for resistance (Afanasiev, 1956) (Schneider, et al., 1961), but none was highly resistant and most were susceptible. The fact that it is a very unpredictable disease makes it more difficult to study. It needs an association of several environmental conditions that does not occur every year.

However, partial genetic resistance to *Aphanomyces* has been described for over 50 years. Although it was shown that resistance to *Aphanomyces* was heritable and dominant, inheritance of resistance is poorly understood (Bockstahler, et al., 1950).

Lines with resistance to *Aphanomyces* have primarily been developed in East Lansing, MI, and several breeding lines (US-H20, EL48 and SP6822 for instance) were derived from selections made under high disease pressure (Yu, 2004), in naturally infested fields. Resistance was measured by harvest yield as an indirect measure of the persistence of sugar beet stands after infection earlier in the season. Greenhouse evaluations were generally found to reflect field performance (Schneider 1954, 1959). Correlations were however inconsistent (Schneider, et al., 1983) presumably due to the difficulty to control variables such as effective inoculum concentration, and to variability in soil conditions (Yu, 2004).

Breeding sugar beet resistance to *Aphanomyces* at both the seedling and plant stages remains an important breeding objective. It will reduce not only production costs but also the potential pollution caused by fungicide application. However, resistance sources are limited: the genetic background of the resistance seems to be narrow, with no more than a few germplasm sources (Yu, 2004).

Another problem for the study of resistance to *Aphanomyces cochlioides* is the lack of a reliable resistance test. Most of them are based on varieties' yield performance in the field with *Aphanomyces cochlioides* epidemics, but reproducibility is low due to interference from many environmental factors (Downie, et al., 1952).

c. Bioassay

A good bioassay should be reliable. Results have to be correlated with what is found in the field and repeatable. A number of factors have to be determined: culture conditions (container, substrate, time of inoculation, culture temperature, water need...), quantifying method (visual, coloration, fluorescence, molecular techniques...), material used (should be discriminant).

d. Techniques of existing bioassays

Inoculum production

Both oospores and zoospores have been used for artificial inoculation of sugar beet seedlings (Coe, et al., 1966), (Schneider, 1954), (Schneider, 1978), (Schneider, et al., 1983). Oospores have the advantage that they can be produced easily, and can be maintained in long term storage. However their disadvantage is that the timing of inoculation of very young seedlings (<2 weeks old) introduces a time lag between oospore germination, zoospore release, infection, ramification and development of symptoms. Zoospores have the advantage of being abundant and easily produced, but the disadvantage of rapidly encysting in adverse conditions (agitation, light, presence of polyvalent cations...). This reduces the effective concentration of active zoospores presented to the seedling. Resistant and susceptible varieties could be discriminated reliably using active zoospores as the inoculum. (Yu, 2004)

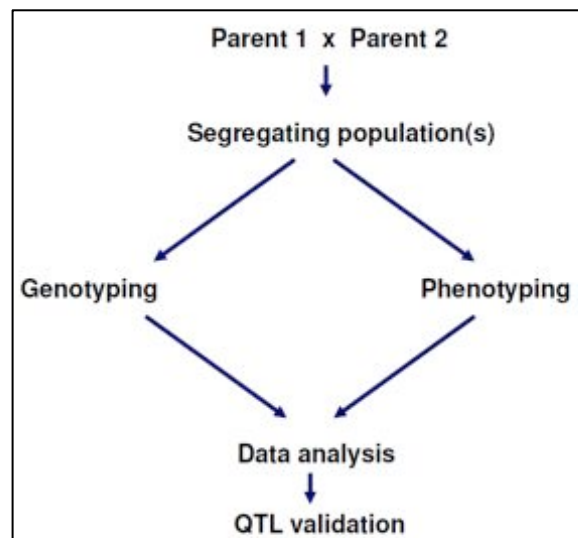


Figure 9 : Summary of the necessary steps in a QTL mapping study ((Buerstmayr, 2011)

Scoring

Most of the bioassays are performed on adult plants and scoring is made on roots (Peterson, et al., 1988), (Rao, et al., 1995), (Davis, et al., 1995), (Infantino, et al., 2006), (Beale, et al., 2002), (Metzger, et al., 2008), (Moliszewska, et al., 2008). Plant can also be scored upon their general appearance (Panella, et al., 2008) (Grünwald, et al., 2003) (Pilet-Nayel, et al., 2002) (Davis, et al., 1995).

A few bioassays with *Aphanomyces cochlioides* have been performed on seedlings. Coe & Schneider (1966) chose to compute a mean disease severity index of different entries by assigning a numerical rating to each surviving plant according to severity of symptoms, totaling the individual plant scores and dividing it by the number of plants inoculated. They also made a numerical rating expressing the foliage vigor of the surviving plants assigned to each entry. Results from the two methods generally correlated well with one another.

Some other technics could be used to avoid a biased score, but they first have to be tested. Thermography and chlorophyll-fluorescence imaging can show a change in photosynthetic efficiency and transpiration (Chaerle, et al., 2004). Real time PCR methods are currently gaining interest. PCR can measure the correlation between amount of pathogen DNA and the reaction of the cultivar (Infantino, et al., 2006), (Weiland, et al., 2000). This method is sensitive enough to detect pathogen in symptomless tissues (Okubara, et al., 2005). SESVanderHave already used real time PCR for cercospora leaf spot detection and it was effective (De Coninck, et al., 2012).

D. Quantitative Trait Loci (QTL) analysis

1. QTL definition

Nowadays molecular tools and statistical methods allow to localize genes implied in quantitative resistance on genetic maps and to estimate their effect. They are called QTL for "quantitative trait loci". QTL are generally spread all over the plant genome. A lot of factors can influence the detection (type and size of the segregating population, detection threshold, heritability, phenotype measurement, map accuracy...). (Kover, et al., 2001).

2. Principle of QTL mapping

a. Principle

QTL mapping is a statistical study of the alleles that occurs at a locus and the phenotypes that they produce. It is based on the segregation of genes and markers via chromosome recombination during the meiosis, thus allowing their analysis in the progeny (Paterson, 1996).

QTL analysis depends on linkage disequilibrium (LD), a situation where genes fail to segregate independently between genetic markers and the QTL. Two main analyses exist: linkage analysis and association analysis. They differ on the type of population used. Different statistical approaches can be used to detect the most likely positions of QTL: simple regression, interval mapping, composite interval mapping and a stepwise approach. These approaches allow the detection of interactions between QTL.

Several steps are necessary in a QTL mapping study (Figure 9). First, parents have to be chosen in order to produce a segregating population. Then this population should be both genotyped and phenotyped, and the data combined to detect and quantify a QTL.

b. Requirements for QTL mapping

Type and size of mapping population. The mapping population should use parental lines that are contrasting phenotypically for the target trait. They should be genetically

divergent, to permit the identification of a large set of polymorphic markers that are well distributed across the genome. The choice of mapping population (F₂, Double Haploid, Recombinant Inbred Lines...) can vary, depending on the objectives of the experiment. The size of mapping population for QTL analysis depends on several factors (Buerstmayr, 2011).

Saturated linkage map based on molecular markers. To perform a whole-genome QTL scan, it is desirable to have a complete coverage of the genome under investigation. In such a map, markers are available on each chromosome from one end to the other, and adjacent markers are sufficiently close that recombination events only rarely occur between them. For practical purposes, it is generally required to have a map distance of about 5 to 10 centiMorgans (cM). To be useful, markers should be polymorphic in the mapping population (Prasanna, 2003) (Buerstmayr, 2011).

Reliable phenotypic screening of mapping population. This is the critical part of the analysis. This screening has to be as precise as possible. Basic phenotypic data are the estimates of phenotypic performance of individuals across environments. The accuracy and precision of phenotyping determines how realistic the QTL mapping results are. An appropriate phenotyping protocol should consist of a representative sample of environments and their optimal location; a number of replications per individual in each environment; an experimental design: randomization provides statistical validity to results and protection from bias. However, there is always some variation left uncontrolled within blocks.

3. Statistical methods for QTL Mapping

a. *Analysis of variance (ANOVA)*

This method is the simplest (Soller, et al., 1976). The aim is to test the H₀ hypothesis for each marker: "there is no difference in the mean of the trait between lines that possess marker allele A from lines that possess the alternative allele B". This hypothesis is tested using t-tests or F-tests (Prasanna, 2003).

This method gives an approximate QTL position, a statistical significance (p-value), a percentage of phenotypic variance explained by each marker and an estimate of additive and dominant effects of single QTL. However, it has some disadvantages because it does not include the map information and each marker is treated as an independent statistical test (Buerstmayr, 2011). It is also difficult to conduct separate estimates of QTL location and QTL effect. A weak QTL effect near the marker can be confused with strong QTL effects far from the marker. Moreover, individuals with missing genotypes often need to be discarded. Last, when the markers are widely spaced and/or unevenly distributed, the QTL may be quite far from neighboring markers, and hence the power for QTL detection will decrease (Lander, et al., 1989), (Manly, et al., April 1999), (Broman, 2001).

The rank sum test of Kruskal Wallis can be regarded as the nonparametric equivalent of the one way analysis of variance. With this test, no assumptions are made about the probability distribution of the quantitative trait. (Van Ooijen, 2009)

b. *Interval mapping method*

Simple Interval Mapping (SIM). This method developed by Lander et al. (1989) includes the genetic map information. The aim is to test the likelihood that a QTL could occur at certain intervals along the genetic map. This is done by performing a likelihood ratio test at every position within the interval. In this approach, the QTL is located within a chromosomal interval, defined by the flanking markers. (Lander, et al., 1989) proposed a simple rule for constructing confidence intervals for QTL position, which uses the logarithm of odds (LOD score).

LOD score is defined as the logarithm of the ratio of two likelihoods: the likelihood for the alternative hypothesis (that there is a QTL) and the likelihood of the null hypothesis (that there is no QTL). A LOD score of three for a given point means the odds are a thousand to one in favor of a QTL being located at that position (Buerstmayr, 2011).

SIM has more power and requires fewer individuals than ANOVA (Lander, et al., 1989), (Knott, et al., 1992), (Zeng, 1994) but it has limitations. First, SIM considers one QTL at a time, ignoring the effects of other (mapped or not yet mapped) QTL. Therefore, SIM can provide a biased identification and estimation of the effect and position of QTL when such multiple QTL are located in the same linkage group (Knott, et al., 1992), (Martinez, et al., 1992), (Zeng, 1994). Second, QTL outside the interval under consideration can affect the ability to find a QTL within it (Zeng, 1994). Third, false identification of a QTL (false positive) can arise if other QTL are linked to the interval of interest.

Composite Interval Mapping (CIM). (Jansen, 1993), (Zeng, 1993) and (Zeng, 1994). It is an expansion of SIM. Markers near the QTL are included as cofactors in the estimation of QTL effects. This minimizes the effects of QTL in the remaining genome when attempting to identify a QTL in a particular region. It increases the power of detection for weak effects' QTL that can be hidden by a QTL of strong effect.

It has the advantage of giving a more precise estimation of the QTL position. This is especially useful when QTL are close to each other in the same linkage group. It must be used carefully because it can lead to calculation artifacts (Buerstmayr, 2011). CIM can be affected by an uneven distribution of markers in the genome, and is not directly extendable to analyze epistasis. The use of tightly linked markers as cofactors can reduce the statistical power to detect a QTL (Zeng, et al., 1999).

Multiple Interval Mapping (MIM). To avoid CIM's limitations, (Kao, et al., 1999) proposed an implemented "multiple interval mapping" (MIM) to map multiple QTL simultaneously. The idea of MIM is to fit multiple putative QTL effects and associated epistatic effects directly in a model to facilitate the search, test and estimation of positions, effects and interactions of multiple QTL. MIM consists of an evaluation procedure designed to analyze the likelihood of the data given a genetic model (number, positions and epistatic terms of QTL). When compared with methods such as SIM and CIM, MIM tends to be more powerful and precise in detecting QTL.

4. Tests for QTL significance

One of the challenges for QTL mapping is the difficulty of determining appropriate significance thresholds (critical values) for the two types of errors: false positive and false negative. Determining appropriate threshold values appears to be difficult because many factors can vary between two experiments and can influence the distribution of the test statistics. These include, but are not limited to, the sample size, the genetic map density, segregation ratio distortions, the proportion of missing data. A permutation test is usually used to determine a threshold value for significance testing of the existence of a QTL effect. Permutation tests generate many different samples from the actual data by randomizing trait values with respect to the marker genotypes to estimate empirically the threshold for a test statistic for detection of a QTL. This approach accounts for missing marker data, actual marker densities, and nonrandom segregation of marker alleles. This permutation analysis is repeated for a number of replicates (usually 1,000 permutations) to obtain a distribution of the maximum test statistics, and from the distribution to obtain the threshold value (Semagn, et al., 2010).

5. Estimated parameters

a. Confidence interval

Lander et al. (1989) suggested to find the location of the estimated QTL using a decrease of one from the peak LOD score.

b. QTL effect

The additive effect represents half of the difference between the averages of both parental alleles in segregation. This effect is expressed in the units of the trait being studied. It has a meaning in reference to one of two parents arbitrarily chosen. In addition, the QTL effect is also estimated by the percentage of the phenotypic variation explained by the QTL.

6. QTL detection in Sugar Beet

In sugar beet, several QTL have been mapped for sucrose yield and quality, yield and some qualitative traits (as nitrogen, potassium...) (Schneider, et al., 2002) (Möhring, et al., 2004), restoration of CMS (Hjerdin-Panagopoulos, et al., 2002), disease resistances (Cercospora leaf spot (Setiawan, et al., 2000), Rhizoctonia root and *Rhizoctonia solani*). A previous study made by SESVanderHave showed that resistance for *Aphanomyces cochlioides* is situated on chromosome 4 and 6 of SESVanderHave map.

Table 1 : Genotype of the parents for experiment 1

Marker	D101_S01	D028_S02	D084_S01	D102_S03	D106_S03	D055_S04	D110_S01	D103_S02	D029_S01	BAC067_S01	F077_S02	F086_S04	F085_S01	F107_S01	F067_S03	F096_S01	F108_S01	F036_S01	
Seed name	38	41	44	46	49	51	51	53	57	5	6	8	9	18	24	25	25	29	
S11T270375001	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
S11T270345001	H	H	H	H	H	H	H	H	H	H	H	H	B	B	H	H	H	H	H
S11T270385001	H	H	H	H	H	H	H	H	H	B	B	B	B	B	B	B	B	B	B
S11T269975001	H	H	H	H	H	B	B	B	B	H	H	H	H	H	H	H	H	H	H
S11T269985001	H	H	H	H	H	B	B	B	B	H	H	H	H	H	H	H	H	H	H
S11T270075001	H	H	H	H	H	B	B	B	B	H	H	H	H	H	H	H	H	H	H
S11T269995001	H	H	H	H	H	B	B	B	B	H	H	B	B	H	H	H	H	H	H
S11T270055001	H	H	H	H	H	B	B	B	B	B	B	B	B	H	H	H	H	H	H
S11T270095001	H	H	H	H	H	B	B	B	B	B	B	B	B	H	H	H	H	H	H
S11T270005001	H	.	.	H	H	.	B	B	B	B	B	B	B	B	B	B	B	B	B
S11T270015001	H	H	H	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B
S11T270185001	H	H	B	B	B	B	B	B	B	H	H	H	H	H	H	H	H	H	H
S11T269945001	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
S11T270105001	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
S11T270155001	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B

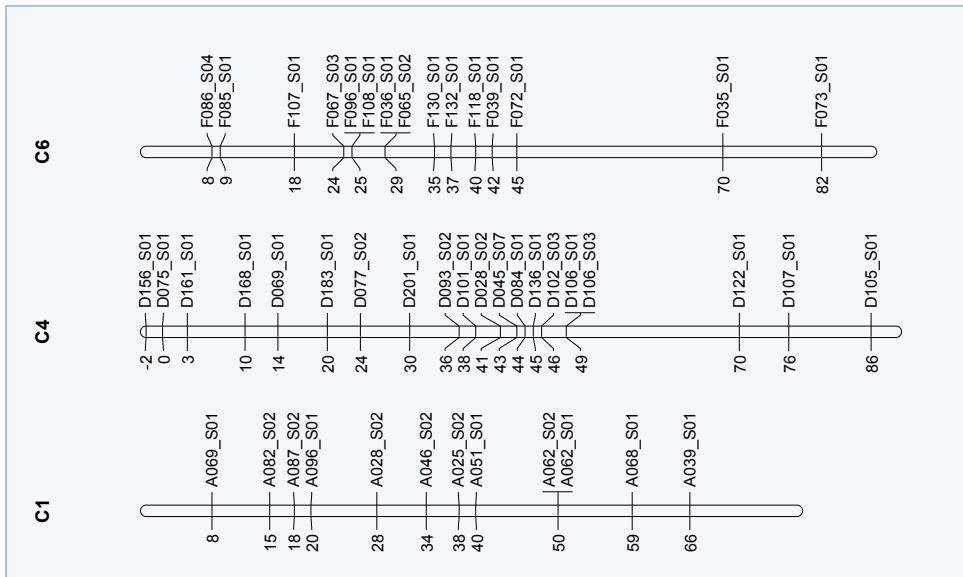


Figure 10 : Genetic map of the used markers

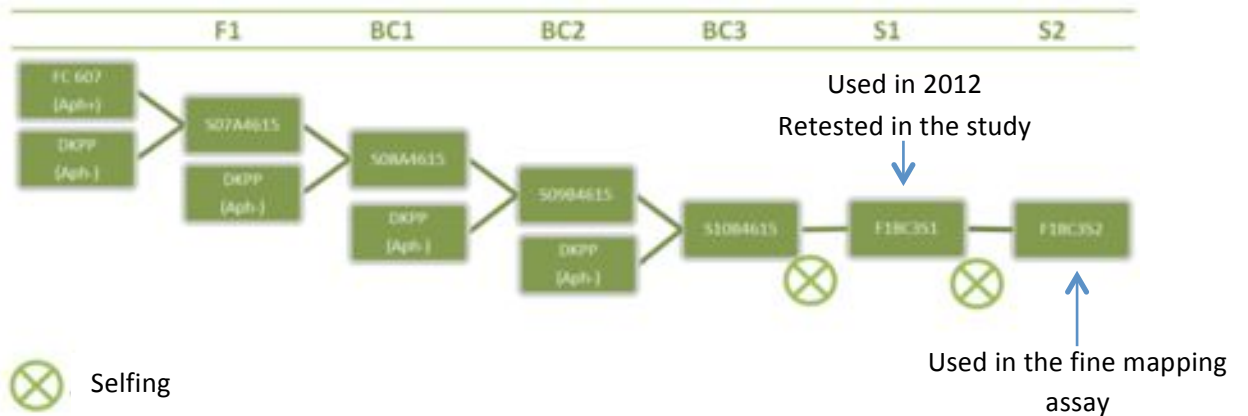


Figure 11: Genealogy of the tested populations

Material and methods

A. Plant material

1. Plant material used to develop the bioassay

The sugar beet lines that have been used for this work were, a line derived from FC607 (abbreviated in the text as FC607), DKPP and their hybrid FC607xDKPP. FC607 is the resistance source and is derived from an American line (Smith, 1980). It is partially resistant to *Aphanomyces cochlioides*. DKPP is an elite pollinator line, susceptible to *Aphanomyces cochlioides* and is a multigerm line.

2. Plant material used in repeating the 2012 assay

Plant material used in the 2012 assay came from an initial cross between FC607 and DKPP. It was a BC3 S1 level (three backcrosses with DKPP as a recurrent parent and one selfing). The genealogy is shown in figure 11. This population was called artificial because those lines were selected for their variability for the resistance character (it was not following the Hardy-Weinberg principle). 16 lines were tested and segregate for the chromosomal region which was expected to carry the character. Three groups of genotypes could be outlined. One which was entirely heterozygous on chromosome 4 (between 38 and 57 cM) (group A), one which was heterozygous between 38 and 49 cM (group B), and the last which was heterozygous between 38 and 41 cM (group C). (Table 1)

Chromosomes were all fixed except chromosomes 1, 4 and 6. (Figure 10)

Three controls have been used, and were the same as those used to develop the bioassay : FC607, DKPP and their hybrid.

3. Plant material used for resistance fine mapping

The lines used here were BC3 S2 lines. They were the product of the same initial cross between FC607 and DKPP than the previous assay with one more selfing (Figure 11). DKPP was the recurrent parent. As it was a backcross line, that meant that some parts of the chromosomes were still unfixed. Because it descended from the previous population, it is also an artificial one.

As it was supposed that QTL for resistance were situated on chromosomes 4 and 6 from a previous bioassay, these parts of the genome were particularly selected during the backcrosses to keep variability in those zones. Among all families, 16 were selected, depending on their genotype. It should allow a good coverage of recombination events to locate the resistance more precisely. We focused here on the 20 to 50 cM zone of chromosome 4. (Figure 12)

The same controls as the previous assay were used.

B. Seed treatment

After the harvest, seeds were dried in a room at 29°C for 3 to 4 days. Then they were cleaned, and dust and small seeds were removed. Before using it in an assay, to prevent them from being already infected with *Aphanomyces cochlioides* or another fungi, seeds were washed with water during 4 hours. A fungicide could not be used to avoid interference with future inoculation.

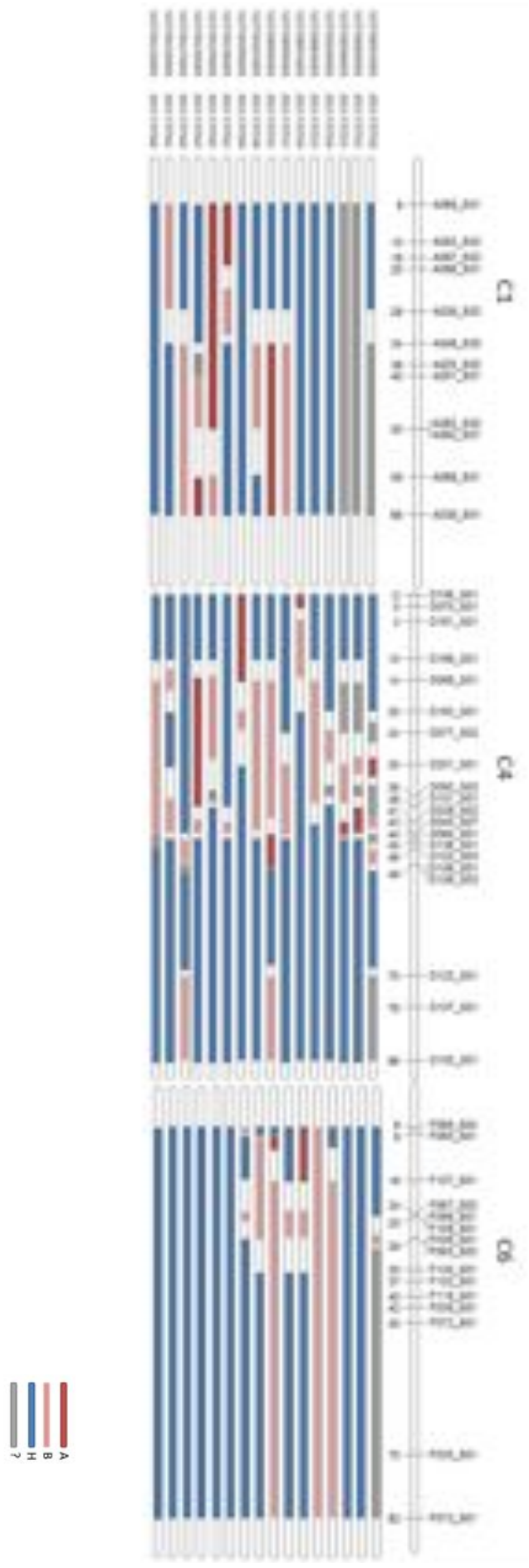


Figure 12 : Genotype of the parents for fine mapping assay

C. Inoculum preparation

Aphanomyces cochlioides was isolated and cultivated on a potato dextrose agar (PDA) medium for 7 days at 24°C. Spores were then cultured in a maltose dextrose solution for 5 days at 24°C. Then they were washed 3 times with autoclaved mineral water of the Spa brand. On the last day, a sample of the solution was diluted 100-fold and the spores were counted to determine their concentration. During the inoculum preparation time the solution was shaken but not too strongly to avoid damage to the flagella. According to the number of spores in the solution, they were diluted to reach the desired concentration. The precise protocol used was the same as the one which is usually used by SESVanderHave.

A mixture of several *Aphanomyces cochlioides* strains were used for this assay. They all came from strains produced by the lab.

D. Growth conditions

During the development of the bioassay, different growth conditions were tested: different growth systems (in tubes or in trays, with compost or with sand), different dates of inoculation and different inoculum concentrations. More information about material and methods used to develop the bioassay can be found in annexes I and II. At the end of the development, it has been decided to keep the following protocol using tubes.(Figure 13)

1. **Sowing**

Seeds were placed in a box containing an accordion-shaped filter paper. Then 38 mL of water were added into the box. First they were put to germinate in the dark in an incubator at 24°C during 3 to 4 days. Then they were put under light in a growth chamber at 18°C during the day (16h light period) and 19°C at night (8h) for 2 to 3 days.

2. **Tube preparation**

Tubes (about 20 cm long and 2 cm in diameter) were filled with sand. At the bottom of it, a piece of glass wool was placed to block the holes. A capillary matting strip was placed from the top to the bottom (through the piece of glass wool) to irrigate the substrate (Figure 14). 2 days before transplantation, the tubes were placed in a water box to humidify the sand.

3. **Transplantation**

Six and seven days after sowing, plants were transplanted into the sand. 10 mL of water was added to each plant at this stage to ensure that the plants were well watered from the beginning.

4. **Growth conditions**

Plants were then placed in a growth chamber (phytotron) at a temperature of 22°C in the day (16h) and 19°C in the night (8h). Those temperatures were chosen as being optimal for the growth of the sugar beet and the pathogen.

Two days after transplanting, plants were irrigated with nutritive solution.

5. **Nutritive solution**

The nutritive solution used here was the Steiner solution (see annex II) diluted 10 times.

6. **Inoculation**

Plants were inoculated 22 days after sowing. 1ml of 2000sp/ml inoculum solution was applied at the bottom of the hypocotyl using a pipette. Care was taken that all plants were well watered prior to infection.

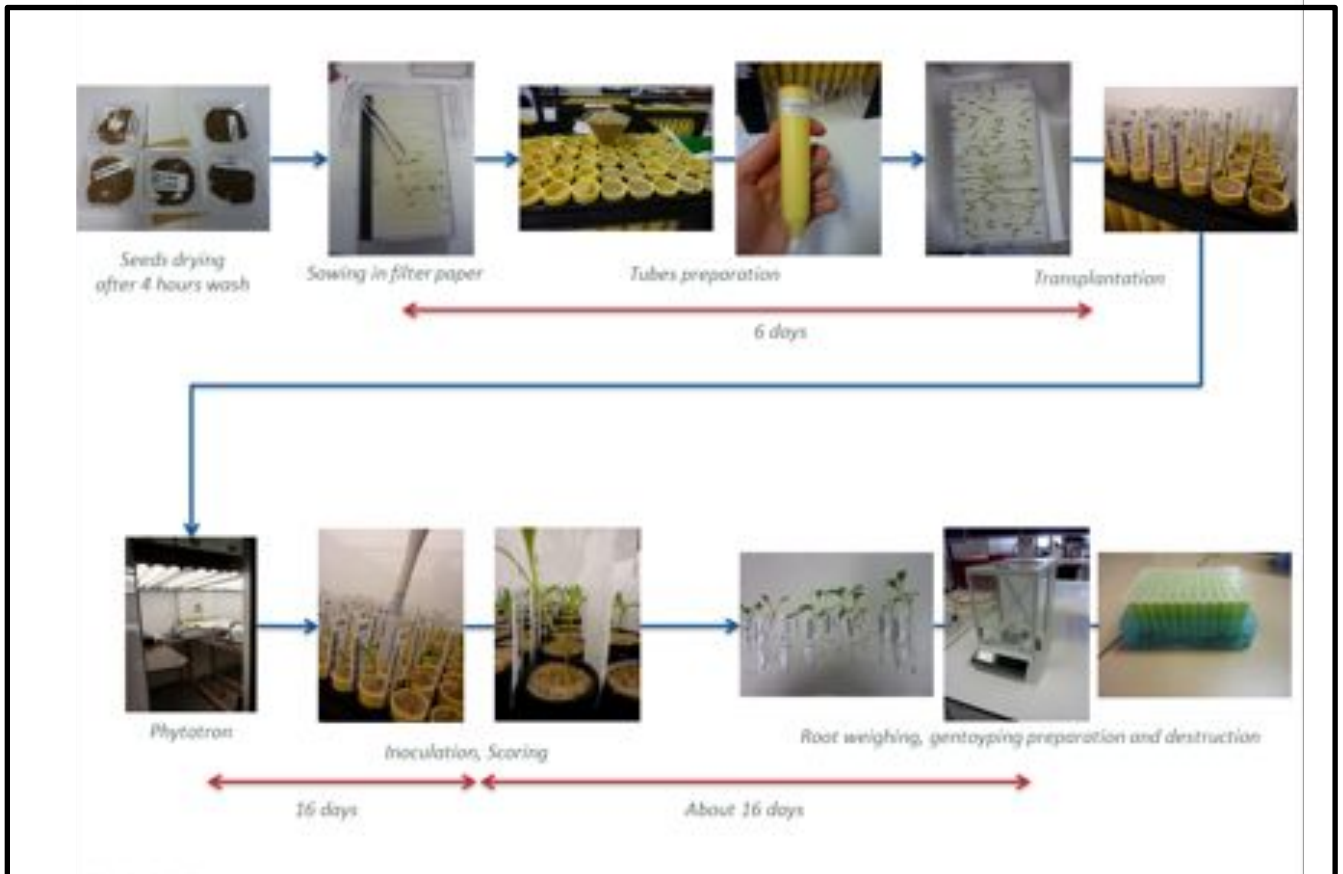


Figure 13 : Protocol's steps followed for the experiments

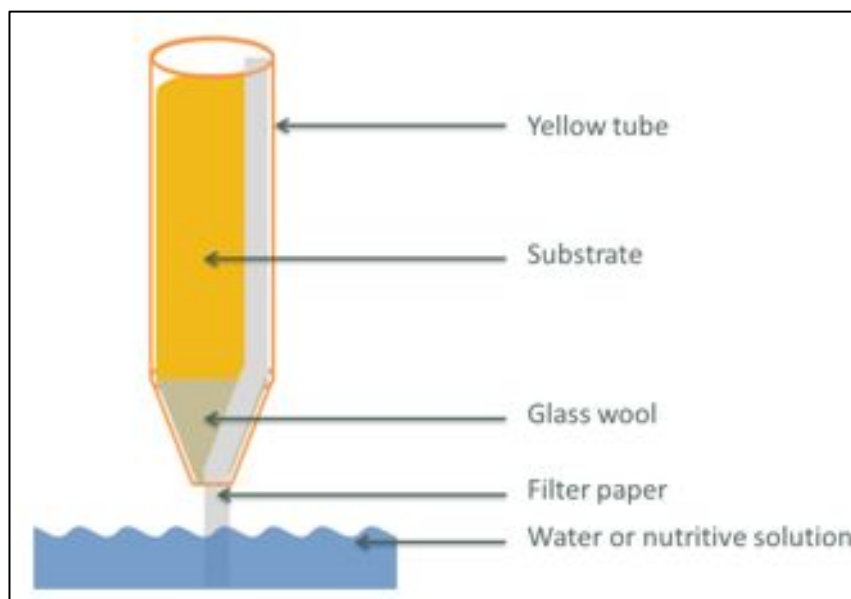


Figure 14 : Tube system

E. Experimental design

In the phytotron where the test was performed, no gradient (temperature, light...) was observed. It could have been easy to make only one randomized complete block. However, 600 plants had to be cultivated and the boxes can just contain 200, so three blocks were still needed. In each block, there were 10 repetitions of each genotype. This make in total 30 repetitions per genotype, except for DKPP which had 20 repetitions in each block or 60 repetitions in total. All repetitions were randomized in the block.

To make it easier, in the rest of the document the repeat of the assay made in 2012 will be called “experiment 1” and the assay for fine mapping will be referred as “experiment 2”

F. Evaluations and analyses

1. **Evaluation of resistance**

A first type of scoring was made during the incubation based on the example used by Yu (2004). The score “0” meant that plant had no or a few symptoms. The score “1” meant that more than 2/3 of hypocotyl and root surface was diseased. “0,5” was used for all other cases which were not 0 nor 1 (Figure 15).

During the bioassay development, on the final scoring day (around 16 days after inoculation) additional evaluations were performed.

Root observations

Root were cut under the hypocotyl and the weight was measured. Statistical analysis on the data were made, but the results were not consistent. The idea came to observe the roots and calculate the infected surface with the help of a software used in an assay for nematodes. But after the first trial, it was noticed that plants were too young and roots were too thin to see anything on it.

qPCR trials

After weighing, root samples were prepared in order to perform a qPCR assessing the quantity of *Aphanomyces* DNA in comparison with the quantity of sugar beet DNA.

The samples were washed to remove remaining substrate, they were put in a 96-well box and put, at least one hour, at -80°C. Then, the samples were put in the freeze-dryer during two days. The dried samples were then grinded. The DNA was extracted with the kit usually used by SESVanderHave, “Nucleomag 96 plant” from Macherey Nagel.. The qPCR is made with specific primers adapted to *Aphanomyces cochlioides* and sugar beet. The PCR technic used is the TaqMan.

The results given were the cycle threshold (Ct) of each sample for both sugar-beet and *Aphanomyces cochlioides*. With those data it was possible to calculate the $2^{-\Delta\Delta Ct}$ which is a common value used to quantify DNA in qPCR. An mean comparison with the Duncan test allowed to compare the three genotypes (controls) used.

Due to bad results for the bioassay development, those evaluations were not executed for experiment 1 and 2.

Pathogen identification

To be sure that the plants were really inoculated with *Aphanomyces cochlioides* a part from an infected hypocotyl was isolated on PDA. A few days later, it was possible to recognize *Aphanomyces cochlioides* under a microscope (Figure 3).



Figure 15 :Yu scoring system (personal photos, Yu, 2004)

2. Watering homogeneity

To evaluate if water was homogeneously distributed in the tubes, they were weighed before and after watering. Two repetitions were made, one for each assay.

3. Statistical analysis

Statistical analyses were made with the software R (R Core Team, 2013). Statistical analyses were made on all the data to assess significant differences between treatments.

a. Analyses of variance

Because this study involved different scoring time points, it was possible to do a kinetic analysis by the mean of the evaluation of the AUDPC (Area Under Disease Progression Curve). AUDPC was calculated from the first day after inoculation until the last day of the experimentation (19dpi for experiment 1 and 15dpi for experiment 2). Besides the AUDPC value, the score at the day were the controls are the most significantly different from one another and where the range between the most susceptible and the most resistant is the maximal was chosen as a second score (it was 7dpi for both experiment).

The evaluation of different effects on 7dpi score and AUDPC was made by an ANOVA. The analysis of variance model was the following: $AUDPC_{ij} = \mu + g_i + \beta_j + \varepsilon$. $AUDPC_{ij}$ is the AUDPC of genotype i in block j , μ is the AUDPC mean, g_i is the genotype effect, β_j is the block effect and ε the residuals effects. Respectively for 7dpi: $7dpi_{ij} = \mu + g_i + \beta_j + \varepsilon$.

b. Mean comparison

This ANOVA was followed by a Duncan test to make groups with individuals that are not significantly different from each other. To be more precise, a Dunnett test allowed to compare all genotypes to each controls.

As no block effects were detected in either of the assays, there was no need to adjust the individual values.

c. Heritability

Heritability was calculated with ANOVA data with the following formula: With σ^2_g as the genetic variance, σ^2_e as the residual variance and n the number of repetitions per tested lines.
$$h^2 = \frac{\sigma^2_g}{\left[\sigma^2_g + \frac{\sigma^2_e}{n} \right]}$$

d. Individual distribution

Individuals' distributions were represented according to the individuals' response to *Aphanomyces cochlioides* infection.

4. QTL detection

The analyses were made with the MapQTL6 software (Van Ooijen, 2009).

a. Genotyping

For the experiment 1 and 2, remaining leaves were sampled to allow genotyping. For experiment 1, only leaves that were still healthy at the end of the experiment were sampled, for experiment 2, as much leaf material as possible were sampled during the assay, as soon as plants have a score of "1".

In this study, SESVanderHave consensus map was used. Markers that were chosen in this study have a segregating genotype at their locus.

At each marker, the genotype "A" was given to plants having the genotype coming from FC607 (Resistance donor plant) and le genotype "B" was given to plants whose genotype come from DKPP (receiver plant). "H" was given to heterozygous plants.

b. Experiment 1

This analysis was made with 16 markers SNP (on chromosomes 4 and 6) with a segregating population of 16 lines (total of 182 individuals sampled).

Comparison of the three genotypes groups

To compare those three groups, the ANOVA model: $Group_i = \mu + G_i + \varepsilon$ was followed. $Group_{ij}$ is the Group named i , μ is the group mean, G_i is the group effect and ε the residuals effects.

Rank sum test of Kruskal-Wallis

Because our population has a non-normal distribution, Kruskal-Wallis test was preferred to ANOVA. No further analysis will be made because this population is not adapted for QTL mapping.

c. Experiment 2

The analysis was made with 53 markers SNP (on chromosomes 1, 4 and 6) with a segregating population of 16 lines (total of 262 individuals sampled).

Comparison of families results

As a first overview of the results, results of each individual were observed. For each, a letter was given: "A" if the scores (7dpi score and AUDPC) were low and seem to show resistance (usually 0 for 7dpi score and less than 11 for the AUDPC), "B" if they are high (usually 1 for 7dpi score and more than 16 for AUDPC) and seems to be susceptible. "H" is for all other cases. In each family, for each letter, the number of individuals was counted and the mean was calculated. Looking at the family results, it is possible to give the same letters to the global family. "A" is given when the family has a lot of "A" and "H" individuals compared to "B", "B" is given when the family has a lot of "B" individuals compared to "A" and "H" and "H" is given for all intermediate families.

With those results in parallel to the genotype data, it might be possible to see a tendency in marker alleles of genotypes that are more resistant.

Rank sum test of Kruskal-Wallis

Like for experiment 1, a Kruskal-Wallis test was made on the data.

Interval mapping

This analysis was made for both traits, 7dpi score and AUDPC. It was made only on chromosomes 1, 4 and 6. For each family, parts that were fixed were treated as missing data otherwise it could have disturbed the analysis.

Because the population used here is an artificial one, it was not possible to make a special map for this assay, this is why the consensus map from SESVanderHave was used.

LOD score

To know if the results are significant, the maximum LOD score was calculated using a permutation test with 1000 iterations. The genome wide threshold with a P-value of 0.05 (or 5%) was chosen. Genome wide means that it is the maximum LOD value everywhere in the genome including all linkage groups.

MQM (Multiple QTL Mapping)

This analyze was done in the same way as interval mapping, except that some co-factors were manually selected. They were chosen on results obtained in the interval mapping test.

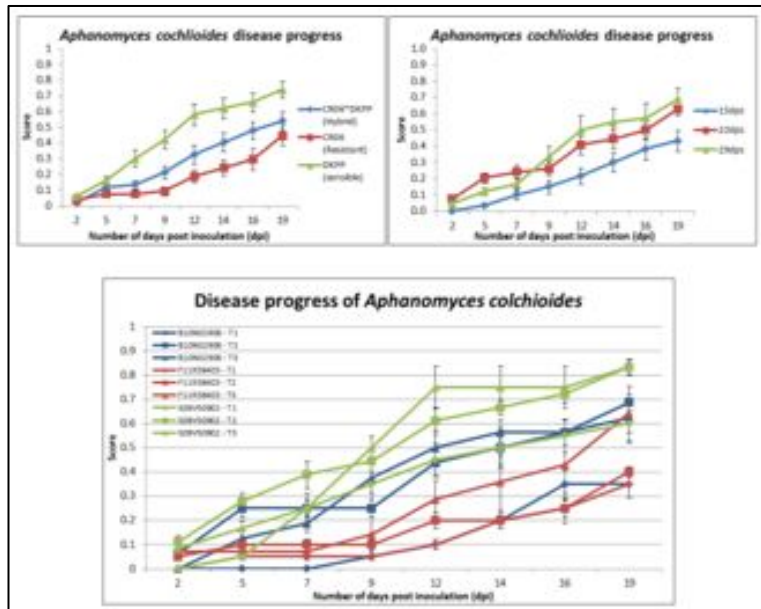


Figure 16: Assay with different inoculation dates

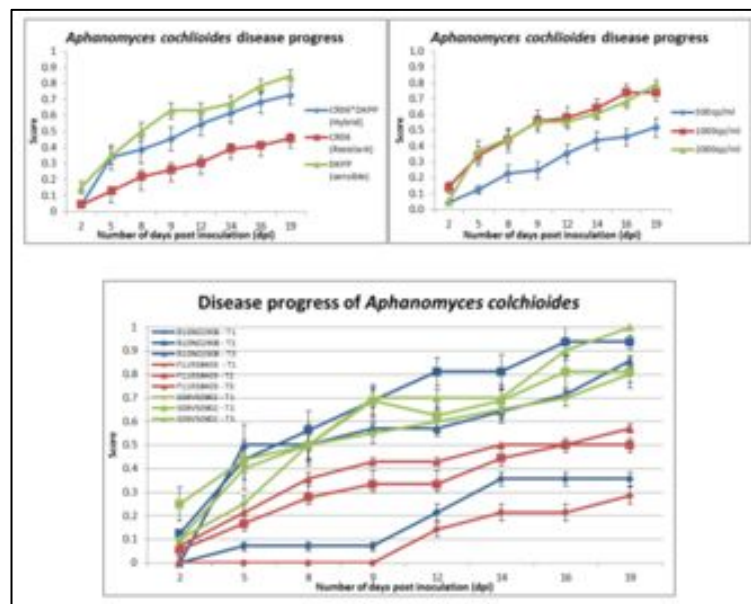


Figure 17 : assay with different inoculum concentrations

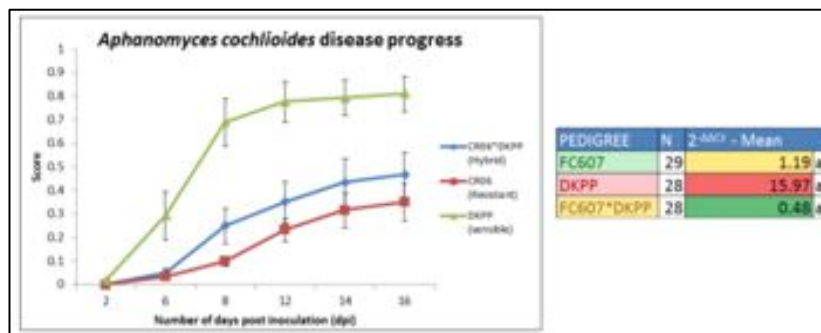


Figure 18 : last assay confirming the two previous assays and qPCR results

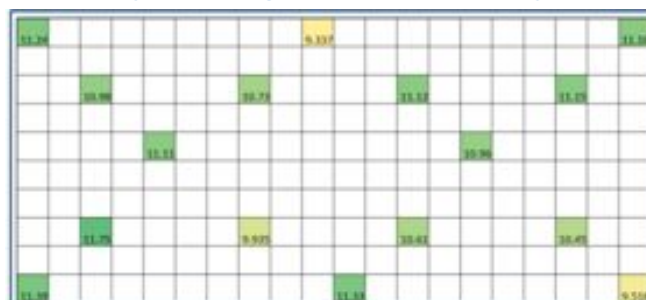


Figure 19 : percentage of water in the tubes (Standard deviation : 0.679)

Results

A. Bioassay development

The assay with different inoculation dates (15, 22 or 29 dps (days post sowing)) showed a significant difference between the treatments ($p=0.0116$). Plants that were inoculated at 15dps showed fewer symptoms than plants inoculated at 22 and 29 dps (these two treatments were not significantly different from 12, 14 and 19 dpi onwards (days post inoculation)). The fact that inoculation at 22 and 29 dps was not significantly different was confirmed by the calculation of AUDPC (Area Under Disease Progress Curve) (Figure 16).

The next assay comparing three inoculum concentrations (500, 1000 or 2000sp/mL) showed a significant difference between the inoculum concentrations used. Plants inoculated with 500sp/ml showed less symptoms than with 1000 or 2000sp/ml, which do not differ from one another from 5dpi onwards. This is confirmed by the calculation of the AUDPC. (Figures 17)

For the 2 first assays, plant mortality after transplantation (before inoculation) was rather high. It was first thought that it was due to pathogens remaining on the plant, but after isolation on petri dishes, this hypothesis was not confirmed. To see if it was possible to disinfect seedlings before transplantation, a small assay was made with 30 plants (3 genotypes). For each genotype, 5 plants were transplanted as usual and 5 others were soaked briefly in 70% alcohol and immediately rinsed with water. A visual observation led us to conclude that there was no difference between disinfected or untreated plants. It was then noticed that the upper quarter of the tube was not wet. Thereafter 10mL of water was added at transplantation, leading to a decrease in plant mortality.

Results of these two first bioassays could be compared for the concentration 2000sp/ml that was common to the two assays. This comparison showed that results were not significantly different; it can be conclude that the assay is reproducible.

A last assay made to confirm the previous results showed a very good discrimination between the resistant and susceptible genotype. Their means were significantly different ($p<0.01$). A qPCR test was attempted and some trends were observed, the three genotypes behaved as expected, but no significant difference was observed between the treatments (Figure 18).

These assays also indicated that scoring could be stopped after 14 or 16 dpi (when scores tend to reach a threshold). (Figures 16, 17 and 18)

The assay in compost failed to show any symptoms. The qPCR for this assay did not give any expected observations. No fungal DNA and Sugar-beet DNA was detected either (see Discussion).

More information about bioassay development's results can be found in annexes I and II.

B. Experimental design validation

1. Pathogen verification

After a culture on agar medium, it is possible to recognize specific structures of *Aphanomyces cochlioides* (figure 3) under the microscope.

2. Homogeneity of watering

The percentage of water in the tubes was not significantly different ($p\text{-value} > 0.05$) at the different locations in the box. (Figure 19)

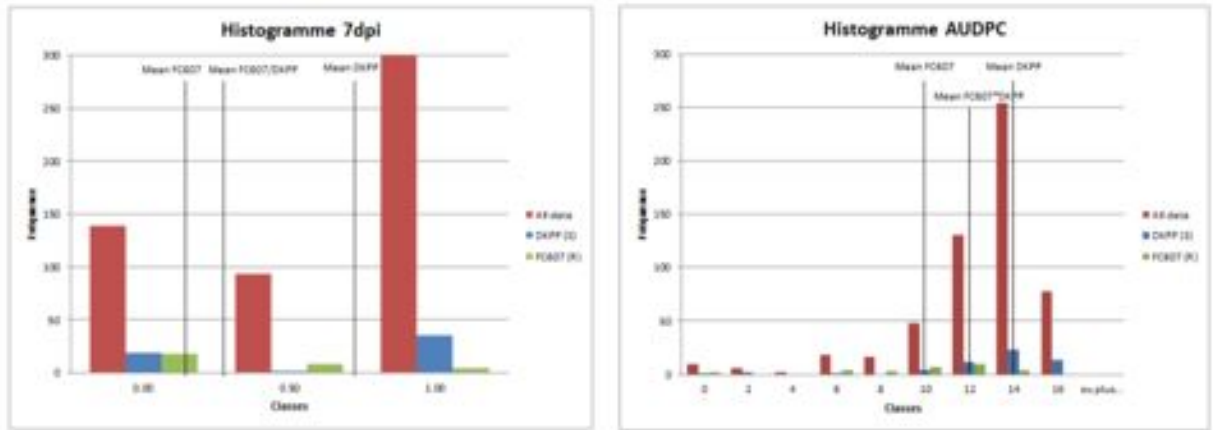


Figure 20: individuals repartition according to 7dpi and AUDPC for experiment 1

Table 2 : 7dpi and AUDPC results for experiment 1: ANOVA, Duncan test

7dpi						AUDPC							
7dpi	Df	Sum Sq	Mean Sq	F value	Pr(>F)	PEDIGREE	N	Mean	St.terr.	grp	Mean	St.terr.	grp
Genotype	18.00	11.75	0.65	3.97	0.00	FC607	30	0.267	0.067	c	9.03	0.655	b
Bloc	1.00	0.10	0.10	0.60	0.44	FC607/DKPP	30	0.350	0.087	bc	10.50	0.617	ab
Genotype*Bloc	17.00	2.82	0.17	1.01	0.45	S11T270345001	30	0.633	0.083	abc	11.93	0.519	a
Residuals	527.00	86.57	0.16			S11T270105001	29	0.638	0.086	abc	11.08	0.717	ab
						DKPP	56	0.643	0.063	ab	12.05	0.447	a
						S11T270055001	30	0.650	0.064	ab	11.50	0.570	ab
						S11T269995001	30	0.667	0.081	ab	11.54	0.546	ab
						S11T270005001	30	0.667	0.077	ab	11.48	0.684	ab
						S11T270375001	30	0.667	0.084	ab	11.28	0.687	ab
						S11T270075001	30	0.683	0.066	ab	11.96	0.391	a
						S11T270415001	30	0.700	0.074	ab	12.34	0.372	a
						S11T270015001	30	0.717	0.075	ab	11.83	0.516	a
						S11T269975001	29	0.741	0.073	ab	11.77	0.575	a
						S11T270385001	30	0.750	0.071	ab	12.22	0.348	a
						S11T270185001	20	0.800	0.084	ab	12.72	0.329	a
						S11T269985001	30	0.833	0.060	ab	12.88	0.245	a
						S11T270095001	30	0.833	0.060	ab	12.57	0.269	a
						S11T269945001	30	0.850	0.049	a	12.54	0.388	a
						S11T270155001	10	0.850	0.107	ab	12.82	0.264	a

7dpi			
Bloc	N	Mean	St.terr. grp
A	197	0.660	0.030 a
B	189	0.680	0.032 a
C	178	0.674	0.031 a

AUDPC			
Bloc	N	Mean	St.terr. grp
A	197	11.810	0.189 a
B	189	11.610	0.241 a
C	178	11.840	0.215 a

Table 3 : 7dpi and AUDPC results without controls for experiment 1: ANOVA, Duncan test

AUDPC					
AUDPC	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	15.00	127.96	8.53	1.15	0.31
Bloc	1.00	5.09	5.09	0.68	0.41
Genotype*Bloc	14.00	52.76	3.77	0.51	0.93
Residuals	417.00	3105.41	7.45		

7dpi					
7dpi	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	15.00	2.49	0.17	1.06	0.39
Bloc	1.00	0.02	0.02	0.15	0.70
Genotype*Bloc	14.00	1.51	0.11	0.69	0.79
Residuals	417.00	65.16	0.16		

Table 4 : Dunnett test for 7dpi's score and AUDPC for experiment 1

7dpi						AUDPC					
PEDIGREE	N	Mean	Dunnett	Mean	Dunnett						
FC607	30	0.27	vs. 0.999	9.03	vs. 0.387						
FC607/DKPP	30	0.35	vs. 0.021	10.50	vs. 0.178						
S11T270105001	29	0.64	0.007	11.08	0.075						
S11T270375001	30	0.67	0.002	11.28	0.032						
S11T270005001	30	0.67	0.002	11.48	0.014						
S11T270055001	30	0.65	0.004	11.50	0.013						
S11T269995001	30	0.67	0.002	11.54	0.011						
S11T269975001	29	0.74	0.001	11.77	0.004						
S11T270015001	30	0.72	0.001	11.83	0.003						
S11T270345001	30	0.63	0.008	11.93	0.002						
S11T270075001	30	0.68	0.001	11.96	0.001						
DKPP	56	0.64	0.021	12.05	0.178						
S11T270385001	30	0.75	0.001	12.22	0.001						
S11T270415001	30	0.70	0.001	12.34	0.001						
S11T269945001	30	0.85	0.001	12.54	0.001						
S11T270095001	30	0.83	0.001	12.57	0.001						
S11T270185001	20	0.80	0.001	12.72	0.001						
S11T270155001	10	0.85	0.002	12.82	0.001						
S11T269985001	30	0.83	0.001	12.88	0.001						

Table 5: Genotype comparison for experiment 1

SEED NAME	MS	7dpi	aupdc
S11T270375001 A	0.667	11.283	
S11T270345001 A	0.633	11.933	
S11T270385001 A	0.750	12.217	
S11T269975001 B	0.741	11.767	
S11T269985001 B	0.833	12.875	
S11T270075001 B	0.683	11.958	
S11T269995001 B	0.667	11.542	
S11T270055001 B	0.650	11.500	
S11T270095001 B	0.833	12.567	
S11T270005001 B	0.667	11.483	
S11T270015001 B	0.717	11.833	
S11T270185001 C	0.800	12.725	
S11T269945001 C	0.850	12.542	
S11T270105001 C	0.638	11.078	
S11T270155001 C	0.850	12.825	

Comparison of the 7dpi's score and AUDPC of the three groups made according to genotype					
7dpi	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotypepage	2.00	0.31	0.16	1.01	0.36
Residuals	415.00	64.05	0.15		

AUDPC					
Genotypepage	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotypepage	2.00	4.86	2.43	0.32	0.73
Residuals	415.00	3161.74	7.62		

Signification :

- A : should be more resistant according to genotyping
- B :
- C : should be more sensible according to genotyping

C. Experiment 1

1. **Statistical analysis**

a. Analysis of variance

As described in Material and Methods, in addition to the AUDPC at 19dpi (end of the assay), studies were also made on the disease score attributed on the 7th day post inoculation because a very good discrimination was noticeable (from a score of 0.267 attributed to the resistant control to a score of 0.850 attributed to the most diseased plant). (Table 2)

Variance analysis postulates were verified (homoscedasticity, normality and independency (Annex IV). Thanks to this analysis, it was possible to observe that there are no block effects. This allowed us to treat all data together. It was possible to observe a significant effect related to the genotypes. This meant that each genotype did not react the same way to *Aphanomyces cochlioides* (Table 2).

b. Individuals distribution

A continuous distribution of the individual 7dpi scores and AUDPC can be observed. This showed a quantitative reaction of the genotype. The response did not follow a normal distribution (figure 20), but no standard transformation (log, ln, ln x+1, ...) succeeded in normalizing it.

It is possible to see that FC607 is the most resistant (7dpi = 0.267 and AUDPC = 9.03), followed by the hybrid FC607*DKPP.DKPP, which is the susceptible line, which has a quite high AUDPC (12.05) and a moderately high 7dpi score (0.643), but some of the genotypes were already more susceptible (for example S11T27015S001 with a 7dpi score of 0.85 and an AUDPC of 12.82).

The fact that FC607's AUDPC was not zero confirmed that it is responsible for a partial resistance because even the "resistant" genotype is contaminated.

c. Mean comparison

The Duncan test allowed the splitting of the accessions into groups (Table 2). On this table, it can be seen that only FC607 is really different from the other genotypes. This is confirmed by the same analysis made without the controls (Table 3). The Dunnett test (Table 4) shows a comparison between each genotype and the controls. In this assay, one can see that all genotypes (except the resistant and hybrid controls) are not significantly different from the susceptible control ($p > 0.05$).

d. Heritability

Heritability (h^2) is quite high for the two tested values (7dpi score and AUDPC). For AUDPC, $h^2=0.65$ and for 7dpi, $h^2=0.75$. Both measures are very close. This value shows that the phenotypic variation due to genetic differences is quite high.

2. **Genetic analysis**

a. Comparison of the three types of genotypes

As explained in Materials and Methods, three types of genotype are expected, demonstrating resistant, intermediate and susceptible phenotypes. However this is not the case. Indeed, one of the genotypes that theoretically should be one of the most susceptible appeared to be the most resistant (S11T27010S001). Moreover, ANOVA did not show any significant difference between the groups ($p > 0.05$) (Table 5).

Table 6 : Kruskal-Wallis results with sampled individuals for experiment 1

Chr	Position (cM)	Locus	K*	Df	Signif	Nr Inf	Meanrank-A	Mean-A	Nr-A	Meanrank-B	Mean-B	Nr-B
C4	38	D101_501	21.758	2	*****	146	50	8.7	43.0	80.4	11.1	78.0
C4	41	D028_502	21.871	2	*****	143	48.7	8.7	41.0	77.9	11.0	78.0
C4	44	D084_501	21.851	2	*****	121	38.2	8.9	31.0	65.2	11.0	66.0
C4	46	D102_503	20.298	2	*****	122	39.2	9.0	30.0	64.9	10.9	68.0
C4	49	D106_503	18.984	2	*****	119	38.4	9.0	28.0	62.7	10.9	67.0
C4	51	D085_504	11.245	2	****	45	13.6	9.1	12.0	24.8	11.2	26.0
C4	51	D110_501	11.108	2	****	43	13.3	9.1	12.0	23.6	11.1	24.0
C4	53	D103_502	8.929	2	**	42	13	9.0	11.0	23.5	11.2	25.0
C4	57	D029_501	8.661	2	**	43	13.3	9.0	11.0	24.1	11.1	26.0
C6	8	F086_504	1.994	2	-	62	34.2	10.8	12.0	33.1	11.1	33.0
C6	9	F085_501	1.638	2	-	92	46.8	10.4	19.0	50.3	11.5	36.0
C6	18	F107_501	0.427	2	-	97	51	10.8	20.0	50.1	11.2	39.0
C6	24	F067_503	0.648	2	-	116	55.2	10.6	25.0	60.9	11.3	57.0
C6	25	F096_501	0.364	2	-	116	55.2	10.6	25.0	60.0	11.2	57.0
C6	25	F108_501	0.897	2	-	114	53.9	10.6	25.0	60.4	11.4	55.0
C6	29	F036_501	3.095	2	-	113	46.8	9.9	23.0	60.6	11.5	57.0

Significance levels:
 * 0.1
 ** 0.05
 *** 0.01
 **** 0.001
 ***** 0.0001

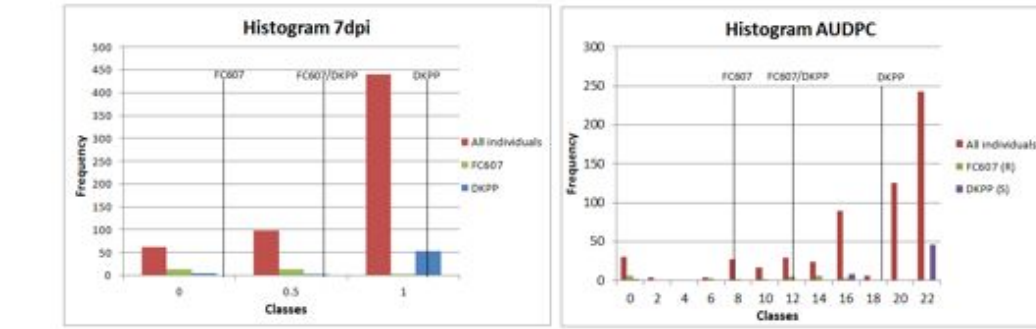


Figure 21 : individuals repartition according to 7dpi's score and AUDPC for experiment 2
 Table 7 : 7dpi and AUDPC results for experiment 2: ANOVA, Duncan test

7dpi					AUDPC						
Genotype	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Genotype	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	18.00	4313.90	234.22	8.63	<2e-16	Genotype	18.00	14.43	0.80	8.89	<2e-16
Bloc	2.00	30.90	15.46	0.57	0.57	Bloc	2.00	0.03	0.02	0.17	0.85
Genotype*Bloc	36.00	836.80	23.25	0.86	0.71	Genotype*Bloc	36.00	2.55	0.07	0.78	0.81
Residuals	543.00	14741.80	27.15			Residuals	543.00	48.96	0.09		

Table 8 : 7dpi and AUDPC results without controls for experiment 2: ANOVA, Duncan test

7dpi					AUDPC						
Genotype	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Genotype	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	15.00	4.01	0.27	2.92	0.00	Genotype	15.00	1501.50	100.10	3.76	0.00
Bloc	2.00	0.03	0.02	0.18	0.84	Bloc	2.00	34.30	17.17	0.64	0.53
Genotype*Bloc	30.00	2.28	0.08	0.83	0.72	Genotype*Bloc	30.00	790.70	26.36	0.99	0.49
Residuals	432.00	39.48	0.09			Residuals	432.00	11512.50	26.65		

Table 9 : Dunnett test for 7dpi's score and AUDPC for experiment 2

		7dpi				AUDPC			
geno	N	Mean	Dunnett	Mean	Dunnett	Mean	Dunnett	Mean	Dunnett
FC607	30	0.30	vs.	8.53	vs.	0.018	vs.	0.000	vs.
FC607/DKPP	30	0.60	0.002	vs.	12.87	0.018	vs.	0.000	vs.
S13T301295001	30	0.65	0.000	1.000	0.002	13.18	0.008	1.000	0.000
S13T300945001	30	0.65	0.000	1.000	0.002	13.73	0.003	1.000	0.000
S13T301265001	30	0.73	0.000	0.562	0.098	14.68	0.000	0.845	0.008
S13T300605001	30	0.85	0.000	0.017	0.996	15.95	0.000	0.206	0.175
S13T300995001	30	0.82	0.000	0.060	0.834	16.40	0.000	0.095	0.374
S13T301015001	30	0.85	0.000	0.017	0.996	16.75	0.000	0.048	0.596
S13T300835001	30	0.82	0.000	0.060	0.834	16.80	0.000	0.043	0.630
S13T301325001	30	0.92	0.000	0.000	1.000	17.22	0.000	0.017	0.887
S13T301165001	30	0.88	0.000	0.004	1.000	17.65	0.000	0.006	0.994
S13T300555001	30	0.90	0.000	0.000	1.000	17.88	0.000	0.003	1.000
S13T300915001	30	0.93	0.000	0.000	1.000	18.32	0.000	0.000	1.000
S13T301045001	30	0.92	0.000	0.000	1.000	18.38	0.000	0.000	1.000
S13T300585001	30	0.90	0.000	0.002	1.000	18.53	0.000	0.000	1.000
DKPP	60	0.91	0.000	0.000	vs.	18.71	0.000	0.000	vs.
S13T301185001	30	0.92	0.000	0.000	1.000	18.72	0.000	0.000	1.000
S13T301095001	30	0.92	0.000	0.000	1.000	18.75	0.000	0.000	1.000
S13T301275001	30	0.93	0.000	0.000	1.000	19.15	0.000	0.000	1.000

b. Kruskal-Wallis test

First, a Kruskal Wallis test was made on parental genotypes (chromosomes 4 and 6) with the mean of 7dpi score and AUDPC of each family. For both scores, no QTL were detectable.

The Kruskal-Wallis test performed with sampled individuals (for which leaves were sampled) clearly showed a significant effect of markers on chromosome 4, indeed, all markers on this chromosome had a significant K-value. By contrast, nothing was detected on chromosome 6. (Table 6)

D. Experiment 2

1. Statistical analysis

a. Analysis of variance

7dpi was also chosen in addition to AUDPC at 15dpi (end of the assay), because of a good discrimination (from a score of 0.3 attributed to the resistant control to a score of 0.933 attributed to the most diseased plant). (Table 7)

Homoscedasticity (equality of variance), normality and independence were verified for the variance analysis (Annex IV). Here again, one sees that there is no block effect and data can be treated together. And as with previous assay, it is possible to see a significant effect of the genotype.

b. Distribution of Individuals

Again, a continuous distribution of 7dpi score and AUDPC is observed, this is consistent with the fact that the resistance is quantitative. The distribution is not normal, but no transformation was found to correct it. (Figure 21)

c. Mean comparison

The Duncan test shows that the experiment worked well because the three controls are clearly discriminated.

With the groups formed by the Duncan test, only genotypes S13T30129S001 and S13T30094S001 are significantly different from the susceptible control DKPP ($p < 0.05$) (Table 7). This is confirmed by the analysis without controls (Table 8). The Dunnett test (Table 9) shows that no genotypes are comparable to the resistant control, but with the 7dpi score, two genotypes (the two cited above) are significantly less infected than the susceptible control. With the AUDPC, besides those two, the genotype S13T30126S001 is also significantly less contaminated than the susceptible control, but again, they are significantly different from the resistant control.

d. Heritability

As in the previous assay, heritability is rather high for the 7dpi score and AUDPC. For AUDPC, $h^2 = 0.73$ and for 7dpi, $h^2 = 0.66$. They are also in the same range as the heritability values calculated in the forgoing assay.

2. Genetic analysis

a. Families result comparison

On table 10, it is possible to see that families with the highest number of individuals that seem to be more resistant (having a lot of A individuals, such as S13T30094S001, S13T30129S001 or S13T30126S001) are also generally more resistant (considering 7dpi

score and AUDPC). It is noticeable that they have in general more markers with genotype coming from FC607, especially on chromosome 1.

b. Kruskal-Wallis test

This test allows the detection of significant effects. For the 7dpi score, a QTL is detected on chromosome 1 between markers A046_S02 and A062_S01, on chromosome 4 between markers D156_S01 and D075_S01 and D201_S01 and D093_S02. For the AUDPC, on chromosome 1, a QTL is detected between markers A046_S02 and A062_S01, on chromosome 4 a QTL is detected between markers D201_S01 and D093_S02. (Table 11)

For both 7dpi score and AUDPC, nothing is detectable on chromosome 6.

As it was explained in the Bibliography, this test gives just a preview of our data and is not the most reliable.

c. Interval mapping

First, we made the study on families, with the use of the parental genotype.

The significant threshold of the LOD was calculated by a permutation test with 1000 iterations. The result was a LOD score of 2.8 for the AUDPC and 3 for 7dpi score. These LOD score looks coherent (a usual value is situated around 3).

Interval mapping, which is more powerful than the previous Kruskal-Wallis test, showed only one significant QTL on chromosome 1 with a LOD score of 4.42 at 53 cM. This QTL is significant between 36 and 58 cM and has A046_S02 and A068_S01 as flanking markers. It is only significant for the 7dpi score. This QTL explains 71% of the phenotypic variance. (Figure 22)

For the AUDPC, a peak is detectable on chromosome 1 but it is not significant.

Then the analysis was made on all sampled individuals (individuals for which leaves were sampled). Only chromosomes 4 and 6 were genotyped.

The LOD score is 1.8 for 7 dpi and 1.9 for AUDPC

Here, there is a significant QTL on chromosome 6. The highest LOD value is 2.28 at the marker F107_S01, for AUDPC (a peak is visible for 7dpi but it is not significant) (Figure 20) (Figure 23). With a further analysis (Table 12) allele B seems to give the resistance. This confirms the analysis with families, saying that FC607 does not give a resistance QTL on chromosome 6.

d. Multiple QTL mapping

As is visible in figure 20, the QTL detected on chromosome 1 is not very precisely located. To improve the precision of the location, marker A062_S01 (50 cM) was put as cofactor, because it was the closest marker to the position of the QTL with interval mapping (Figure 24). Now, the QTL is significant between 50 and 58 cM with the maximum at 52 cM. This QTL explains 72% of the phenotypic variance. Individuals that carry the allele from FC607 (A) are more resistant than those with the allele from DKPP (B) (Table 13).

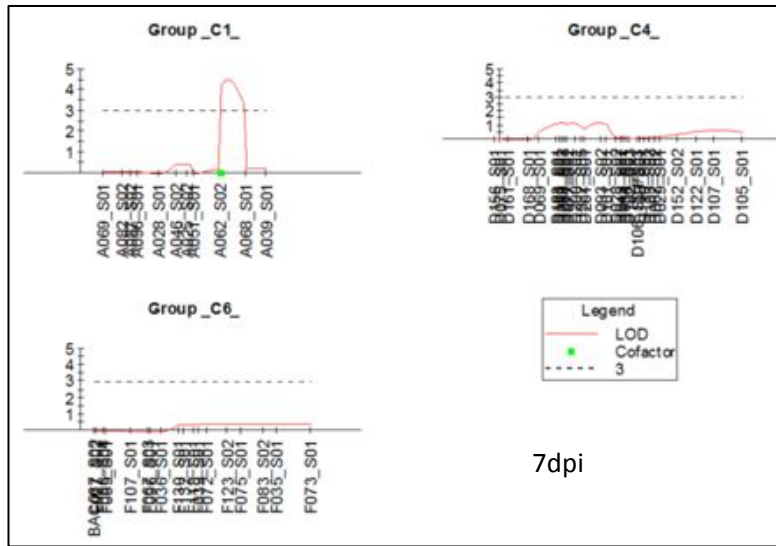


Figure 24 : Multiple QTL mapping for experiment 2 with A062_S01 as cofactor

Table 13 : Means in regard to genotype for marker A025_S02

Values	A	B	H	Total
Mean AUDPC	13.46	17.98	17.43	17.01
Mean 7dpi	0.65	0.90	0.87	0.85
Nb for A025_S02	2	7	3	21

Discussion

A. Bioassay development

a. A poorly understood disease

Aphanomyces cochlioides is a recently discovered disease, first identified by Drechsler (1929). As already discussed (in the bibliography section), it is still not well known and seems to depend a lot on environmental conditions. Thus, it is not sure that the results obtained in the bioassay will always be repeatable.

b. Elimination of the compost assay

As explained in the results, the system with compost was directly set aside because of the difficulty in obtaining reproducible symptoms. The reason why no symptoms can be observed may be linked to the fact that the plants developed much better in compost than in sand. So after 26 days (date of inoculation) they were maybe too strong to be infected by the spore concentrations used (500 sp/ml, 1000 sp/ml and 2000sp/ml). One option could be to try it again with an earlier inoculation (for example 9 or 10 days after sowing) or/and to use a higher concentration of inoculum.

The fact that plants may be too strong to be infected could explain why the qPCR was not effective. It may also be because of some soil remaining after washing that contains inhibitors.

c. Focus on the tube system: an assay that still needs to be improved

The system with tubes and sand was chosen at the end of the bioassay development. With the results from the bioassay development, it can be supposed that sand is probably the best tested substrate to be used. Plants should be inoculated after 22dps for a good outcome. A concentration of 2000sp/ml was used for inoculation. These conditions were tested in the 3rd assay which showed satisfying results. The advantage of this bioassay is the quick access of results (in about 38 days) because of the use of seedlings, and an easy scoring protocol.

However, it is important to remind that sand is not a good retainer of water. A good and stable irrigation has to be ensured throughout the assay. This is true especially during the infection period because *Aphanomyces cochlioides* needs water to propagate. Homogeneity of watering could be a problem in the repeatability of the assay.

Knowing that the environment has an important effect on plant infection by *A. cochlioides*, it is highly likely that it will have an effect on QTL detection (Kover, et al., 2001). It could be interesting to try a wider range of environmental conditions to optimize the inoculation and plant growth. More situations could be tested, including different pH conditions, other substrates... In fact the bioassays' results showed that, despite a significant discrimination of the controls ($P < 0.01$), other genotypes are not discriminating so clearly. It could be worth trying other resistant and susceptible controls to see how they behave in this test.

This assay could be also interesting to try with specific strains of *Aphanomyces cochlioides*; here a mixture of strains was used, but plants may react differently according to the strain used. A QTL could also be specific to one strain.

QTL detection depends a lot on population size, genetic background and environment. The number of plants that could be put in the bioassay limited this study, but increasing the number of genotypes and population size can refine the detection accuracy.

Table 14: comparison of the 2012 assay with experiment 1 (2013)

Genotype	Family	N	Grp	2013		2012			Disease Index
				7dpl mean	AUDPC mean	GPS_A	GPS_H	GPS_B	
S11T270345001	S10D4615^500^044.	30	A	0.633	11.930	6.00	4.54	4.17	0.440
S11T270105001	S10D4615^708^020.	29	C	0.638	11.080	8.50	8.50	7.33	0.160
S11T270055001	S10D4615^706^015.	30	B	0.650	11.500	8.65	7.52	7.69	0.125
S11T270375001	S10D4615^500^047.	30	A	0.667	11.280	7.55	7.70	5.16	0.463
S11T270005001	S10D4615^706^010.	30	B	0.667	11.480	6.40	6.29	4.83	0.325
S11T269995001	S10D4615^706^009.	30	B	0.667	11.540	7.88	8.10	7.53	0.046
S11T270075001	S10D4615^706^017.	30	B	0.683	11.960	6.85	6.90	5.62	0.217
S11T270015001	S10D4615^706^011.	30	B	0.717	11.830	8.51	7.85	6.45	0.319
S11T269975001	S10D4615^706^007.	29	B	0.741	11.770	8.03	7.49	5.55	0.448
S11T270385001	S10D4615^500^048.	30	A	0.750	12.220	8.60	7.69	4.98	0.728
S11T270185001	S10D4615^708^028.	20	C	0.800	12.720	7.95	7.70	7.31	0.086
S11T270095001	S10D4615^706^019.	30	B	0.833	12.570	8.50	8.31	7.79	0.090
S11T269985001	S10D4615^706^008.	30	B	0.833	12.880	7.49	7.52	5.14	0.459
S11T269945001	S10D4615^303^004.	30	C	0.850	12.540	7.13	6.78	5.79	0.230
S11T270155001	S10D4615^708^025.	10	C	0.850	12.820	8.32	7.68	7.98	0.043

QTL detection was made by measuring the evolution of the disease over time. It could be possible to find another way of measuring the disease.

B. Experiment 1

When experiment 1 is compared to the 2012 assay, we see that the results are not correlated. An individual that was more resistant according to the 2012 results was not necessarily more resistant according to experiment 1 results. S11T27038S001 is a good example (Table 14).

The new bioassay may not measure the same effect as those measured in 2012, indeed plants are at a different stage (seedling stage vs. mature plant). This is the main reason that can be suggested to explain this difference. Prioul et al. (2004) showed in a study about resistance to *Mycosphaerella pinodes* in pea that the QTL were different at the seedling and plant stage.

Other reasons can be suggested: inoculation concentration may be too strong for the plants that have a limited growth in sand. The new bioassay may not be discriminating enough and cannot detect small variations in the resistance level. It may give better results if individuals had a homozygous genotype. The detected QTL may appear with epistatic effects that have been lost during the backcrossing process.

Due to the fact that the population tested was not in Hardy-Weinberg equilibrium (because individuals were selected) the genotype coming from DKPP was much more represented than the one coming from FC607. Although the entirely fixed parts within the families were treated as missing data, it may result in biased analyses.

What is encouraging in experiment 1, is that out of 4 families having a majority of fixed markers from DKPP (susceptible source), 3 behave in a susceptible way (unlike in 2012, where 3 out of those 4 families were healthier). It may be interesting to study the S10D4615^708^020 family (here, individual S11T27010S001) more deeply because, although it belongs to the families with a majority of fixed marker from DKPP, it is behaving as expected in both 2012 assay and experiment 1.

C. Experiment 2

Here, only one QTL is detected on chromosome 1. This shows that the initial hypothesis saying that QTL was on chromosome 4 is potentially questionable. This implies that the choice of the families for the test was not optimal to study the resistance because this choice was made to have as much recombination as possible on chromosome 4. Luckily, chromosome 1 also showed a good recombination pattern which placed this QTL. This QTL still needs to be validated by other trials.

This result is interesting because when SESVanderHave detected the QTL on chromosomes 4 and 6, the study was made with an O-type plant. This QTL was introgressed in DKPP (elite pollinator). With the present results, we suppose that the QTL we had on chromosome 4 in O-type does not appear in DKPP, whereas the QTL on chromosome 1 express more in DKPP. Bouchez et al. (2002) observed some digression in predicting QTL effect after the introgression in elite varieties. They concluded that it was due to genotype environment interactions that were not taken into account. In another study about the effect of genetic background and environment on QTL and epistasis for rice panicle number, another team concluded that the effect of genetic background on QTL was even greater than that of the environment (Liao, et al., 2001). This shows the relativity of QTL.

To use this resistance on chromosome 4, breeders faced the problem that the resistance for Rhizomania is situated about at the same place. This resistance to Rhizomania is

recessive and the plant need one allele on each chromosome for a better expression. Even if they were not at the same locus, they are very close and it is difficult to find a plant with a recombination event at this place. But if the resistance to *A. cochlioides* is not expressed on chromosome 4 in the pollinator, this will simplify the introgression of *A. cochlioides* resistance in varieties. At the plant scale, several genetic factors for resistance have to be combined, but there is a risk of emergence of “super races” of the pathogen, permitting them to avoid all resistance genes (Finckh, 2008).

Here we also find a QTL on chromosome 6 with the analysis with all sampled individuals but DKPP seems to bring the resistance. Toojinda, et al. (1998) studied the introgression of QTL for resistance to stripe rust in barley in a QTL-unrelated genetic background. They detected additional resistance QTL. The susceptible parent contributed to resistance for some of these new QTL. They hypothesize that favorable alleles were fixed at these new QTL in the original mapping population. And concluded that genetic background may, therefore, have an important role in QTL-transfer experiments.

Conclusion

The objectives of this work were to study the genetic control of *Aphanomyces cochlioides*, including the development of a relevant bioassay to test this resistance.

We tried to find QTL for *Aphanomyces* resistance that were transferred from a highly resistant line, FC607, into an elite pollinator line, DKPP. The material under study was selected F1Bc2S1 and F1Bc2S2 lines.

The null-hypothesis was the presence of a major QTL responsible for disease resistance being expressed at chromosome 4, between 20 and 50 cM. This hypothesis was developed from programs using the same source of resistance, however introduced in a lines belonging to the female heterotic group. The lines selected for this study were chosen on basis of presence of specific zones from FC607 at chromosome 4.

In this material, prior evidence, obtained from an assay evaluating growth inhibition as the expression of *Aphanomyces* resistance, indicated presence of a QTL at the chromosome 4, explaining a limited part of the resistance. The weakness in the methodology was the possible confusion between QTL for root growth and QTL for disease resistance.

For this reason, we decided to develop a bioassay based on expression of disease symptoms. The development of the bioassay was a success. It could differentiate in a consistent way disease symptoms between the sensible and resistant parental lines and results were quickly available.

We could not confirm the presence of a single major QTL responsible for *Aphanomyces* root rot resistance on chromosome 4 in this population, in the region 20 and 50 cM. That shows the major importance of the genetic background because it can change the expression of QTL to a significant degree.

We discovered a QTL for *Aphanomyces* resistance coming from the susceptible parent at chromosome 6. Is it a QTL that is expressed later in the infection process?

Apart from chromosome 4 and 6, remnant segregation was present at chromosome 1 and allowed to detect a QTL. Although it still has to be validated, this is an interesting thing to study for future uses in breeding program.

Finding QTL is an approach that is now used by the majority of breeding companies because of the ease and quickness of use. Although, breeding resistant varieties with quantitative resistance seems to be more durable, the introgression of multiple QTL is not so easy. It is also relevant to remember that durability of resistance implies the combination of approaches at different levels, in space, in time, genetics and agronomy.

This study shows that there is still a lot to learn about *Aphanomyces cochlioides*. Knowing this pathogen better, on a biological and genetic point of view, could help to control it.

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Abbreviations

AFLP : Amplified Fragment Length Polymorphisms

ANOVA : Analysis Of Variance

AUDPC : Area Under Disease Curve Progress

CIM : Composite Interval Mapping

CMS : cytoplasmic male sterility

Dpi : days post inoculation

Dps : days post sowing

h^2 : heritability

LD : Linkage Disequilibrium

LOD : Logarithm of the Odds

MIM : Multiple Interval Mapping

MQM : Multiple QTL Mapping

PDA : Potato Dextrose Agar

QTL : Quantitative Trait Loci

RAPD : Randomly Amplified Polymorphic DNA

RFLP : Restriction Fragment Length Polymorphisms

SNP : Single Nucleotide Polymorphism

SSR : Simple Sequence Repeats

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ANNEXES

Annex I: Tubes assay – Material and method

Sowing

Before use, seeds were washed with water during four hours to avoid the remaining of fungi on it. Seeds were placed in a box containing an accordion-shaped filter paper. Then 38 mL of water were added into the box. First they were put to germinate in the dark in an incubator at 24°C during 3 to 4 days. Then they were put under light in a growth chamber at 18°C during the day (16h light period) and 19°C at night (8h) during 2 to 3 days.

Tubes preparation

Tubes were filled with sand. At the bottom of it, a piece of glass wool was placed to block the holes. A capillary matting strip was placed from the top to the bottom (through the piece of glass wool) to irrigate the substrate (Figure 1). 2 days before transplantation, the tubes were placed in a water box to humidify the sand.

Transplantation

When they start to germinate, they are transplanted in the substrate. 10 mL of water should be added at this stage to be sure that the plant is well watered in the early stages.

Growth conditions

Plants were then placed in a growth chamber (phytotron) at a temperature of 22°C in the day (16h) and 19°C in the night (8h). Those temperatures have been chosen as being optimal for the growth of the sugar beet and the pathogen.

Two days after transplanting, plants were irrigated with nutritive solution

Nutritive solution

The nutritive solution used here is Steiner (see annex III) diluted 10 times.

Inoculation

Plants were inoculated 22 days after sowing. One milliliter of inoculum solution was applied with a pipette, at the bottom of the hypocotyl. Plants should be well watered on the day of inoculation.

Experimental design

As there was no gradient in the phytotron, the experiment was made of only one randomized complete bloc.

Evaluation and analyses

A first type of scoring was made during the incubation based on the example used by Yu (2004). The score "0" means that plant has no or a few symptoms. The score "1" means that more than 2/3 of hypocotyl and root surface is diseased. "0,5" is used for all other cases (Figure 2).

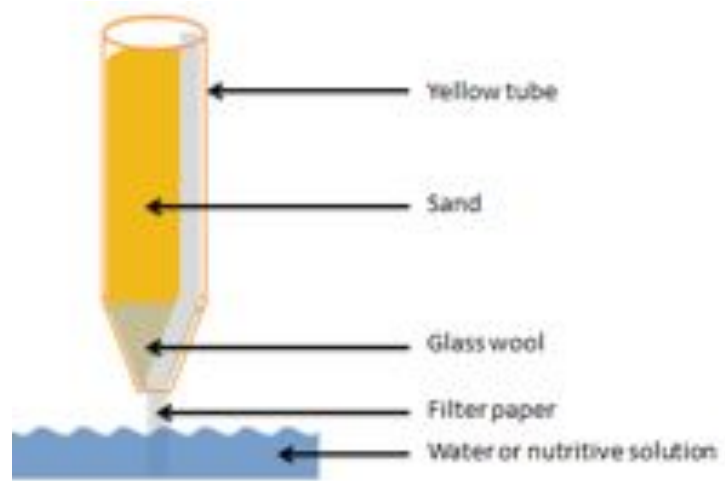


Figure 2 : Tube system



Figure 1 : Yu score (Yu, 2004)

On the final day, a different evaluation was processed. Root weight was observed and a qPCR was made to know the quantity of *Aphanomyces* DNA in comparison with the quantity of sugar beet DNA.

To compare the results we made an ANOVA on the following model: $Score_{ij} = \mu + g_i + T_j + \varepsilon$. $Score_{ij}$ is the score of the genotype i for the treatment j , μ is the score mean, g_i is the genotype effect, T_j is the treatment effect and ε the residuals effects. This ANOVA was followed by a Duncan test to see if the controls and treatments were significantly different.

Root observations

Roots were cut under the hypocotyl and the weight was measured. Statistical analyses on the data were made, but the results were not consistent. The idea came to observe the roots and calculate the infected surface with the help of a software used in an assay for nematodes. But after the first assay, it was noticed that plants were too young and roots were too thin to see anything on it.

qPCR trials

After weighing, root samples were prepared in order to perform a qPCR assessing the quantity of *Aphanomyces* DNA in comparison with the quantity of sugar beet DNA.

The samples were washed to remove remaining substrate, they were put in a 96-well box and put, at least one hour at -80°C . Then, the samples were put in the freeze-dryer during two days. The dried samples need then to be grind. The DNA is extracted with a normal kit usually used by SESVANDERHAVE. The qPCR is made with specific primers for *Aphanomyces cochlioides* and sugar beet. The PCR technic used is the TaqMan.

The results given were the cycle threshold (Ct) of each sample for both sugar beet and *Aphanomyces cochlioides*. With those data it was possible to calculate the $2^{-\Delta\Delta C_t}$, which is a common value used to quantify DNA in qPCR. A mean comparison with the Duncan test allowed comparing the three genotypes (controls) used.

Tested modalities

- Comparison of three dates of inoculation (15/22/29 day post sowing (dps)), ten repetitions were made for each combination.
- Comparison of three inoculum concentration (500-1000-2000 sp/ml), for this test, ten repetitions were made for each combination.

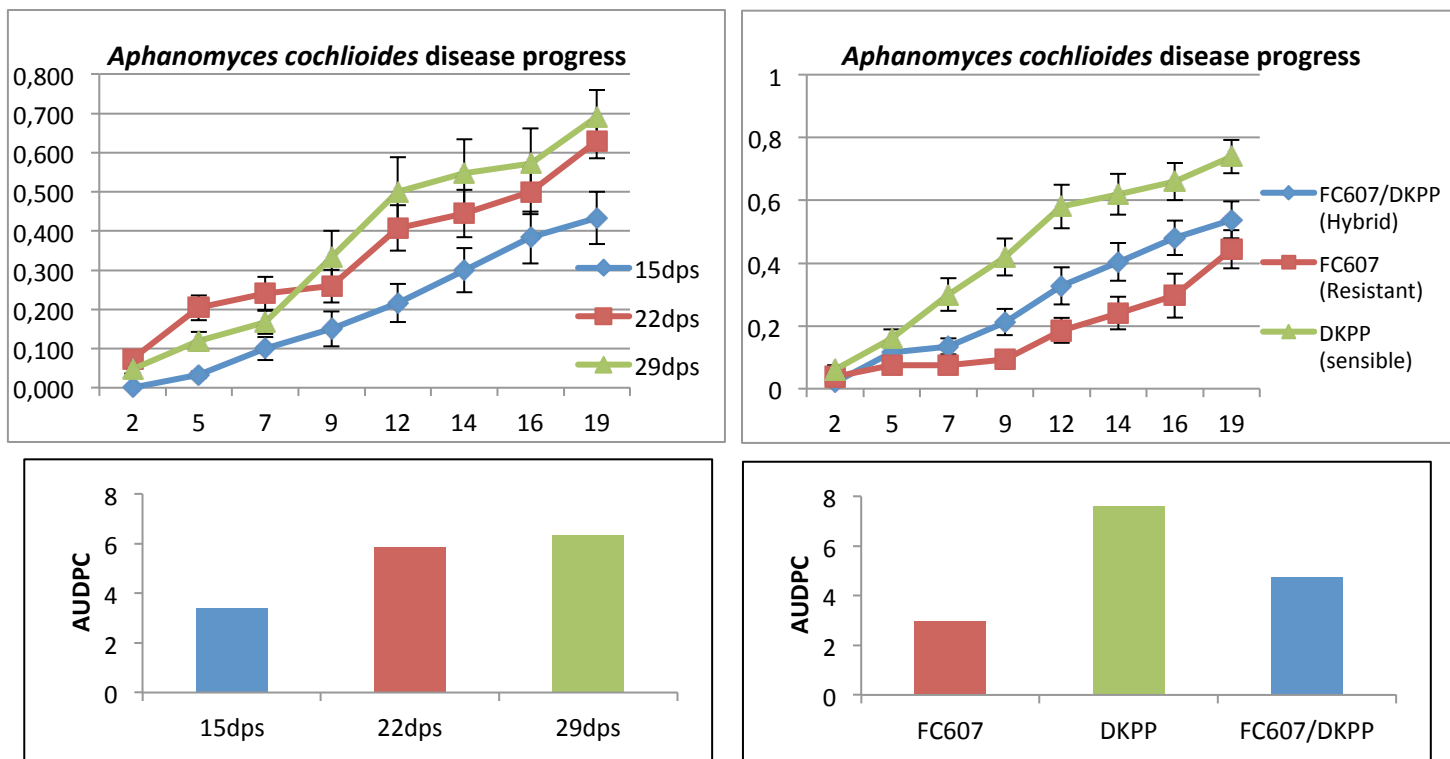
With the results obtained for the 2 first assays, we could make a third assay gathering the best parameters of each.

In this third assay, sand was used as the substrate; plants were inoculated 26 days after sowing with an inoculum of 2000sp/ml. To ensure a good humidity and avoid too much mortality at transplantation, 10ml of water were added in the tubes at transplantation.

Works Cited

Yu, Y., 2004. Genetics of *Aphanomyces* disease resistance in sugarbeet (*Beta vulgaris*), AFLP mapping and QTL analyses, Michigan: Michigan state university.

Figure 3: 1st assay with tubes results (different inoculation days: 15,22,29 dps)



ANOVA

AUDPC	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	2	139.35	69.68	4.75	0.0116452 *
Genotype	2	294.27	147.13	10.04	1.49E-04 ***
Treatment*Genotype	4	29.74	7.43	0.51	0.730623
Residuals	69	1 011.48	14.66		

Duncan test

PEDIGREE	N	AUDPC - Mean	
FC607	27	3.009	b
DKPP	24	7.65	a
FC607/DKPP	26	4.769	b

Treatment	N	AUDPC - Mean	
15dps	30	3.408	b
22dps	27	5.926	a
29dps	20	6.393	a

PEDIGREE	Treatment	N	AUDPC - Mean	
FC607	15dps	10	2.15	b
FC607/DKPP	15dps	10	2.175	b
FC607	22dps	10	2.95	ab
FC607	29dps	7	4.321	ab
DKPP	15dps	10	5.9	ab
FC607/DKPP	29dps	8	6.344	ab
FC607/DKPP	22dps	8	6.438	ab
DKPP	22dps	9	8.778	a
DKPP	29dps	5	8.875	a

Tube assay – Results

Statistical analysis can be found in attachment.

1. 1st assay with tubes (different inoculation days: 15,22,29 dps)

This first assay shows a good discrimination between resistant and sensible individuals with significant results at 5%. It also allows seeing a significant difference between the treatments. Plants that were inoculated at 15 dps have fewer symptoms than plants inoculated at 22 and 29 dps (those two treatments are not significantly different at 12, 14 and 19 dpi). The fact that inoculation at 22 and 29 dps is not significantly different is confirmed by the calculation of AUDPC (Area Under Disease Progress Curve).

2. 2nd assay with tubes (different inoculum concentrations)

This assay also shows a significant difference between resistant and sensible individuals at 5%. It is possible to see a significant difference between the inoculum concentrations used. Plants inoculated with a concentration at 500sp/ml show less symptoms than with 1000 or 2000sp/ml. Those two last concentrations are not significantly different from 5dpi. This is confirmed by the calculation of the AUDPC.

3. 3rd assay with tubes

This last assay shows a very good segregation between the resistant and sensible genotype. Their means are significantly different at 5%.

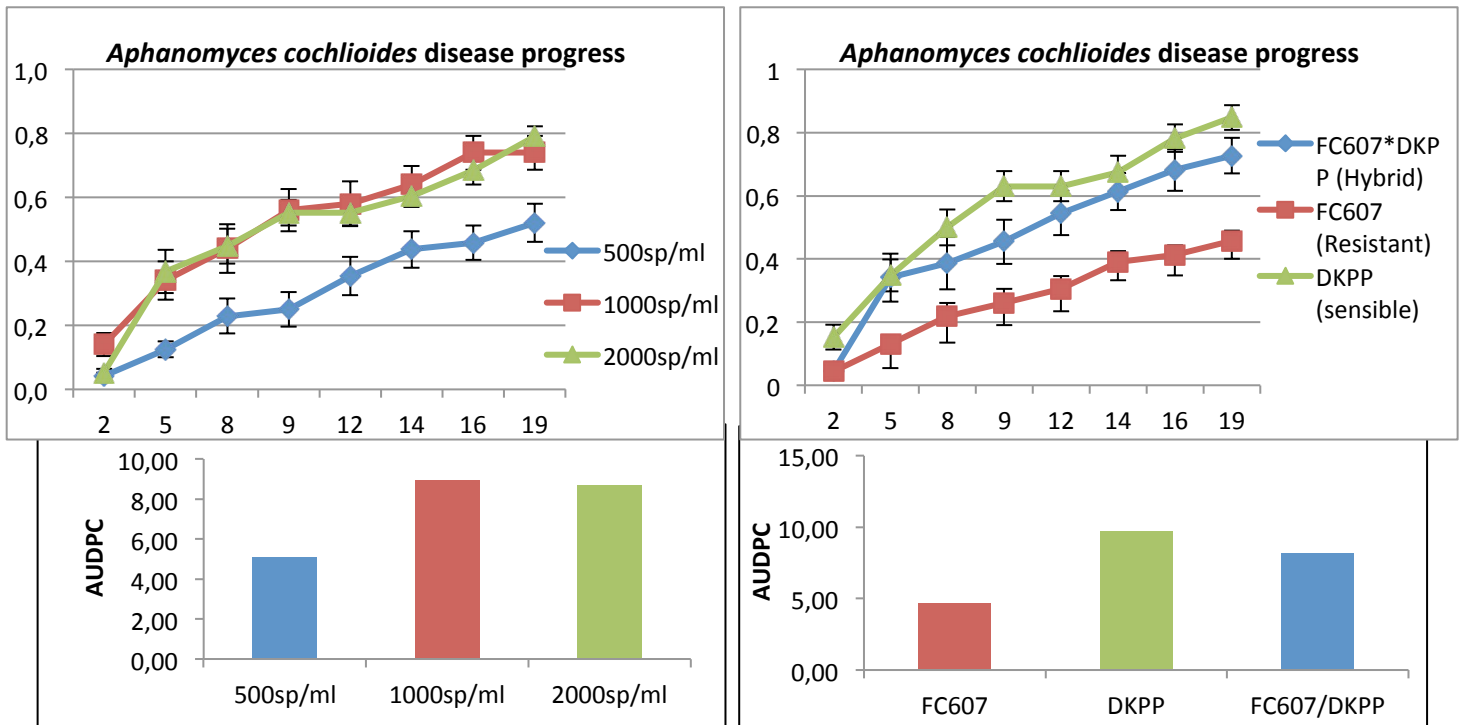
Tubes assay – Discussion

The assay with different inoculation dates (15, 22 or 29 dps (days post sowing)) allows seeing a significant difference at 5% between the treatments. Plants that were inoculated at 15 dps have fewer symptoms than plants inoculated at 22 and 29 dps (those two treatments are not significantly different at 12, 14 and 19 dpi (days post inoculation)). The fact that inoculation at 22 and 29 dps is not significantly different is confirmed by the calculation of AUDPC (Area Under Disease Progress Curve) (Figure 3).

The next assay comparing three inoculum concentrations (500, 1000 or 2000sp/mL) showed a significant difference between the inoculum concentrations used. Plants inoculated with a concentration at 500sp/ml show less symptoms than with 1000 or 2000sp/ml. Those two last concentrations are not significantly different from 5dpi. This is confirmed by the calculation of the AUDPC. (Figures 4)

For the 2 first assays, plant mortality after transplantation was rather high. It was first thought that it was due to pathogens remaining on the plant, but after isolation on petri dishes, this hypothesis was not confirmed. To see if it was possible to disinfect seedlings before transplantation, a small assay was made with 30 plants (3 genotypes). For each genotype, 5 plants were transplanted as usual and 5 other were shortly soaked in 70% alcohol and immediately rinsed with water. A visual observation allowed concluding that there were no difference between disinfected or not plants. Then, we saw that the

Figure 4: 2nd assay with tubes results (different inoculum concentrations)



ANOVA

AUDPC	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	2	224.26	112.13	7.20	0.00159 **
Genotype	2	373.61	186.80	11.99	0.00004 ***
Treatment*Genotype	4	102.07	25.52	1.64	0.17667
Residuals	59	918.97	15.58		

Duncan test

PEDIGREE	N	AUDPC - Mean
FC607	23	4.72 b
DKPP	23	9.84 a
FC607/DKPP	22	8.21 a

Treatment	N	AUDPC - Mean
500sp/ml	24	5.13 b
1000sp/ml	25	9.06 a
2000sp/ml	19	8.72 a

PEDIGREE	Treatment	N	AUDPC - Mean
FC607	500sp/ml	7	1.75 c
FC607/DKPP	500sp/ml	7	3.18 bc
FC607	1000sp/ml	9	5.58 abc
FC607	2000sp/ml	7	6.57 abc
DKPP	500sp/ml	10	8.85 ab
FC607/DKPP	2000sp/ml	7	9.43 ab
DKPP	1000sp/ml	8	10.50 a
DKPP	2000sp/ml	5	10.75 a
FC607/DKPP	1000sp/ml	8	11.53 a

upper quarter of the tube was not wet. That's why it was decided to add 10mL of water at transplantation. After this, plant mortality decreased.

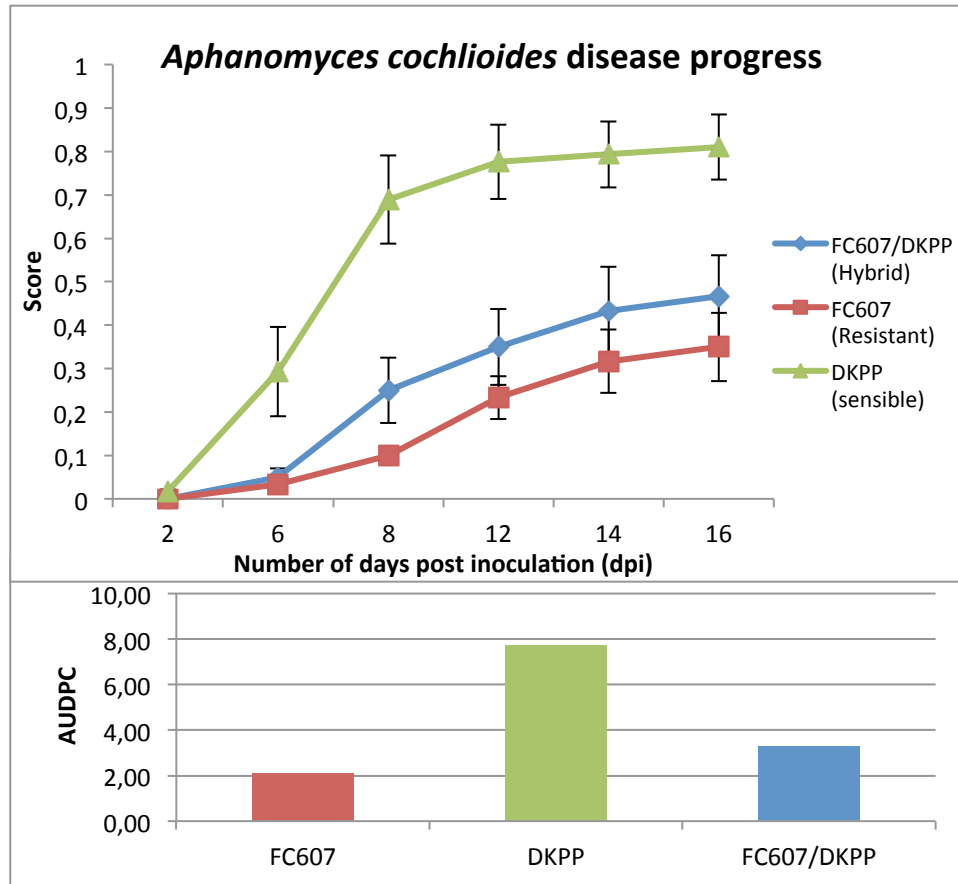
With the above results, it appears that sand is probably the best tested substrate. Plants should be inoculated after 22dps for a good result. A concentration of 200sp/ml ensures the inoculation.

A last assay made to confirm the previous results shows a very good segregation between the resistant and sensible genotype. Their means are significantly different at 5%. A qPCR was tried and some results were observed, the three genotypes behaved in a good way, but no significant difference between the treatments were observed (Figure 5).

We could compare results of these two first bioassays for the modality 2000sp/ml, which is common to the two assays. This comparison showed that results were not significantly different; we can conclude that the assay is reproducible.

These assays also allowed seeing that scoring can be stopped after 14 or 16 dpi (scores tends then to reach a threshold). (Figures 3, 5 and 5)

Figure 5: 3rd assay with tubes results



ANOVA

AUDPC	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	2	518.37	259.19	19.80	0.00000 ***
Residuals	86	1125.68	13.09		

Duncan test

PEDIGREE	N	AUDPC - Mean	
FC607	30	2.08	b
DKPP	29	7.72	a
FC607/DKPP	30	3.28	b

PCR results

PEDIGREE	N	2 ^{-ΔΔCt} - Mean	
FC607	29	1.19	a
DKPP	28	15.97	a
FC607*DKPP	28	0.48	a

Annex II: Compost bioassay - Material and method

Seed treatment

Before use, seeds were washed with water during four hours to avoid the remaining of fungi on it.

Sowing

Seeds were directly sown in 96 plug of compost. The compost used is named “Terreau pour professionnel” from the brand DCM

Growth conditions

They were let to grow in the greenhouse under blue light (to have shorter hypocotyls). When they were big enough (enough roots), plants were randomized.

After inoculation, plants were put in the same phytotron as described for the tests in yellow tubes. Compost was maintained wet, especially the day of inoculation and the days after.

Inoculation

Plants were inoculated 26 days after sowing. One milliliter of inoculum solution was applied at the bottom of the hypocotyl using a pipette. Plants should be well watered on the day of inoculation.

Evaluations

Because no symptoms were visible after 22 dpi and 40 dpi, a qPCR was tried but without any clear result.

Tested modalities

The assay allowed comparing four concentrations of inoculum (0-500-1000-2000 sp/ml). 10 repetitions were made for each combination. The two assays were made the same way, the second to confirm the first one.

Compost bioassay - Result

No clear results were seen, even with qPCR.

Compost bioassay - Discussion

The reason why we can't see any symptoms may come from the fact that the plants develop much better in compost than in sand. So after 26 days they are too strong to be infected by this concentration of spores. It could be an idea to try it again with an earlier inoculation (for example 9 or 10 days after sowing) or/and to use a higher concentration of inoculum.

The fact that plants may be too strong to be contaminated can explain why the qPCR is not effective. It may also be because of some compost remaining after washing that contains inhibitors.

Annex III: Steiner

Confidential information – Phytopathology Department SESVANDERHAVE

Steiner nutrient solution (cf Steiner, 1984)

Solution A:

88,2 g $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ C 5
44,4 g KNO_3 N
1000 ml tap water

Solution B:

13,4 g KH_2PO_4 P 4
15,4 g K_2SO_4 P 6
47,3 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ M 4
20,8 ml H_2SO_4 - 6N (or 12,5 ml of 10 N solution) Z 6
1000 ml tap water

Solution C:

32,85 g NAFE EDTA E 1
1000 ml tap water

Solution D:

0,5 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ Y 1
0,673 g H_3BO_3 B 1
0,127 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ Z 1
0,0195 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ C 1
0,0315 g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ S 1
250 ml tap water

Store solutions A, B, C and D at 4°C in the fridge

1 liter Steiner mother solution:

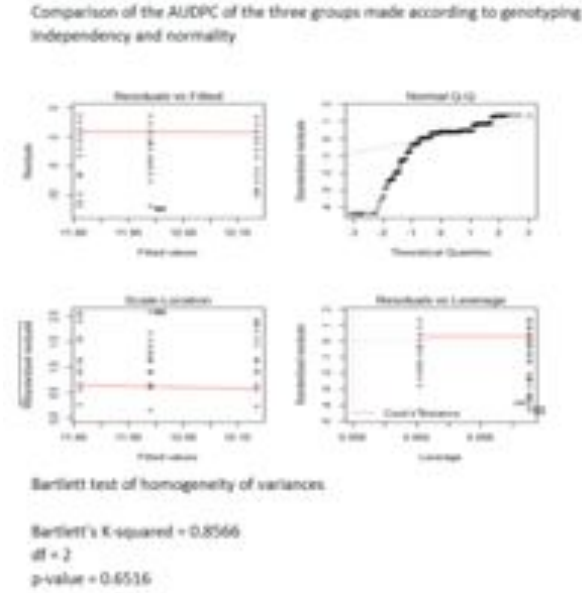
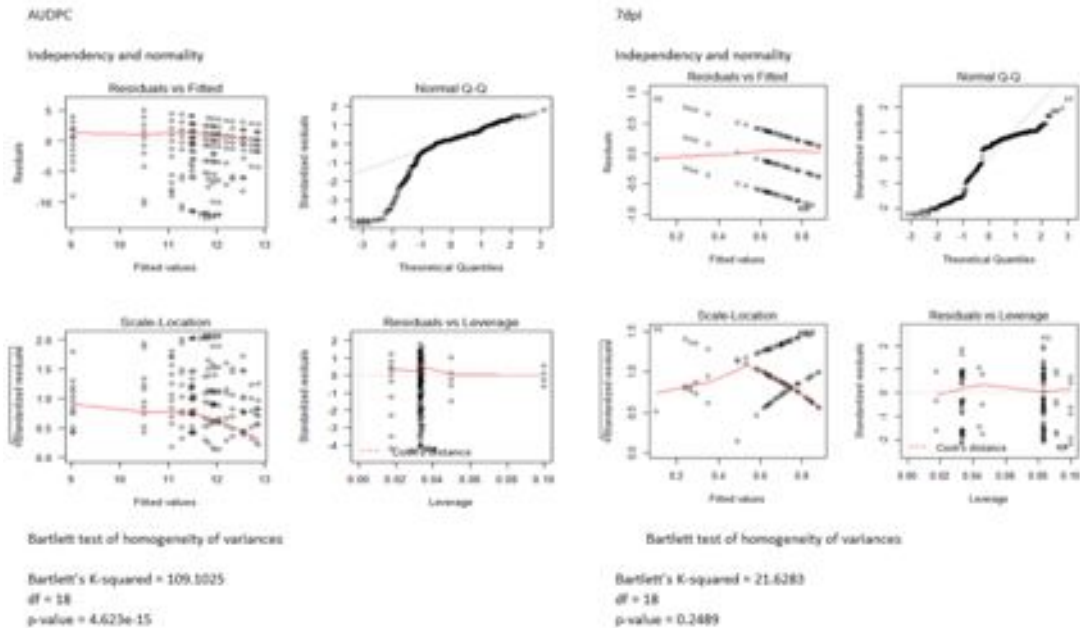
10 ml solution A + 10 ml solution B + 1 ml solution C + 1 ml solution D + 978 ml demi water = solution E

Working solution = 1/10 Steiner solution:

Dilute solution E 1/10 with demi water and adjust to pH=7

Annex IV: Analysis of variance postulates

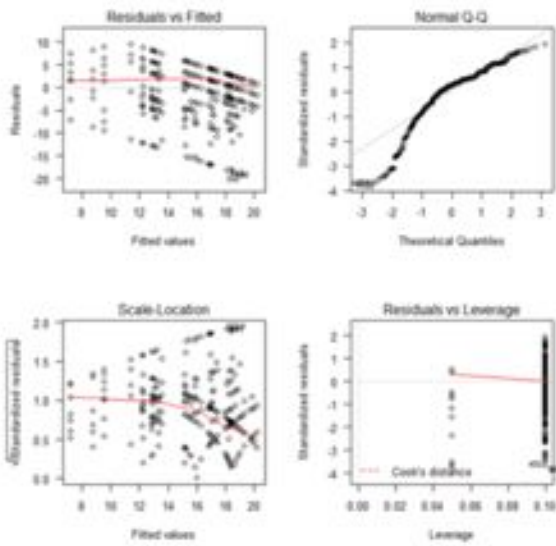
Experiment 1



Experiment 2

AUDPC

Independency and normality

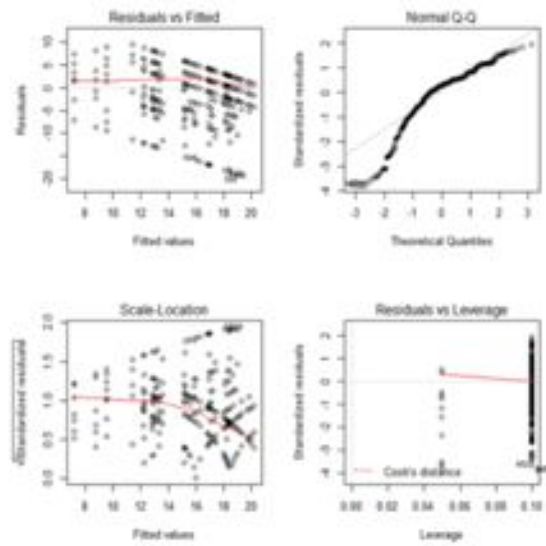


Bartlett test of homogeneity of variances

Bartlett's K-squared = 57.552
df = 18
p-value = 5.056e-06

7dpr

Independency and normality



Bartlett test of homogeneity of variances

Bartlett's K-squared = 43.1494
df = 18
p-value = 0.0007622



Diplôme : Ingénieur de l'Institut Supérieur des Sciences Agronomiques, Agroalimentaires, Horticoles et du Paysage

Spécialité : Ingénieur en Horticulture

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BELGIUM

Maître de stage : Stefaan Horemans

Titre français : Etude du contrôle génétique de la résistance partielle à *Aphanomyces cochlioides* (Drechs.) chez la Betterave sucrière (*Beta vulgaris* L.)

Titre anglais : Studying genetic control of partial resistance to *Aphanomyces cochlioides* (Drechs.) in Sugar-beet (*Beta vulgaris* L.)

Résumé :

Aphanomyces cochlioides est un champignon pathogène critique dans les cultures de betterave à sucre. Il cause de nombreux dommages et des pertes de rendement aux Etats-Unis, en Chine et de plus en plus dans l'est de l'Europe. C'est une maladie très imprévisible. La lutte contre ce champignon est donc un enjeu important et la sélection pour la résistance est un moyen de lutte en développement. Pour connaître mieux le contrôle génétique de la résistance, une analyse QTL de liaison a été réalisée. Les essais précédents menés par SESVanderHave ont été utilisés comme prérequis, des QTLs avaient été trouvés sur les chromosomes 4 et 6. Cependant la technique de phénotypage n'était pas très adaptée et il a fallu d'abord développer un bioessai avant l'analyse QTL. Dans le travail réalisé ici, malgré une bonne discrimination entre témoins, la comparaison des résultats obtenus avec la technique utilisée conventionnellement n'indique aucune corrélation. L'essai de cartographie fine montre quant à lui un QTL sur le chromosome 1. Ces résultats montrent un intérêt pour l'utilisation de la résistance en sélection.

Abstract:

Aphanomyces cochlioides is a fungus whose incidence is rapidly increasing in sugar beet crops. It causes root damage and yield loss in the USA, China and increasingly in eastern Europe. This is a very unpredictable disease. Trying to control it is an important issue and breeding for resistance is the most probable route to a solution. To learn more about the genetic control of the disease, a linkage analysis of QTL was performed. Previous assays made by SESVanderHave were used as a starting point and QTLs were found on chromosomes 4 and 6. However the phenotyping techniques were not well adapted and the development of a suitable bioassay had to be made prior to the QTL analysis. In the present work, new methods were developed but the results showed no correlation with those of the conventional technique, despite a good discrimination of the controls. The fine mapping assay detected a QTL on chromosome 1. These results are of an interest to use in resistance breeding in sugar beet.

Mots-clés : Betterave à sucre, *Aphanomyces cochlioides*, champignon, résistance, analyse de liaison

Key words: Sugar beet, *Aphanomyces cochlioides*, fungus, resistance, linkage mapping



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sugar beet seed

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BELGIUM

Mémoire de Fin d'Études

**Diplôme d'Ingénieur de l'Institut Supérieur des Sciences Agronomiques,
Agroalimentaires, Horticoles et du Paysage**

Année universitaire : 2012-2013

Spécialité : Ingénieur en Horticulture

Spécialisation : Sciences et production végétales, Amélioration des plantes

**Etude du contrôle génétique de la résistance partielle à *Aphanomyces cochlioides*
(Drechs.) chez la Betterave sucrière (*Beta vulgaris* L.)**

Résumé

Mathilde LIORZOU



Volet à renseigner par l'enseignant responsable de l'option/spécialisation*
ou son représentant

Date : .../.../... Signature

Bon pour dépôt (version définitive)

Autorisation de diffusion : Oui Non

Devant le jury :

Soutenu à Rennes le 13/09/2013

Sous la présidence de : Maria Manzanares-Dauleux

Maître de stage : Stefaan Horemans

Enseignant référent : Maria Manzanares-Dauleux

Autres membres du jury (Nom, Qualité) : Mélanie Jubault et Régine Delourme

"Les analyses et les conclusions de ce travail d'étudiant n'engagent
que la responsabilité de son auteur et non celle d'AGROCAMPUS OUEST".

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Genus <i>Beta</i>	
Section <i>Beta</i> (syn. <i>Vulgares</i> Ulbrich)	
2x, 3x, 4x ^a	<i>Beta vulgaris</i> L.
2x, 4x	<i>Beta macrocarpa</i> Guss.
2x	<i>Beta patula</i> Ait.
	<ul style="list-style-type: none"> ssp. <i>vulgaris</i> ssp. <i>maritima</i> (L.) Arcang. ssp. <i>adansensis</i> (Pamuk.) Ford-Ll. et Will.
	<ul style="list-style-type: none"> Leaf beet group^b Garden beet group^c Fodder beet group^d Sugar beet group
Section <i>Corollinae</i> Ulbrich	
2x, 4x	<i>Beta lomatogona</i> Fisc. et May.
2x	<i>Beta macrorhiza</i> Stev.
4x	<i>Beta corolliflora</i> Zos. ex Buttler
4x	<i>Beta intermedia</i> Bunge
4x, 6x	<i>Beta trigyna</i> Waldst. et Kit.
Section <i>Nanae</i> Ulbrich	
2x	<i>Beta nana</i> Boiss. et Heldr.
Section <i>Procumbentes</i> Ulbrich (syn. <i>Patellares</i> Tranzschel)	
2x	<i>Beta procumbens</i> Sm.
2x	<i>Beta webbiana</i> Moq.
4x	<i>Beta patellaris</i> Moq.

^aNumber of chromosomes (2x = 18; 3x = 27; 4x = 36; 6x = 72).
^bAlso named Mangold, Spinach beet, Chard, Swiss chard etc.
^cAlso named red beet.
^dAlso named forage beet.

Figure 1 : Classification de la betterave à sucre (Bosemark, 2006)

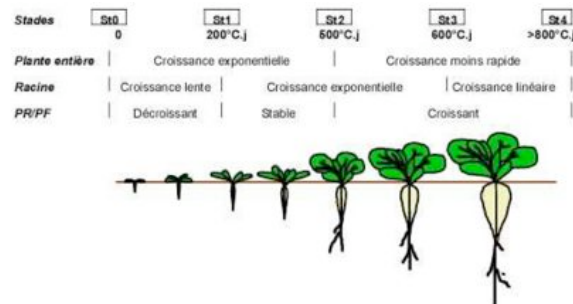


Figure 3 : Stades phénologiques de la betterave à sucre pendant la croissance végétative (ITB)

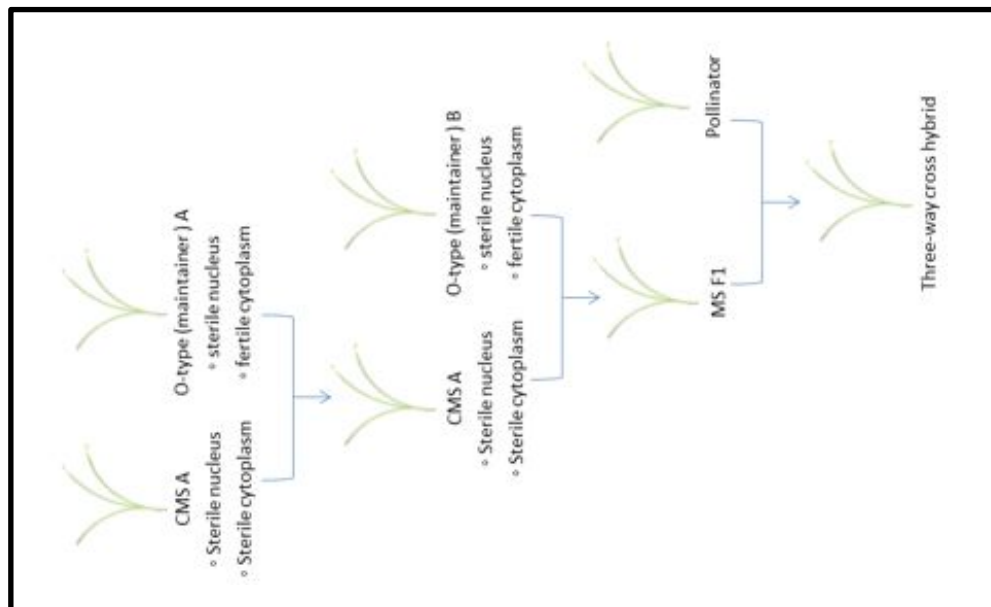


Figure 2 : Création d'un hybride trois voies

Introduction

Dans un monde en constant développement, il est nécessaire de produire plus pour pouvoir nourrir la population toujours croissante. Cela implique d'augmenter les rendements des cultures. Ces rendements sont limités par des facteurs biotiques et abiotiques comme des maladies, des ravageurs et les conditions environnementales.

La betterave à sucre (*Beta vulgaris* spp. *vulgaris* L.) est la deuxième source de production de sucre après la canne à sucre, elle est cultivée dans plus de 50 pays au climat tempéré. En 2011, environ 270 millions de tonnes de betterave à sucre ont été produites dans le monde. Les 5 plus gros producteurs sont la France (31 millions de tonnes), les Etats-Unis (28 millions de tonnes), l'Allemagne (25 millions de tonnes), la Russie (21 millions de tonnes) et l'Ukraine (18 millions de tonnes) (FAO, 2012). La betterave à sucre est une plante dicotylédone appartenant à la famille des Chénopodiacées (Figure 1). Elle a un cycle biennuel (UFS, 2013) constitué d'une phase végétative (Figure 2) et d'une phase reproductive. La température optimale de croissance est entre 20 et 28°C en fonction de la variété (AgroParisTech, 2003).

La betterave à sucre a un génome de neuf chromosomes. Elle est diploïde à l'état naturel. Plusieurs cartes génétiques ont été faites avec différents types de marqueurs moléculaires. Elles montrent en général neuf groupes de liaison. La carte physique de la betterave à sucre a été publiée le 14 février 2012 (Dohm, et al., 2012). Elle est le résultat d'un travail de séquençage réalisé par plusieurs entreprises et laboratoires. Les résultats n'étant pas entièrement publics, SESVanderHave a dû faire son propre séquençage avec ses propres marqueurs.

La plupart des hybrides de betterave à sucre sont diploïdes. Les lignées male stériles (CMS) sont produites à l'aide de de plantes mainteneuses de stérilité (nommées type O) qui portent les mêmes gènes de stérilité que les CMS mais dans un cytoplasme normal (les lignées sont dites équivalentes). La plante femelle d'un hybride est habituellement un hybride d'un CMS avec un autre type O non équivalent. L'hybride produit est un hybride trois voies. (Figure 3)

SESVanderHave est une entreprise internationale spécialisée dans le développement, la production et la commercialisation de semences de betterave à sucre. Cette entreprise existe depuis 2005, lorsque l'entreprise 'Maison Florimond Desprez' a racheté la partie sélection de la betterave à sucre d'Advanta seeds. L'entreprise investit continuellement dans la sélection pour la résistance aux maladies, dont le champignon *Aphanomyces cochlioides* fait partie.

La maladie racinaire *Aphanomyces cochlioides* Drechsler est un oomycète causant la fonte des semis et des pourritures de racines chez la betterave à sucre. (Figure 4). C'est un necrotrophe facultatif (Garrison, 2008). Son cycle de vie est décrit figure 5. Une étude précédente menée par SESVANDERHAVE a montré que la température optimale de croissance du champignon est de 28°C. Le champignon agit sur les zones d'élongation des plantes qui sont plus faibles. Ainsi donc, ce champignon peut attaquer la plante à chaque stade de son développement. Au stade plantule, l'attaque cause le pourrissement et la fonte des semis. Pendant le reste du cycle de la plante, le champignon peut attaquer les racines, causant une réduction de croissance de la racine, la disparition du pivot racinaire et parfois la mort de la plante (Figure 6,7,8). La maladie est omniprésente et cause des pertes de rendement significatives, surtout aux Etats-Unis, dans l'Europe de l'est de du nord (Metzger, et al., 2008) (Moliszewska, et al., 2008) (Piszczek, 2004) (Amein, 2006). Le contrôle d'*A. cochlioides* a été tenté par des méthodes biologiques, chimiques, agronomiques et de sélection, mais à ce jour aucune n'est vraiment efficace. (Payne, et al., 1990), (Whipps, et al., 1993), (Rush, et al., 1993). La méthode chimique la plus utilisée est l'hymexazol

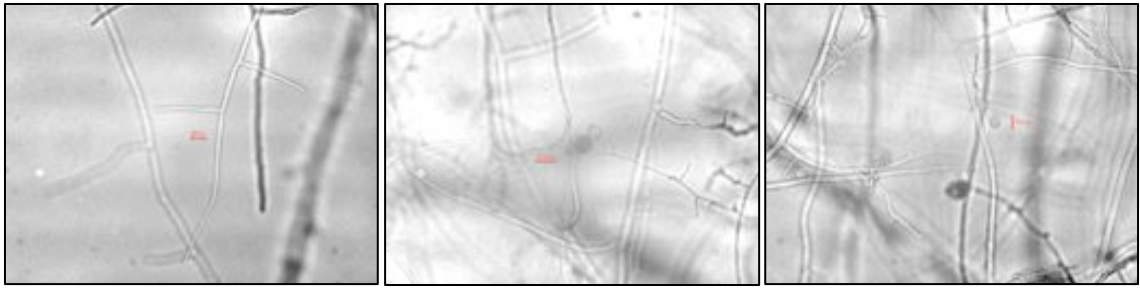


Figure 4 : *Aphanomyces cochlioides* (photographie personnelle)

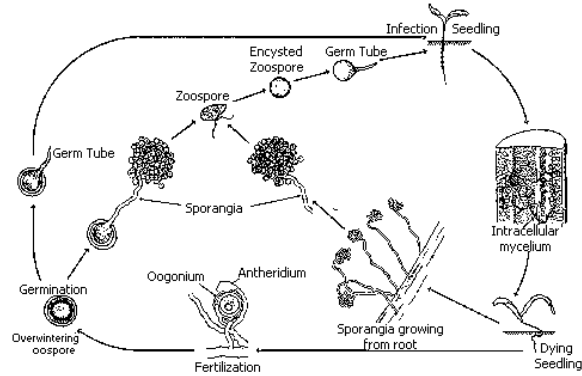


Figure 1. Life cycle of *Aphanomyces cochlioides* on sugarbeet. When root rot occurs later in the season, older plants should be substituted for seedlings in this illustration.

Figure 5: Cycle de vie d'*Aphanomyces cochlioides* (Windels & Engelkes, 1995)



Figure 6: Fonte des semis (SESVANDERHAVE)



Figure 8: symptômes sur le pivot racinaire (SESVANDERHAVE)

Figure 7: Symptômes aériens (SESVANDERHAVE)

(Tachigarin, 3-hydroxy-5-methyl-isoxazole), mais son efficacité reste variable, c'est un produit cher et qui n'est plus efficace après le stade plantule. La meilleure approche semble être la sélection pour la résistance (Yu, 2004). Jusqu'à maintenant, seule une résistance partielle à *Aphanomyces cochlioides* a été trouvée (Bockstahler, et al., 1950) mais c'est un mécanisme encore peu compris (Campbell, et al., 1993). De plus, *A. cochlioides* est l'une des maladies avec le moins de progrès du fait du manque de source très résistante et de la difficulté de créer des conditions d'infection uniformes pour le screening en champ ou en labo (Yu, 2004).

La sélection pour la résistance peut être menée par des tests moléculaires qui sont probablement plus précis que les tests conventionnels. Pour utiliser ces tests moléculaires, il faut d'abord savoir comment la résistance partielle à *Aphanomyces cochlioides* est contrôlée génétiquement. L'objectif est de rechercher des QTL (Quantitative trait loci) qui sont potentiellement impliqués dans la résistance partielle à *Aphanomyces cochlioides*. En 2012, une étude menée par SESVANDERHAVE a permis de situer la résistance partielle sur les chromosomes 4 et 6 de la carte génétique de SESVANDERHAVE. Le but de cette étude était de la localiser plus précisément et de tenter de trouver des marqueurs flanquants. Le problème majeur ici est d'évaluer et de quantifier la résistance à *A. cochlioides*. Des tests existent déjà dans la littérature (Windels, et al., 1989) (Peterson, et al., 1988) (Rao, et al., 1995) (Davis, et al., 1995) (Infantino, et al., 2006) (Panella, et al., 2008). SESVANDERHAVE utilise un test basé sur l'inhibition de la croissance de la plante lié à la croissance du champignon dans la racine. Le paramètre testé est le poids frais de la racine, mais il y a une confusion entre la capacité inhérente de croissance de la plante et l'effet réel de la maladie. C'est pour cela qu'il a été proposé de créer une nouvelle méthode de screening basée sur l'observation directe des symptômes pour éviter cette confusion. Ce test a été développé sur des plantules afin d'avoir des résultats plus rapides et de réduire l'hétérogénéité parmi les plantes. Plusieurs paramètres ont été testés comme le substrat, la date d'inoculation et la concentration de l'inoculum. Une méthode de notation a aussi été développée. Une fois cette méthode établie, l'essai de 2012 a été testé de nouveau avec ce nouveau protocole pour comparer les résultats obtenus avec les deux techniques. Ensuite, un set de lignées recombinantes a été testé pour évaluer leur niveau de résistance. Avec les données de marqueurs SNP (Single Nucleotide Polymorphism) disponibles, les données phénotypiques et génotypiques ont été analysées par des méthodes statistiques afin de découvrir des loci potentiellement impliqués dans la résistance.

Dans ce rapport, nous développerons le matériel et méthodes utilisés, incluant l'explication de la nouvelle méthode de screening. Les résultats seront suivis d'une discussion.

Table 1 : Genotype des parents de l'experimentation 1

Marker	D101_S01	D028_S02	D084_S01	D102_S03	D106_S03	D055_S04	D110_S01	D103_S02	D029_S01	BAC067_S01	F077_S02	F086_S04	F095_S01	F107_S01	F067_S03	F096_S01	F108_S01	F036_S01
Seed name	38	41	44	46	49	51	51	53	57	5	6	8	9	18	24	25	25	29
S11T270375001	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
S11T270345001	H	H	H	H	H	H	H	H	H	H	H	H	B	B	H	H	H	H
S11T270385001	H	H	H	H	H	H	H	H	H	B	B	B	B	B	B	B	B	B
S11T269975001	H	H	H	H	H	B	B	B	B	H	H	H	H	H	H	H	H	H
S11T269985001	H	H	H	H	H	B	B	B	B	H	H	H	H	H	H	H	H	H
S11T270075001	H	H	H	H	H	B	B	B	B	H	H	H	H	H	H	H	H	H
S11T269995001	H	H	H	H	H	B	B	B	B	B	B	B	B	H	H	H	H	H
S11T270055001	H	H	H	H	H	B	B	B	B	B	B	B	B	H	H	H	H	H
S11T270095001	H	H	H	H	H	B	B	B	B	B	B	B	B	H	H	H	H	H
S11T270005001	H	.	.	H	H	.	B	B	B	B	B	B	B	B	B	B	B	B
S11T270015001	H	H	H	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B
S11T270185001	H	H	B	B	B	B	B	B	B	H	H	H	H	H	H	H	H	H
S11T269945001	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
S11T270105001	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
S11T270155001	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B

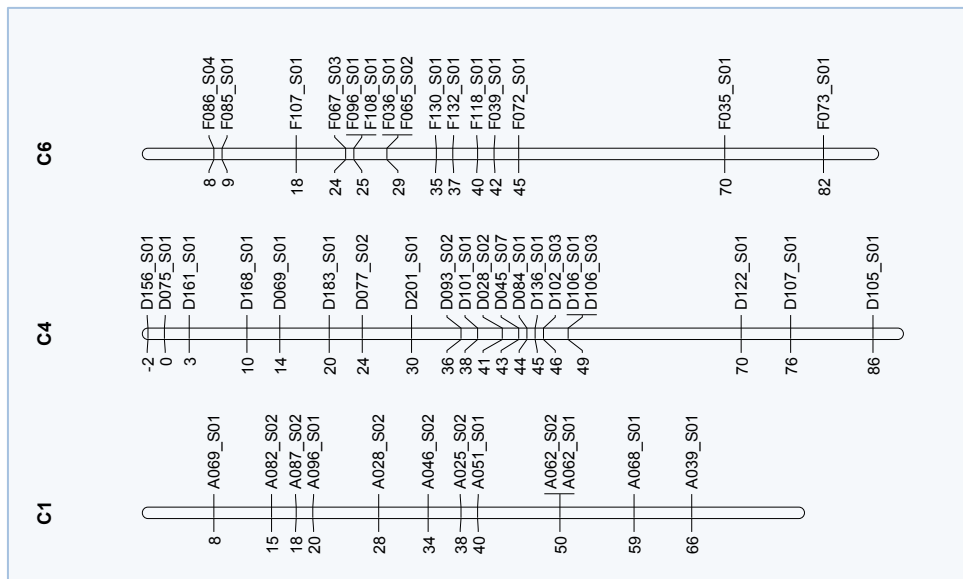


Figure 9 : carte génétique des marqueurs utilisés

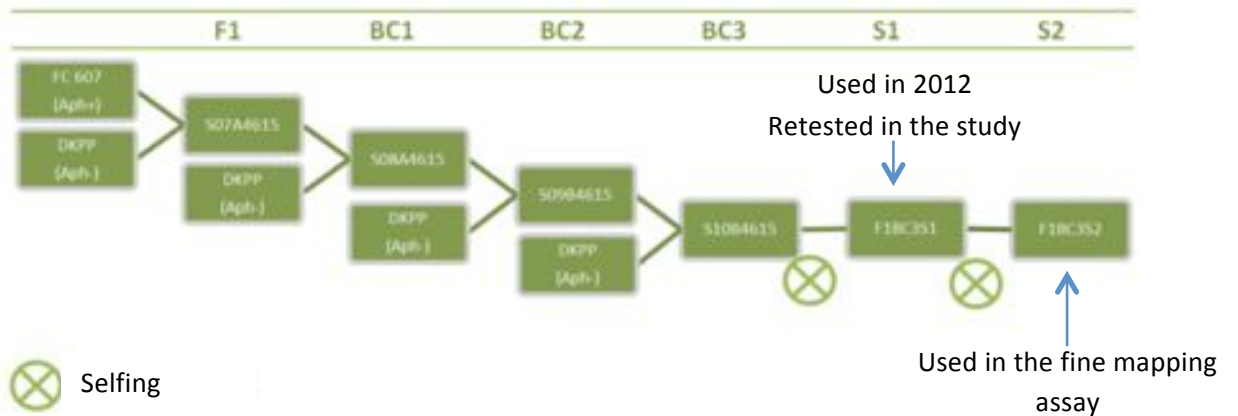


Figure 10: Généalogie des populations testées

Matériel et méthode

A. Matériel végétal

1. **Matériel végétal utilisé pour le développement du bioessai**

Les lignées utilisées pour cette étude étaient une lignée dérivée de FC607 (FC607 est le nom utilisé dans le texte), DKPP et leur hybride FC607xDKPP. FC607 est la source de résistance et est dérivée d'une lignée américaine (Smith, 1980). Elle est partiellement résistante à *Aphanomyces cochlioides*. DKPP est une lignée élite pollinisatrice multigerme, sensible à *A. cochlioides*.

2. **Matériel végétal utilisé dans la répétition du bioessai de 2012**

Le matériel végétal ayant été utilisé provient d'un croisement initial entre FC607 et DKPP. Le type de population est BC3 S1 (trois backcross avec DKPP en tant que parent récurrent et une autofécondation). La généalogie est disponible figure 10. Cette population est appelée artificielle car ces lignées ont été sélectionnées pour leur variabilité au caractère de résistance (elle ne suit pas le principe de Hardy-Weinberg). 16 lignées ont été testées. Trois groupes de génotypes ont pu être mis en évidence sur le chromosome 4. L'un est entièrement hétérozygote (entre 38 and 57 cM) (groupe A), l'un est hétérozygote entre 38 et 41cM (groupe B), le dernier groupe est hétérozygote entre 38 et 49 cM (group C). (Table 1)

Les chromosomes étaient tous fixés sauf les chromosomes 1, 4 et 6 (Figure 9)

Les trois témoins utilisés sont les mêmes lignées que celles utilisés pour développer le bioessai : FC607, DKPP et leur hybride.

3. **Matériel végétal utilisé pour la cartographie fine de la résistance**

Les lignées ayant été utilisées ici sont des lignées BC3 S2. Elles sont le produit du même croisement entre FC607 et DKPP que l'essai précédent, avec une autofécondation supplémentaire (Figure 10). DKPP est le parent récurrent. Puisqu'il s'agit d'une lignée de backcross, cela signifie que certaines parties des chromosomes ne sont pas fixées.

Comme, grâce à un précédent essai, il est supposé que la résistance est située sur les chromosomes 4 et 6, ces parties du génome ont été particulièrement sélectionnées au cours des backcross pour garder la variabilité dans ces zones. Parmi toutes les familles, 16 ont été sélectionnées, en fonction de leur génotype. Les familles choisies permettent une bonne couverture de recombinaisons pour localiser précisément la résistance. Ici, la zone entre 20 et 50 cM du chromosome 4 a été privilégiée (Figure 11).

Les mêmes témoins que dans l'essai précédent ont été utilisés.

B. Traitement des semences

Après la récolte, les semences ont été séchées dans une chambre à 29°C pendant 3 ou 4 jours. Elles ont ensuite été nettoyées, les poussières et petites graines ont été enlevées. Avant leur utilisation dans les bioessais, pour éviter qu'elles ne soient déjà infectées par *Aphanomyces cochlioides* ou un autre champignon, les semences ont été lavées avec de l'eau pendant 4 heures. Cette étape ne peut être faite avec un fongicide puisque cela pourrait perturber l'inoculation à venir.

C. Préparation de l'inoculum

Le protocole précis est celui utilisé habituellement par SESVANDERHAVE

Un mélange de souches d'*Aphanomyces cochlioides* a été utilisé dans cet essai.

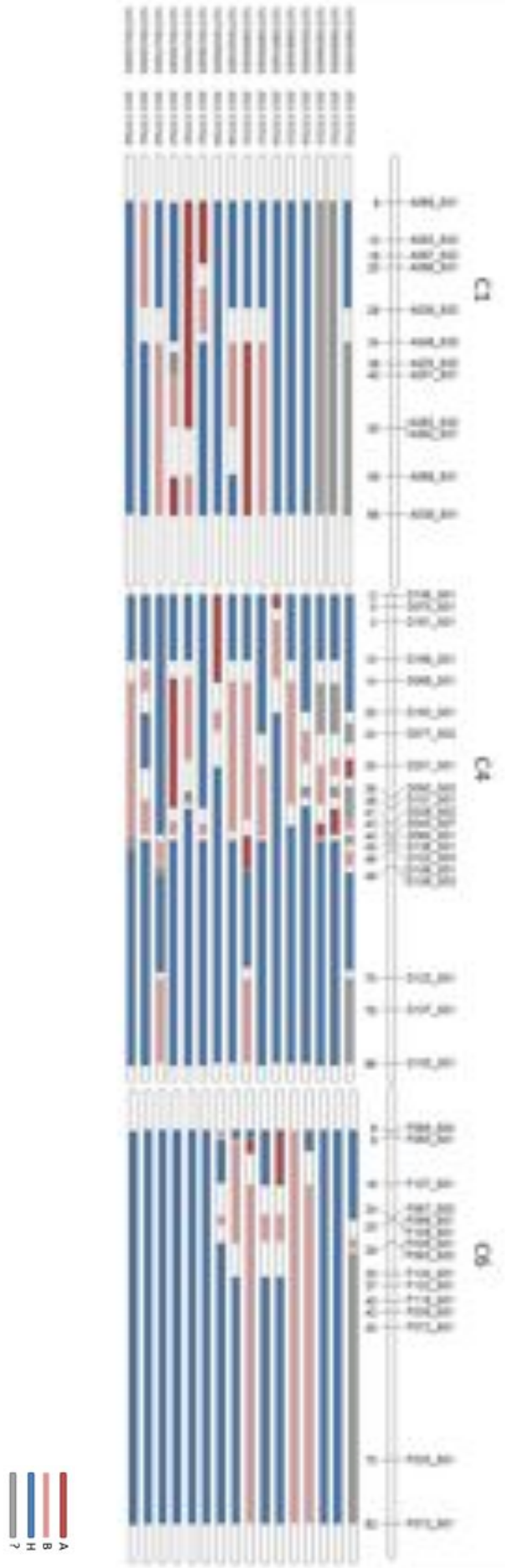


Figure 11 : aénovpe des parents pour l'essai de cartographie fine

D. Conditions de croissance

Pendant le développement du bioessai, différentes conditions de croissance ont été testées. Plus d'informations peuvent être trouvées en annexes I et II. A l'issue du développement, il a été décidé de garder le protocole suivant utilisant les tubes. (Figure 12)

1. Semis

Les semences ont été placées dans une boîte contenant un papier filtre plié en accordéon, 38 mL d'eau ont été ajoutés dans la boîte. Elles ont d'abord été mises à germer à l'obscurité dans un incubateur à 24°C pendant 3 à 4 jours. Puis elles ont été mises sous la lumière dans une chambre de culture à 18°C pendant la journée (16h de lumière) et 19°C pendant la nuit (8h), pendant 2 à 3 jours.

2. Préparation des tubes

Les tubes ont été remplis de sable, à l'extrémité inférieure, un morceau de laine de verre a été placé pour boucher les trous. Une bande de tissu permettant la capillarité a été placée du haut vers le bas (à travers le morceau de laine de verre) pour irriguer le substrat (figure 13). Deux jours avant la transplantation, les tubes ont été placés dans un bac d'eau pour humidifier le sable.

3. Transplantation

Six et sept jours après le semis, les plantes ont été transplantées dans le sable. A ce stade, 10 mL d'eau ont été ajoutés à chaque plante pour assurer une bonne irrigation.

4. Conditions de croissance

Les plantes ont été placées dans une chambre de culture (phytotron) à la température de 22°C pendant le jour (16h) et 19°C pendant la nuit (8h). Ces températures ont été choisies car elles sont optimales pour la croissance de la betterave à sucre et du pathogène.

Deux jours après la transplantation, l'eau des bacs a été remplacée par la solution nutritive.

5. Solution nutritive

La solution nutritive utilisée ici est le Steiner dilué 10 fois (voir en annexe II).

6. Inoculation

Les plantes ont été inoculées 22 jours après le semis. 1 mL de la solution d'inoculum à 2000sp/mL a été appliqué à la base de l'hypocotyle à l'aide d'une pipette. Les plantes doivent être bien irriguées le jour de l'inoculation.

E. Design expérimental

Aucun gradient n'a été décelé dans le phytotron. Cependant, les bacs dans lesquelles les plantes ont été cultivées ne peuvent contenir que 200 plantes sur les 600 au total. Trois blocs ont donc été nécessaires. Dix répétitions de chaque génotype se trouvaient dans chaque bloc. Cela fait au total 30 plantes par génotype, excepté pour DKPP qui a été répété 20 fois dans chaque bloc, soit 60 fois au total.

Pour faciliter la compréhension, dans la suite du rapport, l'essai fait en 2012 sera nommé "expérimentation 1" et l'essai de cartographie fine "expérimentation 2".

F. Evaluations et analyses

1. Evaluation de la résistance

La notation a été faite pendant l'incubation basé sur l'exemple de Yu (2004). Le score "0" signifie que la plante n'a pas ou peu de symptômes. Le score "1" signifie que plus de 2/3 de l'hypocotyle et de la surface racinaire sont attaqués. "0.5" est utilisé pour tous les autres cas qui ne sont ni 0, ni 1 (Figure 14).

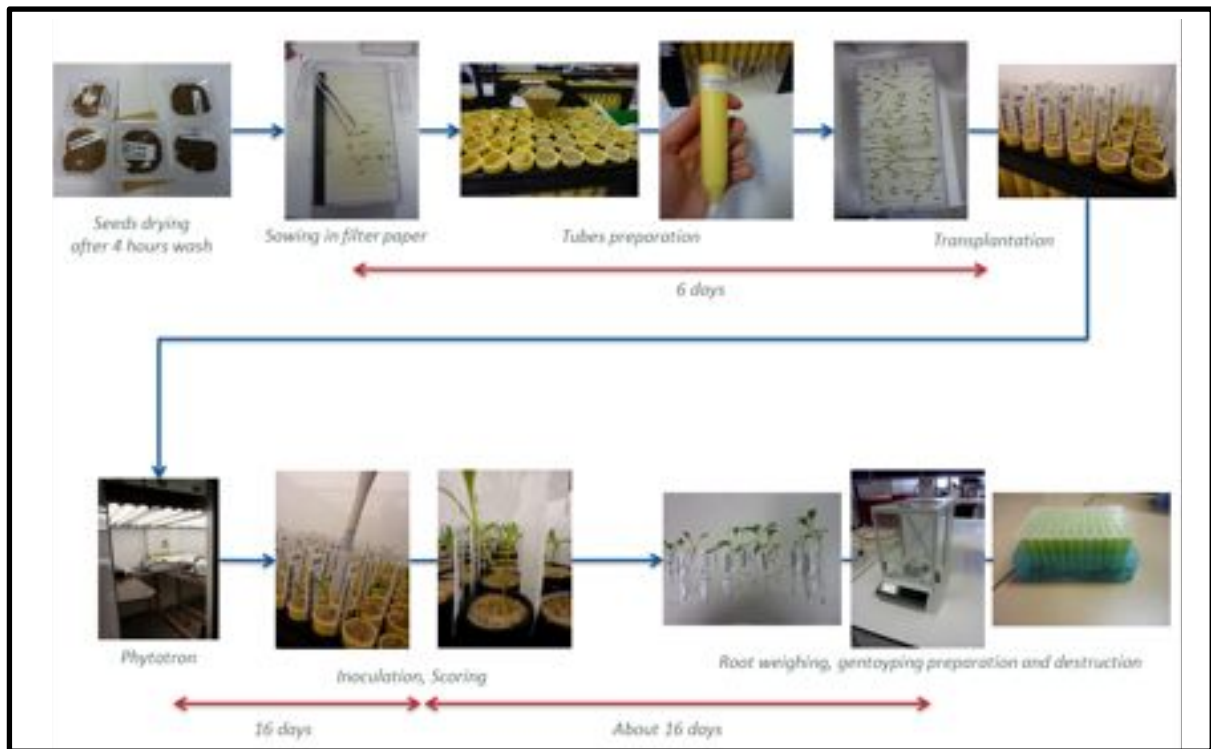


Figure 12 : Etapes du protocole suivi pour les expérimentations

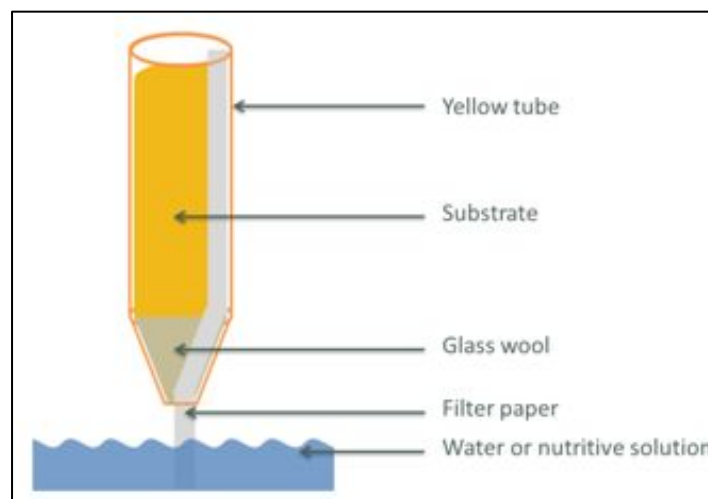


Figure 13 : le système des tubes



Figure 14 : Méthode de notation de Yu (personal photos, Yu, 2004)

Pendant le développement du bioessai, d'autres évaluations ont été réalisées (voir annexes I et II). Du fait des résultats non concluants, elles n'ont pas été répétées lors des deux derniers essais de cartographie de QTL.

Identification du pathogène

Pour être sûr que les plantes ont bien été inoculées par *Aphanomyces cochlioides*, une partie de l'hypocotyle infecté a été isolée sur PDA. Quelques jours plus tard, il était possible de reconnaître *Aphanomyces cochlioides* sous le microscope (Figure 3).

2. Homogénéité d'irrigation

Pour évaluer l'homogénéité de distribution de l'eau dans les tubes, ils ont été pesés avant et après humidification. Deux répétitions ont été faites, une pour chaque essai.

3. Analyses statistiques

Les analyses statistiques ont été faites avec le logiciel R (R Core Team, 2013).

Les études statistiques ont été faites sur toutes les données afin d'estimer les différences significatives entre les traitements.

a. Analyses de variance

Du fait que l'étude implique une notation à différentes dates dans le temps, il est possible d'effectuer une analyse cinétique par le biais d'une évaluation de l'AUDPC (Area Under Disease Progression Curve). L'AUDPC a été calculée depuis le premier jour après inoculation jusqu'au dernier jour d'expérimentation (19 jours pour l'expérimentation 1 et 15 pour l'expérimentation 2). En plus de l'AUDPC, le score du jour pour lequel les témoins étaient les plus significativement différents et pour lequel la gamme entre l'individu le plus sensible et le plus résistant était la plus large, a été choisi comme second score (7 jours après inoculation (7dpi) pour chaque essai).

L'évaluation des différents effets pour le score à 7dpi et l'AUDPC a été faite par une ANOVA. L'analyse de variance a suivi le modèle $AUDPC_{ij} = \mu + g_i + \beta_j + \varepsilon$. $AUDPC_{ij}$ est l'AUDPC du génotype i du bloc j , μ est la moyenne de l'AUDPC, g_i est l'effet du génotype, β_j est l'effet du bloc et ε est l'effet résiduel. Respectivement pour 7dpi : $7dpi_{ij} = \mu + g_i + \beta_j + \varepsilon$.

b. Comparaison de moyennes

L'ANOVA a été suivie d'un test de Duncan pour faire des groupes d'individus non significativement différents les uns des autres. Pour être plus précis, un test de Dunnett a permis de comparer chaque génotype aux témoins.

Puisque qu'aucun effet bloc n'a été détecté dans les deux essais, il n'y a pas eu besoin d'ajuster les valeurs des individus.

c. Héritabilité

L'héritabilité a été calculée avec les valeurs de l'ANOVA à l'aide de la formule : $h^2 = \frac{\sigma_g^2}{\left[\sigma^2_g + \frac{\sigma^2_e}{n} \right]}$
Avec σ^2_g la variance génétique, σ^2_e la variance résiduelle et n le nombre de répétitions par lignées testées.

d. Distribution des individus

La distribution des individus a ensuite été représentée en fonction de leur réponse à l'infection par *Aphanomyces cochlioides*.

4. Détection de QTL

Les analyses ont été réalisées avec le logiciel MapQTL6 (Van Ooijen, 2009).

a. Génotypage

Pour les deux expérimentations, des feuilles ont été échantillonnées. Pour l'expérimentation 1, seul les feuilles restant à la fin de l'essai ont été récoltées, pour l'expérimentation 2, autant de feuilles que possible ont été échantillonnées, dès que la plante atteignait le score de "1".

Dans cette étude, la carte consensus a été utilisée. Les marqueurs ayant été choisis ont un génotype ségrégant à leur locus.

Pour chaque marqueur, le génotype "A" a été donné aux plantes ayant le génotype de FC607 (le donneur de résistance) et le génotype "B" a été donné aux plantes dont le génotype provient de DKPP (plante receveuse). "H" a été donné aux plantes hétérozygotes.

b. Expérimentation 1

Cette analyse a été faite avec 17 marqueurs SNP (sur les chromosomes 4 et 6) avec une population en ségrégation de 16 lignées (total de 165 individus échantillonnés)

Comparaison des trois groupes de génotypes

Pour comparer les trois groupes, le modèle d'ANOVA suivant a été utilisé : $\text{Group}_i = \mu + G_i + \varepsilon$. Group_i est le groupe de génotypes i , μ est la moyenne du groupe, G_i est l'effet du groupe et ε l'effet des résidus.

Test des rangs sommés de Kruskal-Wallis

Puisque notre population ne suit pas une distribution normale, le test non paramétrique de Kruskal-Wallis a été préféré à l'ANOVA. C'est un test qui permet un aperçu des données, mais qui ne permet pas de conclure car il n'est pas le plus précis.

Aucune analyse supplémentaire ne sera faite car cette population n'est pas adaptée pour la cartographie de QTL.

c. Expérimentation 2

L'analyse a été faite avec 37 marqueurs SNP (sur les chromosomes 4 et 6) avec une population en ségrégation de 16 lignées (total de 262 individus échantillonnés).

Comparaison des résultats des familles

Comme premier aperçu des données, les résultats de chaque individu ont été observés. Pour chacun, une lettre a été donnée : "A" si les scores (7dpi et AUDPC) étaient faibles et semblent montrer une résistance (en général 0 pour le score à 7dpi et moins de 11 pour l'AUDPC), "B" si ils sont élevés et semblent être sensibles (habituellement 1 pour le score à 7dpi et plus de 16 pour l'AUDPC). "H" est utilisé dans les autres cas. Pour chaque famille, et pour chaque lettre, le nombre d'individus a été compté et la moyenne a été calculée. En observant le résultat des familles, il est possible de donner les mêmes lettres à la famille entière. "A" est donné quand la famille a beaucoup d'individus "A" et "H" comparés aux "B", "B" est donné aux familles ayant beaucoup d'individus "B" comparé aux "A" et "H". "H" est donné à toutes les familles intermédiaires.

Avec ces résultats, en parallèle des données génotypiques, il est possible de voir une tendance dans les allèles des marqueurs des génotypes les plus résistants.

Test des rangs sommés de Kruskal-Wallis

Comme pour l'expérimentation 1, un test de Kruskal-Wallis a été fait sur les données.

Cartographie d'intervalles



Figure 15 : pourcentage d'eau dans les tubes (Erreur standard : 0.679)

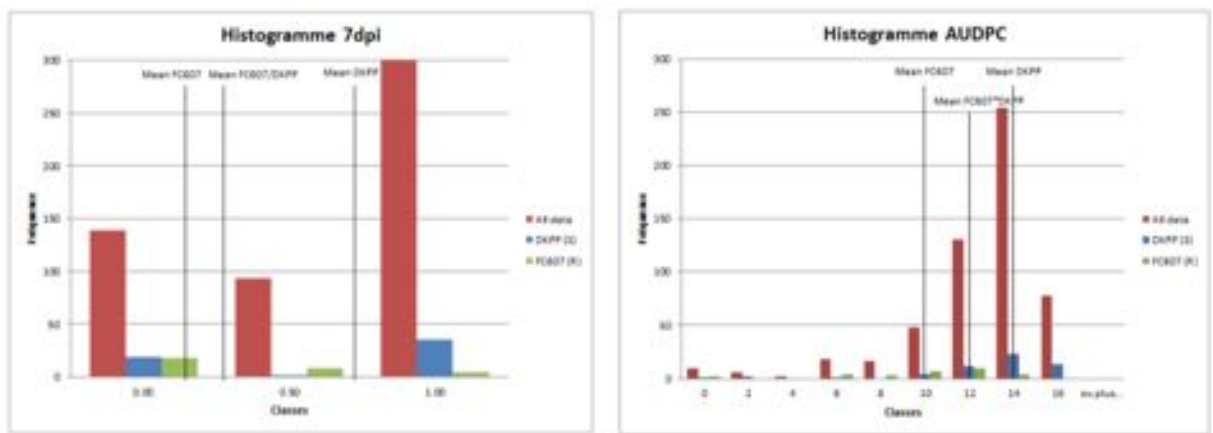


Figure 16: répartition des individus en 7dpi et l'AUDPC pour l'expérimentation 1

Table 2 : résultats pour 7dpi and AUDPC pour l'expérimentation 1: ANOVA, Duncan test

7dpi					7dpi					7dpi						
7dpi	Df	Sum Sq	Mean Sq	F value	Pr(>F)	PEDIGREE	N	Mean	St.terr. grp	AUDPC	Mean	St.terr. grp	Bloc	N	Mean	St.terr. grp
Genotype	18.00	11.75	0.65	3.97	0.00	FC607	30	0.267	0.067 c	9.03	0.655 b	A	197	0.660	0.030 a	
Bloc	1.00	0.10	0.10	0.60	0.44	FC607/DKPP	30	0.350	0.087 bc	10.50	0.617 ab	B	189	0.680	0.032 a	
Genotype*Bloc	17.00	2.82	0.17	1.01	0.45	S11T27034S001	30	0.633	0.083 abc	11.93	0.519 a	C	178	0.674	0.031 a	
Residuals	527.00	86.57	0.16			S11T27010S001	29	0.638	0.086 abc	11.08	0.717 ab					
						DKPP	56	0.643	0.063 ab	12.05	0.447 a					
						S11T27005S001	30	0.650	0.064 ab	11.50	0.570 ab					
						S11T26999S001	30	0.667	0.081 ab	11.54	0.546 ab					
						S11T27000S001	30	0.667	0.077 ab	11.48	0.684 ab					
						S11T27037S001	30	0.667	0.084 ab	11.28	0.687 ab					
						S11T27007S001	30	0.683	0.066 ab	11.96	0.391 a					
						S11T27041S001	30	0.700	0.074 ab	12.34	0.372 a					
						S11T27001S001	30	0.717	0.075 ab	11.83	0.516 a					
						S11T26997S001	29	0.741	0.073 ab	11.77	0.575 a					
						S11T27038S001	30	0.750	0.071 ab	12.22	0.348 a					
						S11T27018S001	20	0.800	0.084 ab	12.72	0.329 a					
						S11T26998S001	30	0.833	0.060 ab	12.88	0.245 a					
						S11T27009S001	30	0.833	0.060 ab	12.57	0.269 a					
						S11T26994S001	30	0.850	0.049 a	12.54	0.388 a					
						S11T27015S001	10	0.850	0.107 ab	12.82	0.264 a					

Cette analyse a été faite pour les deux traits mesurés, 7dpi et l'AUDPC. Elle a été faite uniquement sur les chromosomes 4 et 6. Pour chaque famille, les parties fixées ont été traitées comme données manquantes car elles auraient pu perturber l'analyse.

Puisque la population est artificielle, il n'est pas possible de faire une carte spécifiquement pour cet essai, pour cela, la carte consensus de SESVANDERHAVE a été utilisée.

LOD seuil

Pour savoir si les résultats sont significatifs, le LOD seuil a été calculé en utilisant un test de permutation avec 1000 itérations. Le seuil "genome-wide" avec une p-value de 0.05 a été choisi. "Genome-wide" signifie qu'il s'agit de la valeur maximale de LOD partout sur le génome, incluant tous les groupes de liaison.

Cartographie multiple de QTL

Cette analyse a été faite de la même façon que la cartographie d'intervalles en ajoutant des cofacteurs. Ceux-ci ont été sélectionnés manuellement en fonction des résultats de la cartographie d'intervalles.

Résultats

A. Development du bioessai

Voir annexes I et II.

B. Validation du design expérimental

1. Vérification de l'agent pathogène

Après culture sur gel, il était possible de reconnaître les structures spécifiques à *Aphanomyces cochlioides* sous le microscope (Figure 3).

2. Homogénéité d'irrigation

Le pourcentage d'eau dans le tube n'était pas significativement différent à 5% entre les différentes localisations dans le bac (Figure 15).

C. Expérimentation 1

1. Analyses statistiques

a. Analyse de variance

Comme il l'a été décrit dans la partie Matériel et méthode, en plus de l'AUDPC à 19dpi, les analyses ont aussi été faites sur le score à 7dpi du fait d'une bonne ségrégation (d'un score de 0.267 attribué au témoin résistant à un score de 0.850 à la plante la plus atteinte). (Tableau 2)

Les postulats de l'analyse de variance ont été vérifiés (homoscedasticité, normalité et indépendance) (voir annexe IV). Avec cette analyse, il est possible de voir qu'il n'y a pas d'effet bloc. Cela permet de traiter toutes les données ensemble. L'ANOVA montre aussi un effet significatif du génotype. Cela signifie aussi que chaque génotype ne réagit pas de la même façon lorsqu'il est confronté à *Aphanomyces cochlioides* (Tableau 2)

b. Distribution de individus

Une distribution continue des individus est observée, à la fois pour le score à 7dpi et l'AUDPC. Cela montre une réaction quantitative du génotype. Cette répartition ne suit pas

Table 4 : résultats pour 7dpi and AUDPC sans les témoins pour l'expérimentation 1: ANOVA, Duncan test

Table 3 : Test de Dunnett pour 7dpi et l'AUDPC pour l'expérimentation 1

AUDPC	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	15.00	127.96	8.53	1.15	0.31
Bloc	1.00	5.09	5.09	0.68	0.41
Genotype*Bloc	14.00	52.76	3.77	0.51	0.93
Residuals	417.00	3105.41	7.45		

7dpi	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	15.00	2.49	0.17	1.06	0.39
Bloc	1.00	0.02	0.02	0.15	0.70
Genotype*Bloc	14.00	1.51	0.11	0.69	0.79
Residuals	417.00	65.16	0.16		

PEDIGREE	N	7dpi		AUDPC	
		Mean	St.err. grp	Mean	St.err. grp
S11T270345001	30	0.613	0.083	11.933	0.519
S11T270105001	29	0.618	0.086	11.078	0.717
S11T270055001	30	0.650	0.064	11.500	0.570
S11T269995001	30	0.667	0.081	11.542	0.546
S11T270005001	30	0.667	0.077	11.483	0.684
S11T270175001	30	0.667	0.084	11.283	0.687
S11T270075001	30	0.683	0.066	11.958	0.391
S11T270415001	30	0.700	0.074	12.342	0.377
S11T270015001	30	0.717	0.075	11.833	0.516
S11T269975001	29	0.741	0.073	11.767	0.575
S11T270185001	30	0.750	0.071	12.217	0.348
S11T270185001	20	0.800	0.084	12.725	0.329
S11T269985001	30	0.811	0.060	12.875	0.245
S11T270095001	30	0.811	0.060	12.567	0.269
S11T269945001	30	0.836	0.049	12.542	0.388
S11T270155001	10	0.850	0.107	12.825	0.264

PEDIGREE	N	7dpi		AUDPC	
		Mean	Dunnett	Mean	Dunnett
FC607	30	0.27	vs. 0.999	9.03	vs. 0.387
FC607/DKPP	30	0.35	vs. 0.021	10.50	vs. 0.178
S11T270105001	29	0.64	0.007	0.079	1.000
S11T270375001	30	0.67	0.002	0.035	1.000
S11T270005001	30	0.67	0.002	0.035	1.000
S11T270055001	30	0.65	0.004	0.054	1.000
S11T269995001	30	0.67	0.002	0.035	1.000
S11T269975001	29	0.74	0.000	0.004	0.976
S11T270015001	30	0.72	0.000	0.008	0.999
S11T270345001	30	0.63	0.008	0.083	1.000
S11T270075001	30	0.68	0.001	0.021	1.000
DKPP	56	0.64	0.001	0.021	vs. 12.05
S11T270385001	30	0.75	0.000	0.002	0.948
S11T270415001	30	0.70	0.001	0.013	1.000
S11T269945001	30	0.85	0.000	0.000	0.234
S11T270095001	30	0.83	0.000	0.000	0.333
S11T270185001	20	0.80	0.000	0.002	0.770
S11T270155001	10	0.85	0.002	0.011	0.770
S11T269985001	30	0.83	0.000	0.000	0.333

Table 5 : comparaison des types de génotypes pour l'expérimentation 1

SEED NAME	MS	7dpi	audpc
S11T270375001	A	0.667	11.283
S11T270345001	A	0.613	11.933
S11T270385001	A	0.750	12.217
S11T269975001	B	0.741	11.767
S11T269985001	B	0.813	12.875
S11T270075001	B	0.683	11.958
S11T269995001	B	0.667	11.542
S11T270055001	B	0.650	11.500
S11T270095001	B	0.833	12.567
S11T270005001	B	0.667	11.483
S11T270015001	B	0.717	11.833
S11T270185001	C	0.800	12.725
S11T269945001	C	0.850	12.542
S11T270105001	C	0.638	11.078
S11T270155001	C	0.850	12.825

Comparison of the 7dpi's score and AUDPC of the three groups made according to genotype

7dpi	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	2.00	0.31	0.16	1.01	0.36
Residuals	415.00	64.05	0.15		

PEDIGREE	N	7dpi	
		Mean	St.err. grp
A	90	0.68	0.046
B	238	0.72	0.025
C	90	0.77	0.040

AUDPC	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	2.00	4.86	2.43	0.32	0.73
Residuals	415.00	3161.74	7.62		

PEDIGREE	N	AUDPC	
		Mean	St.err. grp
A	90	11.83	0.309
B	238	11.94	0.176
C	90	12.14	0.284

Signification :
 A : should be more resistant according to genotyping
 B :
 C : should be more sensible according to genotyping

Table 6 : Résultats du test de Kruskal-Wallis avec les individus échantillonnés pour l'expérimentation 1

Chr	Position (cM)	Locus	K*	Df	Signif.	Nr inf.	7 dpi			AUDPC					
							Meanrank-A	Mean-A	Nr-A	Meanrank-B	Mean-B	Nr-B			
C4	38	D101_501	21.758	2	*****	146	50	8.7	43.0	80.4	11.1	78.0	92.5	11.6	25
C4	41	D028_502	21.871	2	*****	143	48.7	8.7	41.0	77.9	11.0	78.0	92.7	11.7	24
C4	44	D084_501	21.851	2	*****	121	38.2	8.9	31.0	65.2	11.0	66.0	79.0	11.8	24
C4	46	D102_503	20.298	2	*****	122	39.2	9.0	30.0	64.9	10.9	68.0	79.8	11.8	24
C4	49	D106_503	18.984	2	*****	119	38.4	9.0	28.0	62.7	10.9	67.0	77.6	11.8	24
C4	51	D055_504	11.245	2	****	45	13.6	9.1	12.0	24.8	11.2	26.0	31.4	11.2	7
C4	51	D110_501	11.108	2	****	43	13.3	9.1	12.0	23.6	11.1	24.0	31.4	11.2	7
C4	53	D103_502	8.929	2	**	42	13	9.0	11.0	23.5	11.2	25.0	28.8	11.0	6
C4	57	D029_501	8.665	2	**	43	13.3	9.0	11.0	24.1	11.3	26.0	29.0	11.0	6
C6	8	F086_504	1.994	2	-	62	34.2	10.8	12.0	33.1	11.1	33.0	26.4	9.5	17
C6	9	F085_501	1.638	2	-	92	46.8	10.4	19.0	50.3	11.5	36.0	42.6	9.9	37
C6	18	F107_501	0.427	2	-	97	51	10.8	20.0	50.1	11.2	39.0	46.8	10.2	38
C6	24	F067_508	0.648	2	-	116	55.2	10.6	25.0	60.9	11.3	57.0	56.8	10.6	34
C6	25	F096_501	0.364	2	-	116	55.2	10.6	25.0	60.0	11.2	57.0	58.4	10.6	34
C6	25	F108_501	0.897	2	-	114	53.9	10.6	25.0	60.4	11.4	55.0	55.5	10.6	34
C6	29	F036_501	3.095	2	-	113	46.8	9.9	23.0	60.6	11.5	57.0	57.8	10.7	33

Significance levels:
 *0.1
 **0.05
 ***0.01
 ****0.005
 *****0.001
 *****0.0005
 *****0.0001

une loi normale (figure 16), mais aucun changement de variable (log, ln, ln x+1, ...) n'a permis de la normaliser.

Il est possible de voir que FC607 est le plus résistant (7dpi = 0.267 et AUDPC = 9.03), suivi par l'hybride FC607*DKPP. DKPP, la lignée sensible, a une AUDPC plutôt haute (12.05) et un score à 7dpi modérément élevé (0.643), mais certains génotypes ont déjà des réactions plus sensibles (par exemple S11T27015S001 avec un score à 7dpi de 0.85 et une AUDPC de 12.82).

Le fait que l'AUDPC de FC607 ne soit pas zéro confirme que c'est une résistance partielle puisque même le génotype résistant est contaminé.

c. Comparaison de moyennes

Le test de Duncan permet de séparer les individus en groupes (tableau 2). Le tableau montre que seul FC607 est vraiment différent des autres individus. Cela est confirmé par la même analyse faite sans les témoins (tableau 3). Le test de Dunnett (tableau 4) montre la comparaison entre chaque génotype et les témoins. On peut voir que tous les génotypes (sauf le résistant et l'hybride) ne sont pas significativement différents du témoin sensible ($p > 0.05$).

d. Héritabilité

L'héritabilité (h^2) est plutôt élevée pour les deux valeurs testées. Pour l'AUDPC, $h^2 = 0.65$ et pour 7dpi, $h^2 = 0.75$. Les deux valeurs sont proches. Cette valeur montre que les variations phénotypiques dues à la génétique sont plutôt hautes.

2. Analyses génétiques

a. Comparaison des trois types de génotypes

Comme il l'a été expliqué dans le matériel et méthode, trois types de génotypes sont attendus montrant des phénotypes résistants, intermédiaires et sensibles. Cependant ce n'est pas le cas. En effet, un des génotypes qui devrait être l'un des plus sensibles, apparaît comme étant le plus résistant (S11T27010S001) (Tableau 5). De plus, les résultats de l'ANOVA ne montrent aucune différence significative entre les groupes ($p > 0.05$).

b. Le test de Kruskal-Wallis

Le test de Kruskal-Wallis a été d'abord été réalisé avec les génotypes parentaux chromosomes 4 et 6). Pour les deux scores, aucun QTL n'est détectable.

Le même test réalisé avec les individus échantillonnés montre clairement un effet sur le chromosome 4, en effet, tous les marqueurs de ce chromosome ont une K-value significative. En revanche rien n'est détectable sur le chromosome 6 (Tableau 6).

D. Expérimentation 2

1. Analyse statistique

a. Analyse de variance

Comme il l'a été indiqué dans la partie matériel et méthode, en plus de l'AUDPC à 15dpi (fin de l'essai), les études ont aussi été menées sur la note attribuée le 7^e jour après l'inoculation du fait d'une bonne ségrégation des témoins (d'un score de 0.3 attribué au témoin résistant à un score de 0.908 attribué au témoin sensible). (Tableau 7)

L'homoscedasticité, la normalité et l'indépendance ont été vérifiées pour l'analyse de variance (voir annexe IV). Ici encore, aucun effet bloc n'est remarqué et les données peuvent être traitées ensemble. Comme pour l'essai précédent, l'effet du génotype est significatif.

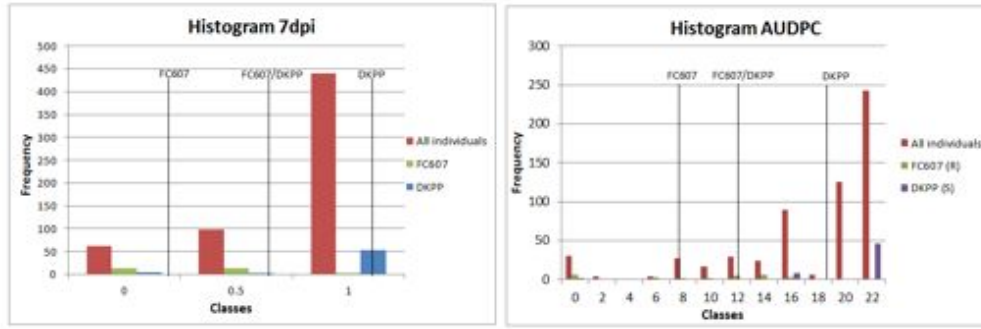


Figure 17 : répartition des individus en 7dpi et l'AUDPC pour l'expérimentation 2

Table 7 : résultats pour 7dpi and AUDPC pour l'expérimentation 2: ANOVA, Duncan test

AUDPC						7dpi						7dpi			AUDPC							
Genotype	Df	Sum Sq	Mean Sq	F value	Pr(>F)	PEDIGREE	N	Mean	St. err	grp	Mean	St. err	grp	Mean	St. err	grp	Mean	St. err	grp			
Genotype	18.00	4315.90	234.72	8.63	2e-16 ***	FC607	30	0.300	0.057	d	8.533	1.043	e									
Bloc	2.00	30.90	15.46	0.57	0.57	FC607/DKPP	30	0.600	0.096	c	12.847	0.915	de									
Genotype*Bloc	36.00	836.80	23.25	0.86	0.71	S13T300945001	30	0.450	0.073	bc	13.733	1.204	bcd									
Residuals	543.00	14741.80	27.15			S13T301295001	30	0.650	0.073	bc	13.183	1.323	c									
						S13T301265001	30	0.733	0.067	ab	14.683	1.164	abc									
						S13T300835001	30	0.817	0.061	ab	16.800	0.981	abcd									
						S13T300995001	30	0.817	0.066	ab	16.400	1.057	abc									
						S13T300605001	30	0.850	0.054	ab	15.950	0.945	abcd									
						S13T301015001	30	0.850	0.054	ab	16.750	0.917	abc									
						S13T301165001	30	0.883	0.057	ab	17.650	1.028	abc									
						S13T300555001	30	0.900	0.044	ab	17.883	0.760	ab									
						S13T300585001	30	0.900	0.050	ab	18.533	0.795	a									
						DKPP	60	0.908	0.035	ab	18.750	0.672	a									
						S13T301045001	30	0.917	0.042	ab	18.383	0.568	ab									
						S13T301095001	30	0.917	0.048	ab	18.750	0.951	a									
						S13T301185001	30	0.917	0.048	ab	18.717	0.977	a									
						S13T301325001	30	0.917	0.035	ab	17.217	0.689	abcd									
						S13T300915001	30	0.933	0.046	a	18.317	0.804	ab									
						S13T301275001	30	0.933	0.040	a	19.150	0.495	a									

Table 8 : résultats pour 7dpi and AUDPC sans les témoins pour l'expérimentation 2: ANOVA, Duncan test

Table 9 : Test de Dunnett pour 7dpi et l'AUDPC pour l'expérimentation 2

7dpi						AUDPC						7dpi						AUDPC						
Genotype	Df	Sum Sq	Mean Sq	F value	Pr(>F)	PEDIGREE	N	Mean	St. err	grp	Mean	St. err	grp	Mean	St. err	grp	Mean	St. err	grp	Mean	St. err	grp		
Genotype	15.00	4.01	0.27	2.92	0.00	S13T300945001	30	0.650	0.073	b	13.733	1.204	bc											
Bloc	2.00	0.03	0.02	0.18	0.84	S13T301295001	30	0.650	0.073	b	13.183	1.323	c											
Genotype*Bloc	30.00	2.28	0.08	0.83	0.72	S13T301265001	30	0.733	0.067	ab	14.683	1.164	abc											
Residuals	432.00	39.48	0.09			S13T300835001	30	0.817	0.061	ab	16.800	0.981	abcd											
						S13T300995001	30	0.817	0.066	ab	16.400	1.057	abc											
						S13T300605001	30	0.850	0.054	ab	15.950	0.945	abcd											
						S13T301015001	30	0.850	0.054	ab	16.750	0.917	abc											
						S13T301165001	30	0.883	0.057	ab	17.650	1.028	abc											
						S13T300555001	30	0.900	0.044	ab	17.883	0.760	ab											
						S13T300585001	30	0.900	0.050	ab	18.533	0.795	a											
						S13T301045001	30	0.917	0.042	ab	18.383	0.568	a											
						S13T301095001	30	0.917	0.048	ab	18.750	0.951	a											
						S13T301185001	30	0.917	0.048	ab	18.717	0.977	a											
						S13T301325001	30	0.917	0.035	ab	17.217	0.689	abcd											
						S13T300915001	30	0.933	0.046	a	18.317	0.804	ab											
						S13T301275001	30	0.933	0.040	a	19.150	0.495	a											

Table 10 : Comparaison des résultats des familles au génotypes pour l'expérimentation 2

Genotype	FC607	DKPP	S13T300945001	S13T301295001	S13T301265001	S13T300835001	S13T300995001	S13T300605001	S13T301015001	S13T301165001	S13T300555001	S13T300585001	S13T301045001	S13T301095001	S13T301185001	S13T301325001	S13T300915001	S13T301275001
FC607	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
DKPP	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
S13T300945001	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
S13T301295001	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
S13T301265001	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
S13T300835001	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
S13T300995001	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
S13T300605001	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
S13T301015001	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
S13T301165001	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
S13T300555001	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
S13T300585001	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
S13T301045001	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
S13T301095001	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
S13T301185001	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
S13T301325001	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
S13T300915001	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93
S13T301275001	0.30	0.60	0.45	0.65	0.73	0.82	0.82	0.85	0.85	0.88	0.90	0.90	0.92	0.92	0.92	0.92	0.93	0.93

Table 11 : Résultats du test de Kruskal-Wallis pour l'expérimentation 2

Value	Chr	Markers	cM	K-Value max
	C1	A046_S02 to A062_S01	34 to 50	5.856
	C4	D156_S01 to D075_S01	-2 to 0	3.119
7dpi	C4	D201_S01 to D093_S02	30 to 36	6.706
	C1	A046_S02 to A062_S01	34 to 50	5.984
AUDPC	C4	D201_S01 to D093_S02	30 to 36	6.523

b. Distribution des individus

Une répartition continue du score à 7dpi et de l'AUDPC est observée, cela correspond avec le fait que la résistance est quantitative. La répartition n'est pas normale, mais aucune transformation n'a permis de la normaliser. (Figure 17)

c. Comparaison de moyennes

Avec les groupes formés par le test de Duncan, il est visible que seuls les génotypes S13T30129S001 et S13T30094S001 sont significativement différents du témoin sensible DKPP (à 5%) (Tableau 7). Cela est confirmé par l'analyse sans les témoins (Tableau 8). Le test de Dunnett (Tableau 9) montre qu'aucun génotype n'est comparable au témoin résistant, mais avec le score à 7dpi, deux génotypes (les deux cités précédemment) sont significativement moins contaminés que le témoin sensible. Avec l'AUDPC, en plus de ces deux génotypes, le génotype S13T30126S001 est aussi moins contaminé, mais de nouveau, ces génotypes sont significativement différents du témoin résistant.

d. Héritabilité

Comme pour l'essai précédent, l'héritabilité est plutôt haute pour le score à 7dpi et l'AUDPC. Pour l'AUDPC, $h^2=0.73$ et pour 7dpi, $h^2=0.66$. Les valeurs sont aussi dans la même gamme d'héritabilité que celles trouvées dans l'essai précédent.

2. Analyse génétique

a. Comparaison des résultats des familles

On voit sur le tableau 10 que les familles avec le plus grand nombre d'individus semblant être résistants (A) semblent être aussi plus résistantes en général (par exemple S13T30094S001, S13T30129S001 ou S13T30126S001). Ces familles ont en général plus de marqueurs avec le génotype provenant de F607, spécialement sur le chromosome 1.

b. Test de Kruskal-Wallis

Ce test détecte des effets significatifs. Pour le score à 7dpi, un QTL est détecté sur le chromosome 1 et deux sur le chromosome 4. Pour l'AUDPC, deux QTL sont détectés, sur les chromosomes 1 et 4. (Table 11) Pour les deux scores, rien n'est détecté sur le chromosome 6.

Comme expliqué précédemment, ce test n'est qu'un aperçu et n'est pas le plus fiable.

c. Cartographie d'intervalles

D'abord nous avons fait l'étude sur les familles, avec l'utilisation des génotypes parentaux.

Le seuil de significativité du LOD a été calculé par un test de permutation avec 1000 itérations. Le résultat est un LOD de 2.8 pour l'AUDPC et de 3 pour le score à 7dpi. Ces LOD seuils semblent cohérent (un LOD score classique est en général de 3).

La cartographie d'intervalles, qui est plus puissante que le test de Kruskal-Wallis, montre seulement un QTL significatif sur le chromosome 1 avec un LOD de 4.43 à 53cM. Ce QTL est significatif entre 36 et 58 cM et a A046_S02 et A068_S01 pour marqueurs flanquants. Il est seulement significatif pour le score à 7dpi. Ce QTL explique 71% de la variance phénotypique (Figure 18). Pour l'AUDPC, un pic est détecté, mais il n'est pas significatif.

L'analyse a ensuite été faite sur les individus échantillonnés (pour lesquelles, des feuilles ont pu être prélevées). Seuls les chromosomes 4 et 6 ont été génotypés.

Le LOD seuil est 1.8 pour 7 dpi et 1.9 pour l'AUDPC.

Ici, un QTL significatif est situé sur le chromosome 6 (Figure 19). La plus haute valeur de LOD est 2.28 au marqueur F107_S01 pour l'AUDPC. En observant les génotypes (Tableau 12), c'est l'allèle B qui semble apporter la résistance. Cela confirme l'analyse précédente

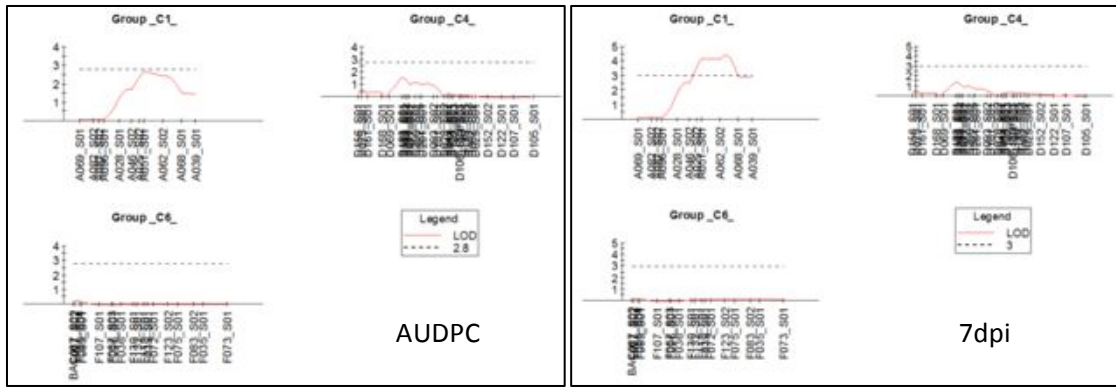


Figure 18 : résultats de la cartographie d'intervalle pour l'expérimentation 2 avec les valeurs des familles et le génotype parental

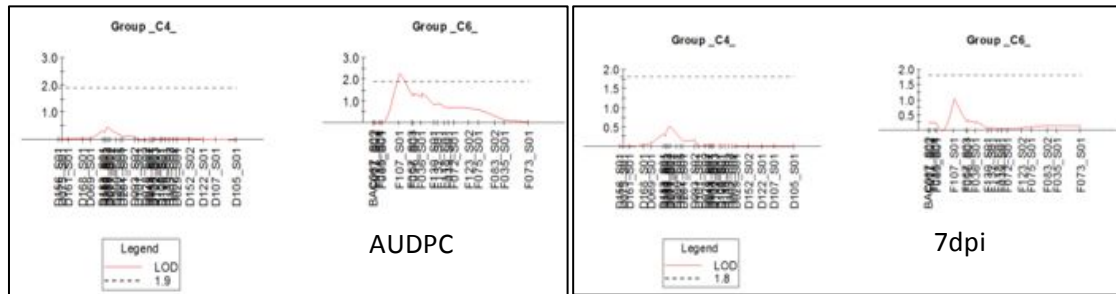


Figure 19 : résultats de la cartographie d'intervalle pour l'expérimentation 2 avec les individus échantillonnés

Table 12 : Moyenne en fonction du génotype au marqueur F107_S01

Values	A	B	H	Total
Mean AUDPC	17.17	12.36	15.31	15.32
Mean 7dpi	0.83	0.61	0.75	0.76
Nb for F107_S01	18	18	40	142

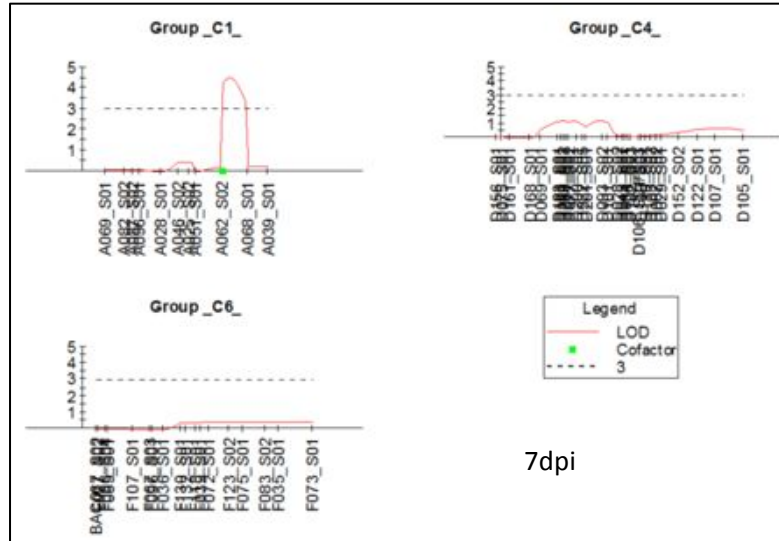


Figure 20 : Cartographie multiple de QTL pour l'expérimentation 2 avec A062_S01 comme cofacteur

Table 13 : Moyenne en fonction du génotype au marqueur A025_S02

Values	A	B	H	Total
Mean AUDPC	13.46	17.98	17.43	17.01
Mean 7dpi	0.65	0.90	0.87	0.85
Nb for A025_S02	2	7	3	21

avec les familles qui indiquait que les allèles provenant de FC607 n'apportaient pas de résistance sur le chromosome 6.

d. Cartographie multiple de QTL

Comme il est observable sur la figure 18, le QTL détecté sur le chromosome 1 n'est pas précisément localisé. Pour améliorer la précision de sa localisation, le marqueur A062_S01 (50 cM) a été indiqué comme cofacteur car c'est le plus proche marqueur de la position du QTL obtenue par cartographie d'intervalles (Figure 20). Maintenant le QTL est significatif entre 50 et 58 cM avec un maximum à 52 cM. Ce QTL explique 72% de la variance phénotypique. Les individus portant l'allèle de FC607 (A) sont plus résistants que ceux avec l'allèle de DKPP (B) (Table 13).

Table 14 : comparaison de l'essai de 2012 avec l'expérimentation 1 (2013)

Genotype	Family	N	Grp	2013		2012			Disease Index
				7dpi mean	AUDPC mean	GPS_A	GPS_H	GPS_B	
S11T270345001	S10D4615^500^044.	30	A	0.633	11.930	6.00	4.54	4.17	0.440
S11T270105001	S10D4615^708^020.	29	C	0.638	11.080	8.50	8.50	7.33	0.160
S11T270055001	S10D4615^706^015.	30	B	0.650	11.500	8.65	7.52	7.69	0.125
S11T270375001	S10D4615^500^047.	30	A	0.667	11.280	7.55	7.70	5.16	0.463
S11T270005001	S10D4615^706^010.	30	B	0.667	11.480	6.40	6.29	4.83	0.325
S11T269995001	S10D4615^706^009.	30	B	0.667	11.540	7.88	8.10	7.53	0.046
S11T270075001	S10D4615^706^017.	30	B	0.683	11.960	6.85	6.90	5.62	0.217
S11T270015001	S10D4615^706^011.	30	B	0.717	11.830	8.51	7.85	6.45	0.319
S11T269975001	S10D4615^706^007.	29	B	0.741	11.770	8.03	7.49	5.55	0.448
S11T270385001	S10D4615^500^048.	30	A	0.750	12.220	8.60	7.69	4.98	0.728
S11T270185001	S10D4615^708^028.	20	C	0.800	12.720	7.95	7.70	7.31	0.086
S11T270095001	S10D4615^706^019.	30	B	0.833	12.570	8.50	8.31	7.79	0.090
S11T269985001	S10D4615^706^008.	30	B	0.833	12.880	7.49	7.52	5.14	0.459
S11T269945001	S10D4615^303^004.	30	C	0.850	12.540	7.13	6.78	5.79	0.230
S11T270155001	S10D4615^708^025.	10	C	0.850	12.820	8.32	7.68	7.98	0.043

Discussion

A. Development du bioessai

a. Une maladie encore mal comprise

Aphanomyces cochlioides est une maladie récemment découverte par Drechsler (1929). Comme il l'a été discuté, elle est toujours mal connue et semble dépendre énormément des conditions environnementales. Pour cela, il n'est pas sûr que les résultats obtenus dans le bioessai soient répétables.

b. Le système des tubes : un essai qui doit encore être amélioré

Le système de tubes avec du sable a donc été choisi à la fin de la mise au point du bioessai. L'avantage de ce bioessai est la rapidité de résultats (environ 38 jours) du fait de l'usage de plantules et de la facilité de notation.

Cependant, il est important de rappeler que le sable n'est pas un bon rétenteur d'eau. Une irrigation bonne et stable doit être maintenue au cours de l'essai. Cela spécialement au cours de la période d'inoculation car *Aphanomyces cochlioides* a besoin d'eau pour se propager. L'homogénéité d'irrigation peut être un problème dans la répétabilité de l'essai.

Sachant que l'environnement a un effet important sur la contamination par *Aphanomyces cochlioides* il est quasiment sûr qu'il a un effet sur la détection de QTL (Kover, et al., 2001). Il pourrait être intéressant d'essayer une plus large gamme de conditions environnementales pour optimiser l'inoculation et la croissance de la plante. Plus de situations pourraient être testées, comme les conditions de pH, d'autres substrats... En fait, les résultats des bioessais montrent que malgré une ségrégation significative des contrôles ($p < 0.01$), les autres génotypes ne ségrégent pas si clairement. Il pourrait être intéressant d'essayer le bioessai avec d'autres témoins résistants et sensibles pour voir comment ceux-ci se comportent.

La détection de QTL dépend aussi de la taille de la population, du fond génétique et de l'environnement. Cette étude a été limitée par le nombre de plantes qui ont pu être traitées dans l'essai, mais la précision de détection peut être affinée en augmentant le nombre de génotypes utilisés et la taille de la population.

La détection de QTL a été faite en mesurant l'évolution de la maladie dans le temps. Il est possible de trouver d'autres moyens de mesurer la maladie.

B. Expérimentation 1

Lorsque l'on compare cet essai à celui réalisé en 2012, les résultats ne sont pas corrélés. Un individu qui était plus résistant en 2012 n'est pas forcément plus résistant ici. L'individu S11T27038S001 est un bon exemple. (Table 14)

Plusieurs raisons peuvent être suggérées pour justifier cette différence. Le nouveau bioessai ne mesure peut être pas les mêmes paramètres que ceux mesurés en 2012, en effet, les plantes sont à des stades de croissance différents (plantules et plantes adultes). Prioul et al. (2004) ont montré dans une étude sur la résistance à *Mycosphaerella pinodes* chez le pois que les QTL étaient différents au stade plantule et au stade adulte.

D'autres raisons peuvent être suggérées : la concentration d'inoculation est peut-être trop élevée pour des plantes ayant une croissance limitée dans le sable. Le nouveau bioessai n'est peut-être pas assez discriminant et n'est pas capable de détecter des petites variations du niveau de résistance. Il donnerait peut être de meilleurs résultats avec des individus au génotype homozygote. Le QTL détecté sur le chromosome 4 pouvait être soumis à un effet d'épistasie qui aurait disparu ou encore les QTL ont peut-être été perdus au cours du processus de backcross.

Le fait que la population testée ne suit pas la loi de Hardy-Weinberg ne rend pas l'analyse plus facile et peut aboutir à une analyse biaisée.

Ce qui est encourageant ici est que parmi les 4 familles ayant une majorité de marqueurs provenant de DKPP (source sensible), 3 se comportent de manière sensible (à la différence de l'essai de 2012 où 3 parmi les 4 sensibles étaient moins contaminés). Il peut être intéressant d'étudier plus la famille S10D4615^708^020 (ici le génotype S11T27010S001) car, malgré le fait qu'elle appartient à une famille avec une majorité de marqueurs ayant le génotype de DKPP, elle se comporte de façon résistante dans les deux expérimentations.

C. Expérimentation 2

Ici, un QTL est détecté seulement sur le chromosome 1. Cela montre que l'hypothèse de départ supposant que le QTL était sur le chromosome 4 est remise en question. Cela implique que le choix des familles pour le test n'est pas optimal pour étudier la résistance puisque les familles ont été choisies pour avoir le maximum de recombinaisons sur le chromosome 4. Par chance, le chromosome 1 possède aussi un bon panel de recombinaisons qui a permis de placer ce QTL. Cependant, ce dernier doit encore être validé par d'autres essais.

Ce résultat est intéressant car le QTL détecté auparavant par SESVANDERHAVE sur le chromosome 4 a été trouvé dans des plantes "type O". Ce QTL a été introgressé dans la pollinisateur élite DKPP. Avec les résultats de la présente étude, on suppose que le QTL trouvé sur le chromosome 4 du "type O" n'apparaît pas chez DKPP, alors que le QTL sur le chromosome 1 s'exprime plus chez DKPP. Bouchez et al. (2002) ont observé des digressions dans la prédiction de QTL après l'introggression dans les variétés élites. Ils concluent que cela était dû à des interactions génotype environnement qui n'ont pas été prises en compte. Dans une autre étude réalisée au sujet de l'effet du fond génétique et de l'environnement sur les QTL et l'épistasie pour le nombre de panicules chez le riz, une autre équipe a conclu que les effets du fond génétique sur les QTL étaient encore plus importants que ceux de l'environnement (Liao, et al., 2001). Cela montre bien la relativité des QTL.

Pour l'utilisation de cette résistance, les sélectionneurs ont été confrontés au problème de la résistance à la Rhizomanie qui est située au même endroit, ou très proche sur le chromosome 4. La résistance à la rhizomanie est récessive et la plante a besoin d'un allèle sur chaque chromosome pour une meilleure expression. Même si elles n'étaient pas au même locus, les deux résistances seraient très proches et il sera très difficile de trouver une plante avec un événement de recombinaison à cet emplacement. Mais si la résistance à *Aphanomyces cochlíoides* ne se trouve pas sur le chromosome 4 chez le pollinisateur, cela simplifiera l'introggression de la résistance à *Aphanomyces cochlíoides* dans les variétés. A l'échelle de la plante, plusieurs facteurs génétiques de résistance doivent être combinés, mais il y a toujours un risque d'émergence de "super races" de l'élément pathogène qui éviteraient tous les gènes de résistance (Finckh, 2008).

Ici on trouve aussi un QTL sur le chromosome 6 avec l'analyse utilisant les individus échantillonnés, mais c'est DKPP qui semble apporter la résistance. Toojinda, et al. (1998) ont étudiés l'introggression d'un QTL de résistance à la rouille de l'orge dans un fond génétique non lié au QTL. Ils ont détecté de nouveaux QTL de résistance, dont certains étaient apportés par le parent susceptible. Ils ont émis l'hypothèse que l'allèle favorable était fixé à ces QTL dans la population de cartographie. Ils concluent que le fond génétique pouvait avoir un rôle important dans les processus de transferts de QTL.

Conclusion

Les objectifs du présent travail étaient d'étudier le contrôle génétique d'*Aphanomyces cochlioides* en incluant le développement d'un biotest fiable.

Nous avons essayé de trouver des QTL pour la résistance à *Aphanomyces cochlioides* ayant été transférés d'une lignée très résistante, FC607, dans une lignée pollinisatrice élite, DKPP. Le matériel étudié était des lignées sélectionnées F1Bc2S1 et F1Bc2S2.

L'hypothèse nulle était la présence d'un QTL majeur responsable de la résistance à la maladie s'exprimant sur le chromosome 4, entre 20 et 50 cM. Cette hypothèse a été développée à partir de programmes utilisant la même source de résistance, mais introduite dans des lignes appartenant au groupe hétérotique femelle. Les lignées sélectionnées pour cette étude ont été choisies sur la base de la présence de zones spécifiques à FC607 sur le chromosome 4.

Dans ce matériel, la présence d'un QTL a précédemment été indiquée sur le chromosome 4, expliquant une part limitée de la résistance. Ce résultat a été obtenu grâce à un essai évaluant l'inhibition de la croissance comme expression de la résistance à *Aphanomyces cochlioides*. La faiblesse de cette méthode est la confusion possible entre le QTL de la croissance racinaire et le QTL de résistance à la maladie.

Pour cette raison, nous avons décidé de développer un bioessai basé sur l'expression des symptômes de la maladie. Le développement du bioessai est un succès. Il permet de différencier de façon consistante l'intensité des symptômes des lignées parentales résistantes et sensibles.

Nous n'avons pas pu confirmer la présence d'un seul QTL majeur responsable de la résistance à *Aphanomyces cochlioides* dans cette population, dans la région 20 à 50 cM. Cela montre l'importance du fond génétique car il peut changer de façon significative l'expression des QTL.

Nous avons découvert un QTL de résistance à *Aphanomyces cochlioides* provenant du parent sensible sur le chromosome 6. Est-ce un QTL qui s'exprime plus tardivement dans le processus d'infection ?

En dehors des chromosomes 4 et 6, une ségrégation était présente sur le chromosome 1 et permet de détecter un QTL. S'il nécessite encore d'être validé, c'est quelque chose qu'il pourrait être intéressant d'étudier pour un usage futur dans les programmes de sélection.

Trouver des QTL est la stratégie maintenant la plus utilisée dans les entreprises de sélection du fait de la facilité et rapidité d'utilisation. Bien que sélectionner des variétés avec des résistances quantitatives semble être plus durable, l'introgression de QTL multiples n'est pas si simple. Il est aussi intéressant de rappeler que la durabilité de la résistance implique la combinaison de plusieurs approches à différents niveaux, dans l'espace, dans le temps, en génétique et en agronomie.

Cette étude montre aussi qu'il y a toujours beaucoup à apprendre sur *Aphanomyces cochlioides*. Connaître mieux ce pathogène, d'un point de vue biologique et génétique pourrait aider à son contrôle.

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Titre français : Etude du contrôle génétique de la résistance partielle à *Aphanomyces cochlioides* (Drechs.) chez la Betterave sucrière (*Beta vulgaris* L.)

Titre anglais : Studying genetic control of partial resistance to *Aphanomyces cochlioides* (Drechs.) in Sugar-beet (*Beta vulgaris* L.)

Résumé :

Aphanomyces cochlioides est un champignon pathogène critique dans les cultures de betterave à sucre. Il cause de nombreux dommages et des pertes de rendement aux Etats-Unis, en Chine et de plus en plus dans l'est de l'Europe. C'est une maladie très imprévisible. La lutte contre ce champignon est donc un enjeu important et la sélection pour la résistance est un moyen de lutte en développement. Pour connaître mieux le contrôle génétique de la résistance, une analyse QTL de liaison a été réalisée. Les essais précédents menés par SESVanderHave ont été utilisés comme prérequis, des QTLs avaient été trouvés sur les chromosomes 4 et 6. Cependant la technique de phénotypage n'était pas très adaptée et il a fallu d'abord développer un bioessai avant l'analyse QTL. Dans le travail réalisé ici, malgré une bonne discrimination entre témoins, la comparaison des résultats obtenus avec la technique utilisée conventionnellement n'indique aucune corrélation. L'essai de cartographie fine montre quant à lui un QTL sur le chromosome 1. Ces résultats montrent un intérêt pour l'utilisation de la résistance en sélection.

Abstract:

Aphanomyces cochlioides is a fungus whose incidence is rapidly increasing in sugar beet crops. It causes root damage and yield loss in the USA, China and increasingly in eastern Europe. This is a very unpredictable disease. Trying to control it is an important issue and breeding for resistance is the most probable route to a solution. To learn more about the genetic control of the disease, a linkage analysis of QTL was performed. Previous assays made by SESVanderHave were used as a starting point and QTLs were found on chromosomes 4 and 6. However the phenotyping techniques were not well adapted and the development of a suitable bioassay had to be made prior to the QTL analysis. In the present work, new methods were developed but the results showed no correlation with those of the conventional technique, despite a good discrimination of the controls. The fine mapping assay detected a QTL on chromosome 1. These results are of an interest to use in resistance breeding in sugar beet.

Mots-clés : Betterave à sucre, *Aphanomyces cochlioides*, champignon, résistance, analyse de liaison

Key words: Sugar beet, *Aphanomyces cochlioides*, fungus, resistance, linkage mapping