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GENETIC AND PHENOTYPIC CHARACHERISATION OF YELLOW RUST RESISTANCE IN THE WHEAT CULTIVAR ALCEDO

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ABSTRACT

Yellow rust resistance in the German winter wheat cv. Alcedo has been described as durable, the resistance having remained effective when grown extensively in Germany and Eastern Europe between 1975 and 1989. Genetic characterisation of field resistance in a cross between Alcedo and the yellow rust susceptible UK winter wheat cv. Brigadier identified two major QTL in Alcedo located on the long arms of chromosomes 2D (*QPst.jic-2D*) and 4B (*QPst.jic-4B*). Yellow rust resistance was evaluated by measuring the extent of fungal growth, Percentage infection (Pi) and the necrotic/chlorotic response of the plant to infection, Infection Type (IT). Both *QPst.jic-2D* and *QPst.jic-4B* contributed significantly to the reduction in yellow rust infection (Pi) and were associated with a low, necrotic IT phenotypic score. In addition, two small effect QTL for field yellow rust resistance were identified in Brigadier, *QPst.jic-1B* on the long arm of chromosome 1B and *QPst.jic-5A* on the short arm of chromosome 5A. The influence of *QPst.jic-1B* was primarily seen with the Pi phenotype. *QPst.jic-5A* was only detected using an approximate multiple-QTL model and selecting markers linked to the major effect QTL, *QPst.jic-2D* and *QPst.jic-4B* as co-factors. Seedling yellow rust resistance was also mapped in the cross, which confirmed the location of *Yr17* from Brigadier to the short arm of chromosome 2A. A seedling expressed QTL was also located in Alcedo that mapped to the same location as the field yellow rust resistance *QPst.jic-2D*.

INTRODUCTION

Modern cultivated wheat is believed to have evolved close to the Fertile Crescent in the Transcaucasia corridor (Dvorak *et al.,* 1998). A chance hybridisation between diploid *Aegilops tauschii* and tetraploid *Triticum turidum* resulted in the production of the hexaploid wheat *T. aestivum* (McFadden and Sears 1947). Due to its ability to grow in a diverse range of environments it is now cultivated across the globe, most successfully at the latitudes 30^{o} N to 60^{o} N and 27^{o} S to 40^{o} S (Nuttonson 1955) although it can stretch from within the Arctic Circle to near the equator at high elevations (Curtis 2002).

In the current economic climate of high food and fluctuating fuel prices food security is a significant issue. Wheat provides a greater calorific intake per tonnage than any other cereal for both human and animal consumption (McIntosh *et al.,* 1995). In February 2008 wheat stocks fell to their lowest levels in more than 50 years due to bad harvests and reduced planting area (Leake 2008). It is therefore increasingly important to ensure the maximum possible yields of future crops in order to increase the reducing global wheat stocks.

After extreme environmental conditions, plant pathogens are the most important factors affecting wheat yields. Wheat is subject to attack from a wide spectrum of fungi, bacteria, viruses, nematodes and insect pests. Possibly the most important of the pathogens are fungi, which are responsible for a large range of diseases of wheat, including the three rust diseases stem rust, leaf rust and yellow rust caused by *Puccinia* spp.

The rust fungi are basidiomycete, biotrophic pathogens. The three main rust diseases of wheat; stem or black rust caused by *P. graminis*, leaf or brown rust caused by *P. triticina* and yellow or stripe rust caused by *P. striiformis* are a major problem for wheat growers around the world, each rust having a specific geographical distribution. Yellow rust is most prevalent in cooler, wetter environments. Across the globe yellow rust is found in Northern Europe (Johnson *et al.,* 2000; Hovmoller *et al.,* 2002),

Eastern and Southern Africa (Pretorius *et al.,* 1997), the Middle East (Torabi and Nazari 1998), the Indian sub-continent and China (Brown and Hovmøller 2002), Australia (Wellings *et al.,* 2002), New Zealand (Beresford 1982), the Andean region of South America (Roelfs *et al.,* 1992) and the west and central states of the USA (Line 2002).

Yellow Rust

P. striiformis is spread by the dispersion of a specialised spore structure known as a urediniospore. These are able to travel vast distances, with evidence of viable urediniospores being dispersed over 2000 miles (Staples 2000). Upon landing on host green tissue a urediniospore will germinate within 12 hours (Mares and Cousen 1977). The germ tube will grow perpendicular to the venation of the leaf (Hu and Rijkenberg 1998). When the germ tube locates a stomata it grows between the guard cells and into the sub-stomatal cavity (Mares and Cousen 1977; Garrood 2001). With *P. striiformis* the germ tube tip grows directly into the sub-stomatal cavity. On reaching the sub-stomatal cavity the infection tip swells to form a sub-stomatal vesicle (SSV).

For *P. striiformis* these stages are observed as early as 12 hours after inoculation (hpi) (Mares and Cousen 1977; Moldenhauer *et al.,* 2006). From the SSV two to three infection hyphae (IH) usually develop. When the IH encounter a plant mesophyll cell a septum is formed (Mares and Cousen 1977; Moldenhauer *et al.,* 2006). The septum separates the hyphae from the haustorium mother cell (HMC) that develops at the hyphal tip. A penetration peg develops from the HMC which pierces the cell wall of the mesophyll cell. Once across the cell wall the penetration peg swells and forms a haustorium around which the plant cell plasma membrane invaginates to produce the extrahaustorial membrane. This ensures that the haustorium never becomes truly intracellular. Haustoria formation has been observed from between 36 and 48 hpi (Mares and Cousen 1977; Garrood 2001)

With the successful establishment of haustoria, runner hyphae (RH) develop intercellularly throughout the host, producing further haustoria.

RH can be seen as early as 3 to 4 days post inoculation (dpi; Mares and Cousen 1977; Moldenhauer *et al.,* 2006). After 11 to 14 days hyphae ramify and fuse, followed by the emergence of urediniospore initials and pedicels bearing urediniospores from the leaf. A cluster of upedicels will then form a uredinium seen as the characteristic yellow lesions on the host plant.

Durable resistance

Due to the possible environmental impact of pesticides and the increasing legislation to reduce the numbers of pesticides available to growers, the most desirable way to protect wheat crops from yellow rust infection is through the use of resistant varieties. Genetic resistance to yellow rust in wheat was identified as early as the turn of the 20th Century, when Biffin (1905) identified a resistance gene in the variety Rivet. Since then resistance has been incorporated into new varieties through conventional breeding. While this proved relatively effective at controlling outbreaks of the disease it was noticed that over time some varieties, that had good levels of resistance, would often become susceptible (Johnson 1983). Joss Cambier was a wheat variety released in 1966 that had adequate levels of resistance until an epidemic of the disease in 1971 and 1972 (Walker and Roberts 1974). In contrast some varieties were grown extensively for many years without an increase in disease susceptibility, including Cappelle Desprez (Johnson 1983), Camp Remy (Boukhatem *et al.,* 2002) and Alcedo (Meinel 1997).

The term 'Durable Resistance' was coined and describes resistance that has remained effective in a variety during its widespread cultivation, over a long period of time, in an environment favourable to the disease (Johnson and Law 1973). An aim for breeders was therefore to incorporate durable yellow rust resistance into new varieties through breeding.

Seedling resistance

Seedling resistance genes have been a common source of yellow rust resistance used by wheat breeders. Seedling resistance genes usually

confer a complete resistant phenotype that is effective throughout the development of the plant and so the phenotype is easily selected for in breeding programs. The genetic basis for seedling resistance was described by the Gene-for-Gene hypothesis (Flor 1956; Flor 1971). Flor observed that resistance in the plant was only affective against a sub-set of pathogen isolates. The Gene-for-Gene hypothesis proposes that a host resistance gene (*R*-gene) is only effective against an isolate carrying the corresponding avirulence gene (*avr*), If either the *R*-gene or the corresponding *avr* gene are absent then a compatible interaction results. Mutation or loss of a redundant *avr* gene in the pathogen can then lead to loss of effective resistance in the host. A recent example includes the breakdown of *Yr17* in resistant varieties such as Brigadier, which became ineffective in 1997 after only a few years of cultivation.

Adult plant resistance

Due to the short effective life of many seedling resistance genes breeders have started to source other forms of resistance. Adult plant resistance (APR) is a term used to describe resistance that becomes effective at growth stages beyond the seedling stage. Resistance to disease that is dependant on the developmental stage of the plant has been described in many plant species including wheat, barley, rice, oats, maize, tobacco and Arabidopsis (Panter and Jones 2002). Cultivars which have shown good levels of resistance to yellow rust in the past, such as Cappelle-Desprez (Johnson 1983), Camp Remy (Boukhatem *et al*., 2002) and Alcedo (Meinel 1997) have been found to possess APR to yellow rust, while the APR genes *Yr18* and *Yr29,* which have been used extensively in CIMMYT spring wheat cultivars have remained effective. It is believed that many of these APR may have potential for producing varieties with durable resistance.

Unfortunately APR is often conferred by multiple genes (Singh *et al.,* 2000; Boukhatem *et al.,* 2002; William *et al.,* 2002.; Mallard *et al.,* 2005) and its effectiveness can be influenced by environmental conditions (Qayoum and Line 1985) making it both difficult to study and to incorporate into new varieties. The few studies that have been carried out

on APR have shown that single APR genes often produce only a partial resistance. By combining multiple APR genes however it is possible to achieve an acceptable level of resistance. The rapid cell death response observed with seedling resistance is usually absent and the APR is associated more with a reduced development of the yellow rust fungus within the leaf.

Because APR is often conferred by multiple genes Quantitative Trait Loci (QTL) mapping has been used in combination with Marker Assisted Selection in order to develop varieties with effective APR. QTL mapping allows a phenotype (in this case APR) displaying a continuous distribution in a population to be split into the components (QTL) which are responsible for the phenotype. This is distinct to when a single gene is responsible for a phenotype and the phenotypes will fall into discreet classes. QTL mapping was made possible following the development of genetic maps made up of genetic markers. By comparing the phenotypes and the genotypes of a population the number, effect and location of the resistance QTL can be determined (Lander and Bostein 1989). Once the locations of the QTL are known the flanking markers can be used for MAS. Instead of selecting just on phenotype a genetic test makes it possible to ensure that all the QTL that contribute to the resistance are selected.

Alcedo is a winter wheat variety grown in Germany and eastern European countries between 1975 and 1989. At its peak in 1981 it was cultivated on approximately 47% of the wheat acreage in Germany. During this time the APR to yellow rust possessed by Alcedo remained effective. For this reason the APR of Alcedo has been described as durable (Meinel, 1997). The APR in Alcedo has been found to be conferred by two major QTL (Simon Berry; personal communication) located on chromosomes 2D and 4B. The work in this report describes the further examination of the position of the two QTL. The phenotype of the resistance is described at both at macroscopic and microscopic level at different wheat growth stages. Alcedo was crossed to the yellow rust susceptible variety Brigadier to produce a DH population. Brigadier achieved recommend list status in 1993, maintaining effective yellow rust resistance with the

resistance gene *Yr17* until 1997 when yellow rust isolates virulent to *Yr17* became prevalent in the UK.

GENETIC CHARACTERISATION

Alcedo Resistance

A Doubled Haploid (DH) population generated from a cross between Alcedo and Brigadier was used in field trials to assess the yellow rust resistance in Alcedo. The field trials were carried out in two years in which the DH population and the varieties Brigadier and Alcedo were exposed to yellow rust. The plants were scored for yellow rust infection at two week intervals. The reaction to yellow rust was assessed in two ways; Percentage infection (Pi) is a measure of the area of green leaf tissue that is exhibiting symptoms of yellow rust infection (Figure 1a). The Infection Type (IT) is a qualitative measure that reflects the response of the plant to infection (Figure 1b).

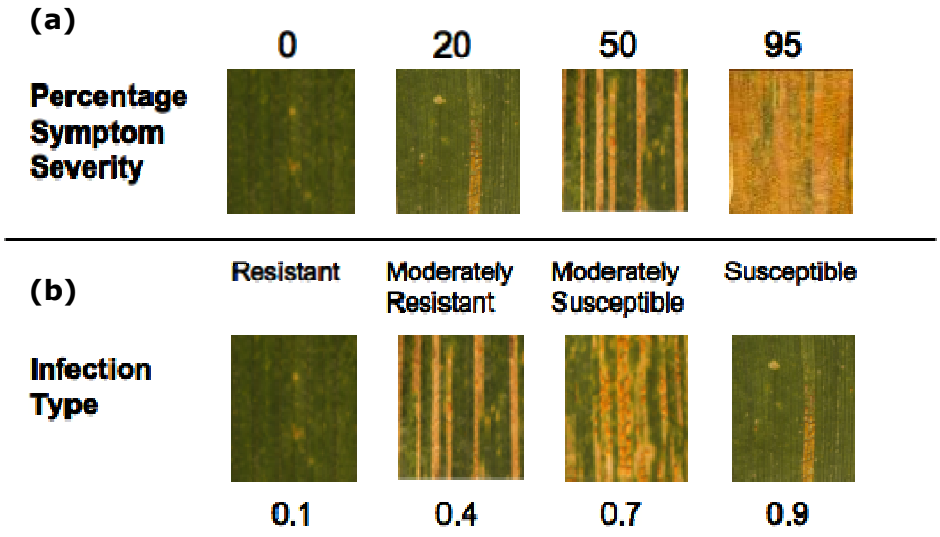


Figure 1. Examples of percentage infection and infection type phenotypes observed. (a) Percentage infection; numbers indicate the percentage of the leaf exhibiting symptoms of the disease (b) Infection type; Resistant = necrotic areas with no sporulation, Moderately resistant = necrotic areas with limited sporulation, Moderately susceptible = chlorotic areas with sporulation and Susceptible, abundant sporulation surrounded by

green plant tissue. Numbers indicate infection type nominal assigned to infection type.

Brigadier exhibited a susceptible phenotype in the field with yellow stripes of pustules (Figures 2a). Alcedo consistently gave a resistant phenotype, showing no signs of infection, exhibiting only healthy green tissue (Figure 2b). This resulted in a large difference in the amount of green tissue seen in the two varieties (Figure 2c).

Figure 2. Field phenotypes of the parents of the DH population (a) Brigadier and (b) Alcedo (c) the parents side by side in the field at a later score date. Clear differences can be seen in the green leaf tissue present on both varieties.

The DH population exhibited a continuous distribution of phenotypes with IT scores ranging from of 0.1 to 0.9 (Figure 3a) and Pi scores ranging from 0% to 100% (Figure 3b). The continuous distribution of scores indicates that multiple QTL are likely to be responsible for the Alcedo resistance. Alcedo had IT and Pi scores of 0.1 and below 10% respectively (Figure 3). Brigadier had IT scores between 0.7 and 0.8. A natural progression of the disease over time was observed in Brigadier which had Pi scores of 50% at the early score date and 80% by the later score date. While Brigadier is classed as susceptible it does not exhibit a completely susceptible phenotype with either scoring system and may therefore contain some small effect resistance QTL.

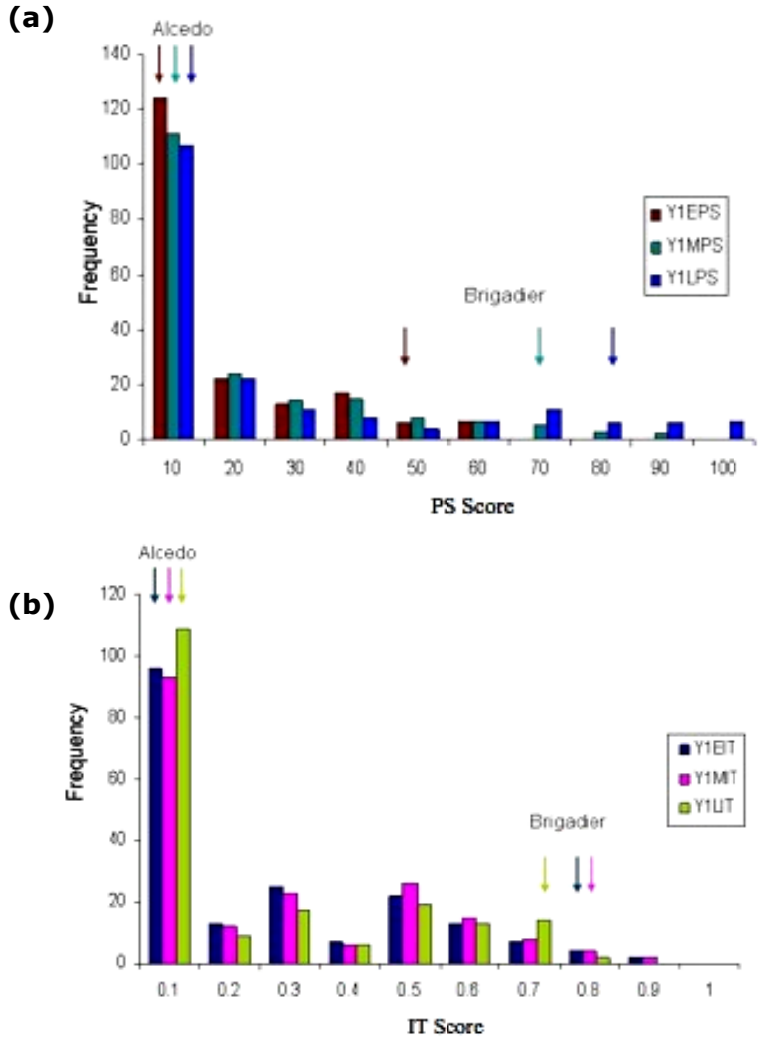


Figure 3. Frequency distributions of (a) Infection Types and (b) Percentage Symptom Severity at consecutive score dates in the first year of scoring of the Alcedo × Brigadier DH population. The parental scores

are indicated with an arrow. EIT = Infection Type at early score date, MIT = Infection Type at middle score date and LIT = Infection Type at late score date. EPS = Percentage Symptom Severity at early score date, MPS = Percentage Symptom Severity at middle score date and LPS = Percentage Symptom Severity at late score date

QTL Mapping

In order to determine how many QTL were responsible for the resistance in Alcedo a technique called QTL mapping was carried out. The technique estimates the number of QTL and the position of the QTL on the chromosomes using a genetic linkage map. The genetic marker alleles present are compared with the phenotype of each individual in order to estimate the likelihood that a marker is linked to a resistance QTL.

It was confirmed that the yellow rust resistance in Alcedo was predominantly conferred by two major QTL located on chromosomes 2D, named *QPst.jic.2D* and chromosome 4B, named *QPst.jic.4B* (Table 1). These QTL contributed to the majority of the resistance (up to 65%) in the DH population (Table 1) and were detected using both types of phenotypic scoring. Brigadier was found to possess a minor resistance QTL detected on chromosome 1B, named *QPst.jic.1B* and another on the short arm of chromosome 5A, *QPst.jic-5A* (Jagger *et al*. 2010a).

The DH lines were divided into 16 genotypes based on the four QTL detected and the mean scores calculated for each genotypic group (Figure 4). Grouping of the DH lines by QTL allowed an assessment of the yellow rust infection phenotypes of each QTL genotype. While alone both *QPst.jic-2D* and *QPst.jic-4B* conferred a high level of yellow rust resistance, when present together an additive effect was observed. The influence of *QPst.jic-1B* and *QPst.jic-5A* on yellow rust resistance was more variable. In year 1 both *QPst.jic-1B* and *QPst.jic-5A* significantly reduced Pi, while in year 2 only an additive effect of *QPst.jic-1B* with *QPst.jic-5A* resulted in significantly less infection than DH lines with no QTL. No additive effect was seen for Pi when *QPst.jic-1B* and *QPst.jic-5A* where present with either *QPst.jic-2D* or *QPst.jic-4B. QPst.jic-2D* and *QPst.jic-4B* both had a significant effect on the necrotic response of the plant. However, only *QPst.jic-5A* in year 1 was seen to influence IT

compared to the no QTL DH lines, *QPst.jic-1B* having no apparent effect on the necrotic response of the plant.

Table 1. Field expressed yellow rust resistance QTL detected in the cross Alcedo × Brigadier by interval mapping

[1]Dataset (LOD threshold)[6]	Chromosome[2]	Parent[3]	Locus[4]	LOD[5]	Expl. % Variance[7]	Phenotypic means[8]	
						Al	Br
Yr1EPi	1B	Br	*Xwmc735*	3.34	7.90	15.81	6.95
(3.2)	2D	Al	*Xgwm320*	17.08	34.20	1.22	19.37
	4B	Al	*Xwmc692*	13.76	28.60	2.82	19.42
Yr1MPi	1B	Br	*Xwmc735*	3.88	9.70	21.77	8.78
(3.1)	2D	Al	*Xgwm320*	18.36	36.20	1.33	26.04
	4B	Al	*Xwmc692*	12.04	25.50	4.38	25.17
Yr1LPi	1B	Br	*Xwmc735*	3.87	9.10	28.33	10.71
(3.3)	2D	Al	*Xgpw8086b*	14.36	31.50	1.76	33.97
	4B	Al	*Xwmc692*	13.89	28.90	3.48	34.37
Yr2EPi	1B	Br	*Xwmc735*	4.50	11.10	18.92	6.84
(3.0)	2D	Al	*Xgwm320*	14.71	30.10	1.83	21.56
	4B	Al	*Xwmc692*	11.10	23.80	3.85	21.41
Yr2LPi	1B	Br	*Xwmc735*	4.35	13.10	33.84	12.23
(2.9)	2D	Al	*Xgwm320*	18.43	36.20	3.28	38.96
	4B	Al	*Xwmc692*	12.64	26.60	7.48	38.10
Yr1EIT	2D	Al	*Xgwm320*	21.40	40.90	0.12	0.37
(3.0)	4B	Al	*Xwmc692*	9.20	20.30	0.17	0.35
Yr1MIT	2D	Al	*Xgwm320*	23.56	43.70	0.12	0.39
(3.2)	4B	Al	*Xwmc692*	9.54	20.80	0.17	0.36
Yr1LIT	1B	Br	*Xwmc735*	3.23	9.90	0.31	0.18
(3.1)	2D	Al	*Xgwm320*	18.06	36.70	0.03	0.12
	4B	Al	*Xwmc692*	10.30	22.30	0.03	0.15
Yr2EIT	2D	Al	*Xgwm301*	20.44	41.30	0.09	0.31
(3.2)	4B	Al	*Xwmc692*	9.88	22.00	0.12	0.28
Yr2LIT	2D	Al	*Xgwm301*	27.57	53.10	0.07	0.32
(3.3)	4B	Al	*Xwmc692*	7.98	17.90	0.13	0.27

[1]The early (E), middle (M) and late (L) Percentage infection (Pi) and Infection Type (IT) datasets in year 1 (Yr1) and year 2 (Yr2) analysed by interval mapping using the software MapQTL v. 5.0. [2]Chromosomal location. [3]Parent contributing QTL. [4]Marker locus associated with QTL. [5]Maximum LOD score associated with closest QTL. [6]LOD threshold based on a p-value of 0.05. [7]Percentage phenotype explained. [8]Phenotypic means of allelic classes at QTL. Al, cultivar Alcedo. Br, cultivar Brigadier.

Figure 4

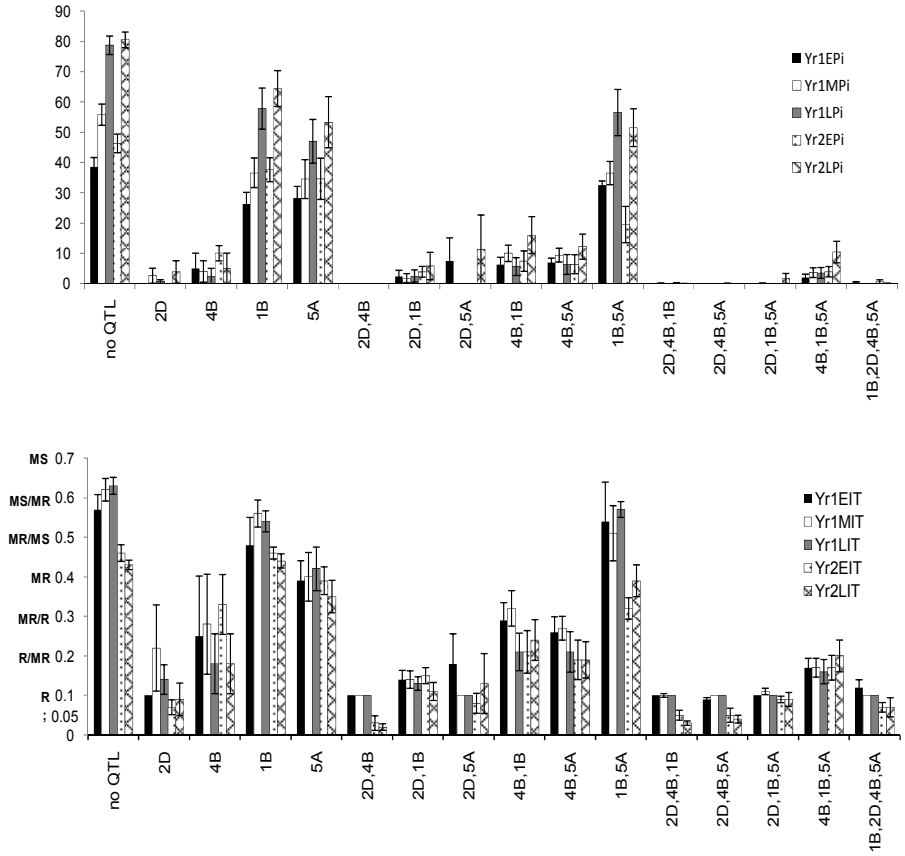


Figure 4. Mean Percentage infection and Infection Type scores for the DH lines of the Alcedo x Brigadier population divided by the four yellow rust resistance QTL identified in this cross. (a) Percentage infection (Pi) and (b) Infection Type (IT) where the Y-axis shows both the IT scores ; - fleck, R - resistant, MR - moderately resistant and MS moderately susceptible and the IT nominal scale 0 to 1.0. The X-axis identifies the QTL present; no QTL, 2D = *QPst.jic-2D*, 4B = *QPst.jic-4B*, 1B = *QPst.jic-1B* and 5A = *QPst.jic-5A*. Error bars show standard errors.

A well as defining how many QTL are responsible for the yellow rust resistance QTL mapping also allows positioning of the QTL onto a genetic map. The QTL mapping placed both *QPst.jic.2D* and *QPSt.jic.4B* at the distil end of their respective chromosomes (Figure 5a). As at the start of this project both the major resistance QTL were not flanked by a marker the precise position of the QTL could not be determined. Knowing the number and exact position of the resistance QTL that confer the yellow rust resistance in Alcedo will help breeders incorporate the resistance into

new wheat varieties. In order to narrow down the position of the QTL and increase the efficiency of marker assisted selection, additional markers were identified in the area surrounding the QTL.

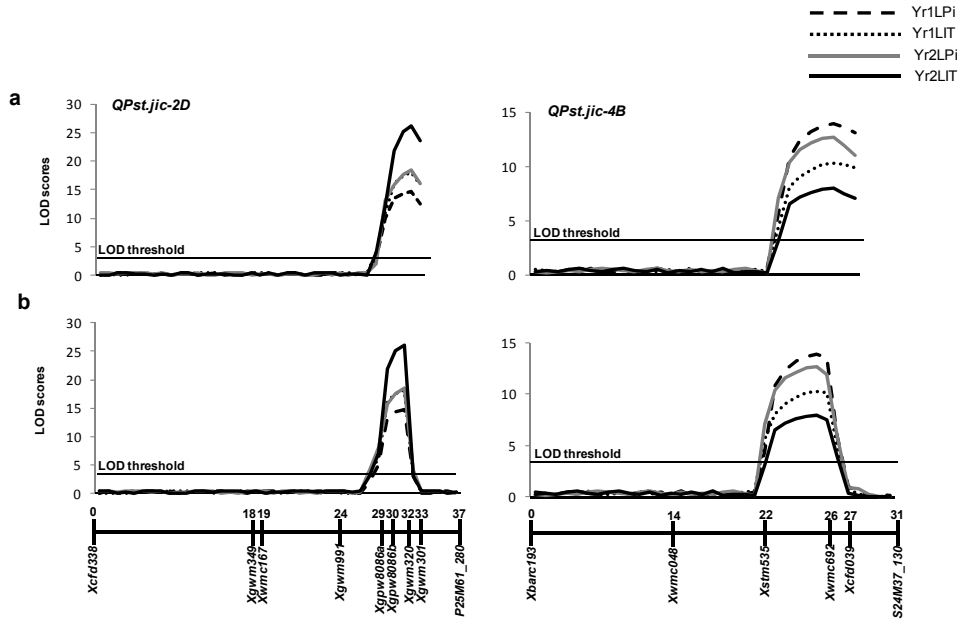


Figure 5. The QTL plots for chromosomes 2D and 4B obtained from QTL mapping using the Percentage infection (Pi) and Infection Type (IT) data sets. The corresponding linkage groups are shown with the relevant marker loci and the map distances in cM (Kosambi) for each plot. The line represents a LOD significance threshold of 3.

Marker Development

A technique known as AFLP was used in order to develop new markers in the regions of the resistance QTL *QPst.jic.2D* and *QPst.jic.4B*. By pooling the DNA of DH lines containing the 2D and 4B QTL individually it is possible to ensure that the DNA pools and any polymorphism will differ only in the regions being targeted.

Polymorphisms were found that segregated with each of the major QTL (Figure 6). An AFLP band linked to the 4B QTL is shown (Figure 6a) where a band is seen in Alcedo, the bulk containing both QTL (R) and the bulk containing just the 4B QTL, but no band is seen in Brigadier, the 2D bulk or the bulk with neither QTL. A band linked to the 2D QTL is shown (Figure 6b) with bands present in Alcedo, the bulk containing both QTL and the bulk containing just 2D.

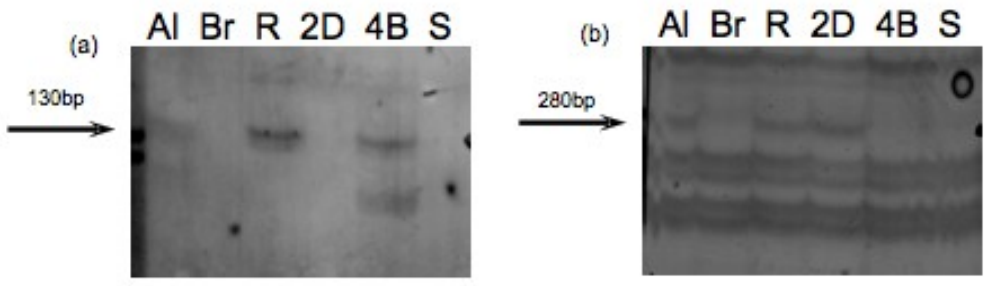


Figure 6. Band patterns observed in the AFLP analysis. a) Band linked to *QPst.jic.4B*. b) Band linked to *QPst.jic.2D*. Al = Alcedo, Br = Brigadier, R = bulk containing both major QTL, 2D = bulk containing *QPst.jic.2D*, 4B = bulk containing *QPst.jic.4B* and S = bulk containing neither of the major QTL. The arrows indicate the polymorphic band, and the size of the band.

The QTL analysis was repeated with the new AFLP markers added to the genetic linkage map (Figure 5b). The resolution of the QTL mapping was increased by including the AFLP markers such that it was found that the SSR markers already flanked both of the major QTL (Figure 5b).

The position of the two Alcedo yellow rust resistance QTL has been further defined by the AFLP makers generated in this study. The defined position of the QTL shows that the pre-existing SSR markers can be used with confidence in order to select for the Alcedo QTL in crosses.

Seedling Resistance

The resistance response of the DH population was also investigated at the seedling stage in glasshouse tests. By using an old yellow rust isolate that has not defeated *Yr17* it is possible to map the position of *Yr17* in the DH population. Seedlings of the DH population were inoculated 14 days after sowing. At the seedling growth stage a modified scoring system was used as illustrated in Figure 6.

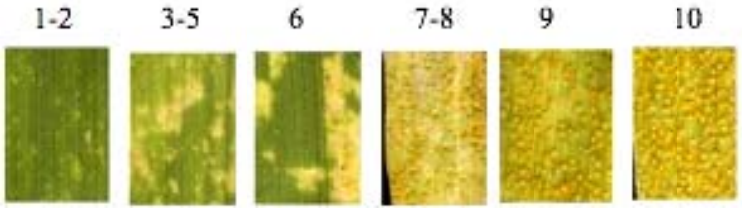


Figure 6. Scoring system used to score yellow rust infection on seedlings. 1-2 = necrotic flecks, 3-5 = necrotic areas without sporulation, 6 = necrotic and/or chlorotic areas with restricted sporulation, 7-8 = moderate sporulation with necrosis and/chlorosis, 9 = sporulation with chlorosis, 10 = abundant sporulation without chlorosis

The DH population exhibited a continuous distribution in the seedling experiment indicating multiple genes were involved in the seedling resistance (Figure 7). Brigadier produced a resistant phenotype of 1 while Alcedo exhibited an intermediate phenotype on the first seedling leaf, with a score between 5 and 6, but showed greater susceptibility on the second leaf with a score of 8 (Figure 7). It is likely that the resistance in Alcedo is effective at the seedling stage as it did not exhibit a fully susceptible phenotype.

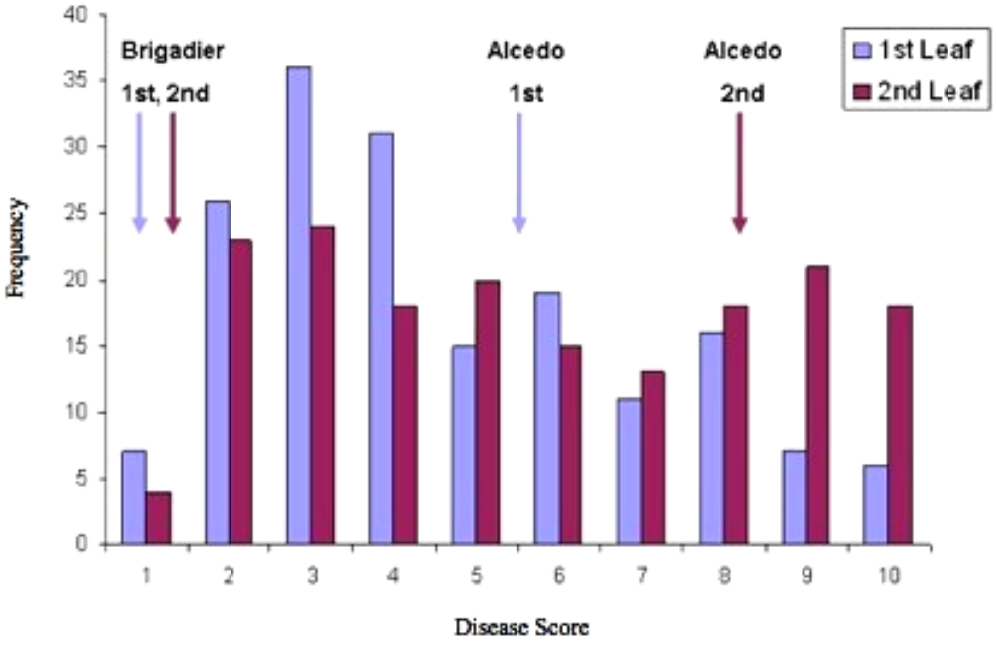


Figure 7. Frequency distribution of yellow rust infection scores on the 1st and 2nd seedling leaf (the mean of 5 seedlings) for the Alcedo × Brigadier DH population. The mean parental Alcedo and Brigadier scores are indicated by the relevant arrows.

When QTL analysis was carried on the seedling yellow rust infection scores two QTL were detected (Table 2). The QTL that contributed the majority of the resistance was located on 2A. *Yr17* is known to be located on chromosome 2A so the QTL is likely to be *Yr17*. A QTL was also found to be located on chromosome 2D donated by Alcedo. This QTL was located to the same region of chromosome 2D as the APR QTL *QPst.jic.2D.* It is possible that this APR QTL also has an effect at the seedling growth stage. However, the phenotype is not as strong as that observed at the adult growth stages, indicating that the effect of *QPst.jic.2D* increases as the plant matures. No such effect was seen for the other major APR QTL found in Alcedo, *QPst.jic.4B*.

[1]Dataset (LOD threshold)[6]	Chromosome[2]	Parent[3]	Locus[4]	LOD[5]	Expl. % Variance[7]	Phenotypic means[8]	
						Al	Br
1st leaf (3.2)	2A	Br	*Xgwm636*	17.37	39.5	5.47	2.64
	2D	Al	*Xgpw8086a*	6.34	15.9	2.98	4.73
2nd leaf (3.2)	2A	Br	*Xgwm636*	22.54	48	7.06	3.13
	2D	Al	*Xgpw8086a*	3.22	8.4	3.92	5.50

Table 2. [1]The 1st and 2nd seedling leaf yellow rust infection phenotypes analysed by interval mapping using the software MapQTL v. 5.0. [2]Chromosomal location. [3]Parent contributing QTL. [4]Marker locus associated with closest QTL. [5]Maximum LOD score associated with QTL. [6]LOD threshold based on a p-value of 0.05. [7]Percentage phenotype explained. [8]Phenotypic means of allelic classes at QTL. Al, cultivar Alcedo. Br, cultivar Brigadier.

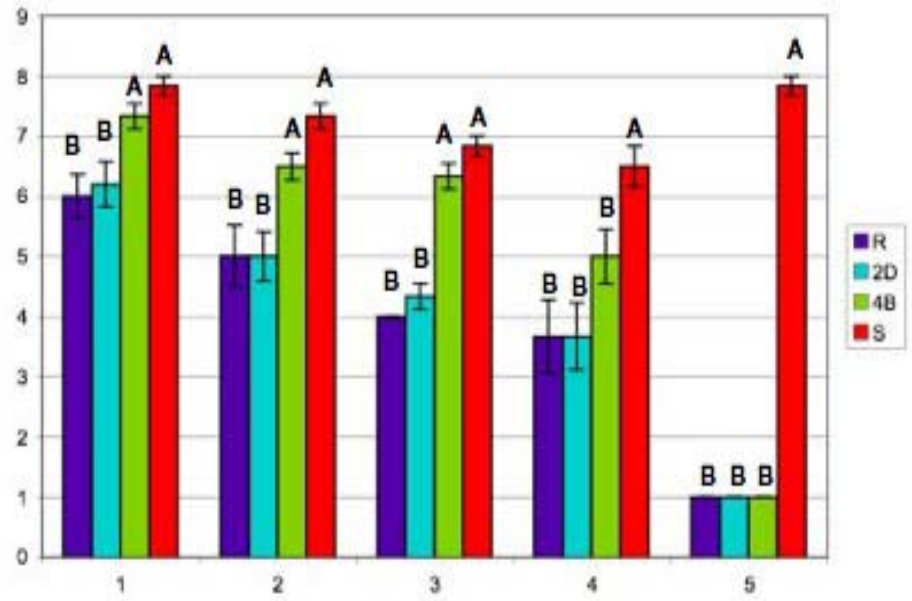
PHENOTYPIC CHARACTERISATION

Growth Stage Analysis

In order to determine when the two major Alcedo QTL became fully effective Brigadier backcross lines, containing each QTL together and individually were inoculated at 5 different growth stages; seedling, vernalised seedling, tillering, booting and at heading. The plants were grown and inoculated under glasshouse conditions.

Significant differences were seen between wheat genotypes, growth stage and for genotype × growth stage interactions. The levels of yellow rust infection on S plants did not differ significantly across the plant growth stages, showing IT scores between 6.5 to 8.0 (Figure 8). Plants containing *QPst.jic.2D* (with or without *QPst.jic.4B)* were significantly more resistant than S plants from the seedling growth stage onwards with the level of resistance increasing at each consecutive growth stage (Figure 8). Full resistance was however only expressed at the heading growth stage, when an average IT score of 1 was observed. Plants carrying *QPst.jic.4B* alone did not show a significant increase in yellow rust resistance compared to S plants until growth stage 41. By growth stage 59 resistance conferred by *QPst.jic-4B* was comparable to that conferred by *QPst.jic-2D*. Brigadier exhibited yellow rust infection scores comparable to S plants at all growth stages, however Alcedo was some what more resistant than the R line at growth stage 41. This could indicate potential genetic components in Alcedo that contribute to yellow rust resistance at specific stages of wheat development and which were not identified in the previous field analysis of yellow rust resistance in Alcedo (Jagger *et al.,* 2010b).

(a)



(b)

(c)

Al[1]	5.33	7.00	3.33	1.67	1.00
Br[2]	8.00	7.67	7.00	7.00	8.00

Figure 8. Average disease scores on 5 different growth stages. (a) Disease scores for R = lines contain *QPst.jic.2D* and *QPst.jic.4B;* 2D = lines contain *QPst.jic.2D;* 4B = lines contain *QPst.jic.4B*; S = lines with neither QTL. (b) The growth stages are pictured below the graph. 1 = seedling (non-vernalised); 2 = vernalised seedling; 3 = tillering; 4 = booting; 5 = heading. (c) Table showing disease scores at each growth stage for parental controls [1]Alcedo; [2]Brigadier. T-tests were carried out for each growth stage individually to determine genotypes significantly different from the S genotype at p = 0.05 (A = genotype or genotypes not significantly different from S at p = 0.05; B = genotypes significantly different from the S genotype at p = 0.05)

Microscopic Analysis

By identifying the phenotype of the Alcedo resistance at the microscopic level it may be possible to determine the mechanisms involved in the resistance. This knowledge could aid in the identification of sources of durable resistance.

Brigadier backcross plants containing both *QPst.jic.2D* and *QPst.jic.4B* together and individually, as well as lines containing neither of the QTL were grown under glasshouse conditions and were inoculated at the heading growth stage. The flourochrome Uvitex-2B was used to stain the yellow rust fungus within the inoculated leaves and the pathogen was visualised using Laser Scanning Confocal Microscopy (LSCM) (Jagger *et al.* 2010b).

Figure 9 shows the normal development of the pathogen within the plant in a compatible interaction. The main structures of the fungus are clearly visible with the exception of the haustoria. The presence of a HMC was taken as an indication that an haustoria was present.

The common resistance response of cell death is also clearly visible using this technique as the dead plant cells autofluoresence. Figure 10 shows the development of the pathogen in the yellow rust resistant lines. In lines with both the QTL the fungus does not progress away from the initial infection site and is surrounded by cell death. In lines with each QTL individually the pathogen would occasionally develop further, but to a lesser extent to that seen in the susceptible line and cv. Brigadier.

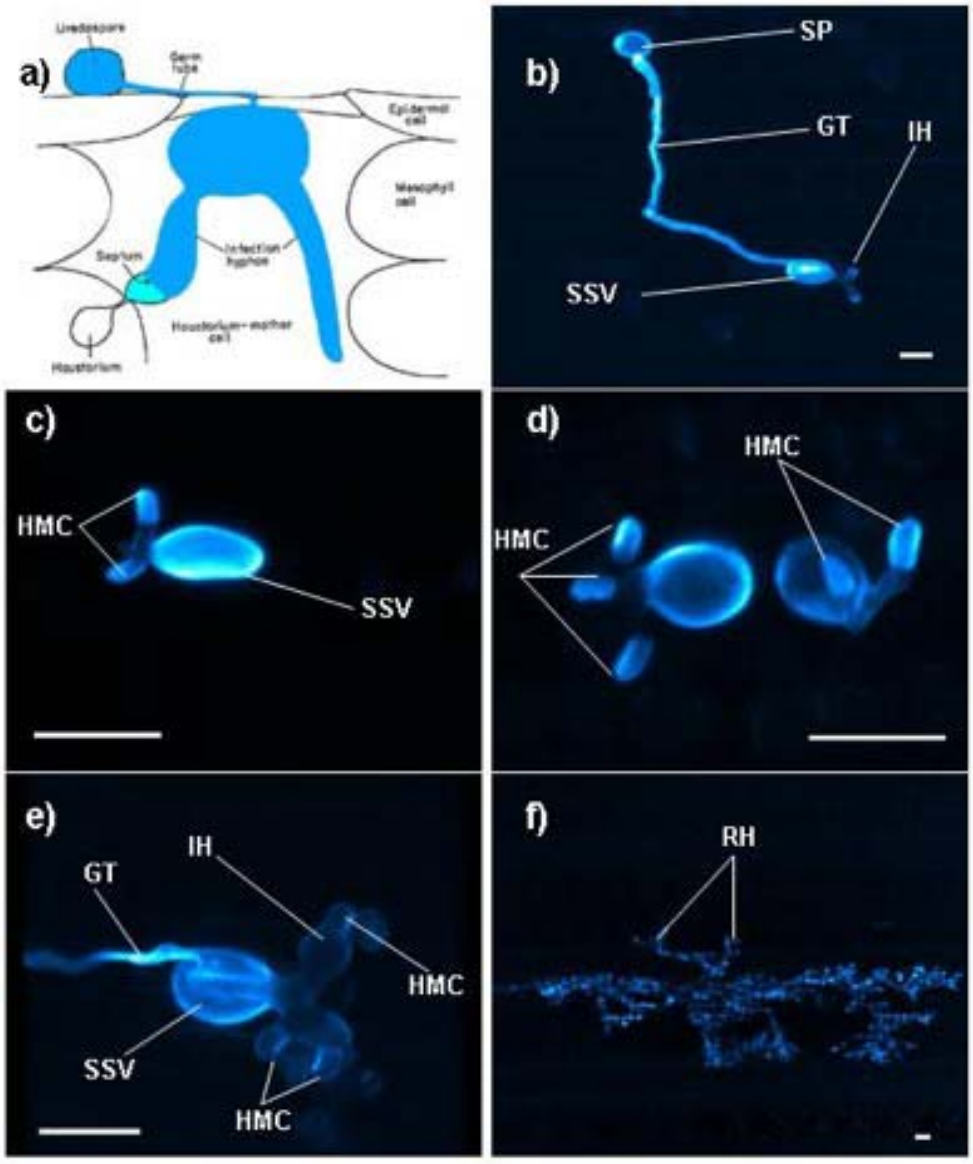


Figure 9. *Puccinia striiformis* (causal agent of yellow rust) infection structures observed using the stain Uvitex-2B **a)** *P. striiformis* infection (adapted from Mares and Cousen, 1977). **b)** Germinating spore with germ tube leading to sub-stomatal vesicle with two infection hyphae. **c)** Detailed view of sub-stomatal vesicle. Two haustoria mother cells can be clearly distinguished at the ends of the infection hyphae. **d)** Two sub-stomatal vesicles developing beneath a single stoma. Two or three infection hyphae can develop from the sub-stomatal vesicle. **e)** Initial establishment of infection, the infection hyphae have begun to swell. **f)** Colonisation occurs when runner hyphae develop throughout the plant tissue. The fungus was stained with Uvitex-2B and observed using confocal laser scanning microscopy (excitation 488 nm, filter 420-480 nm). (Sp = spore, GT = germ tube, SSV = sun-stomatal vesicle, IH = infection hyphae, HMC = haustorial mother cell and RH = runner hyphae). Scale bars represent 20 μm.

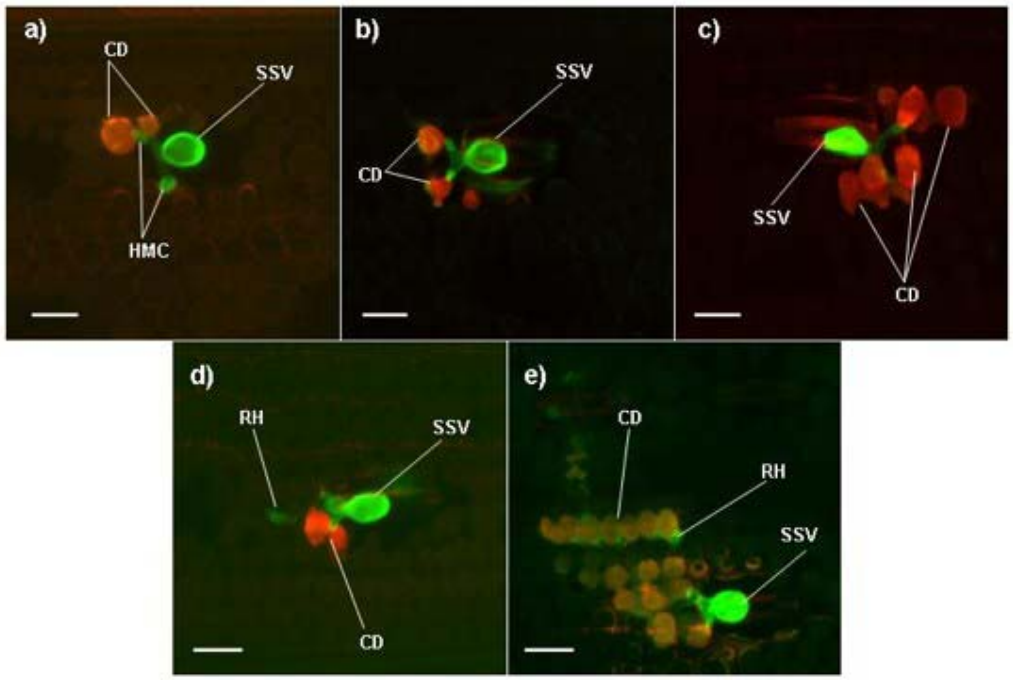


Figure 10. The interaction between *Puccinia striiformis* (the causal agent of yellow rust) and lines carrying both the QTL and each QTL individually. *P. striiformis* development in lines containing both the Alcedo major QTL a) 72 hpi; b) 120 hpi; c) 264 hpi. Cell death is associated with the HMC and by 264 hpi the SSV appears irregular in shape. d) Development of runner hyphae in the 4B line containing *Qst.jic.4B*. e) Runner hyphae development with associated cell death in the 2D lines containing *Qst.jic.2D*. (SSV = sub-stomatal vesicle; IH = infection hyphae; HMC = haustorial mother cell; RH = runner hyphae; CD = host cell death). The Uvitex 2-B stained fungus (excitation 488 nm, filter 420-480 nm, shown in green) and autofluorescing mesophyll cells (excitation 405 nm, shown in red) were observed using confocal laser scanning microscopy. Scale bars represent 20 µm.

DISCUSSION

Two years of field trial data show the yellow rust APR in Alcedo to be conferred by two major QTL located on chromosomes 2D and 4B. Despite a susceptible phenotype in the field, the variety Brigadier appears to contain minor QTL conferring some resistance to yellow rust located on chromosomes 1B and 5A.

The Alcedo, major yellow rust resistance QTL located on 2D and 4B were believed not to be flanked by markers within the pre-existing SSR map. Flanking markers were required to locate more precisely the position of the QTL, to determine the relative effects of each QTL and to have greater confidence when selecting for the QTL in a marker-assisted breeding programme.

By identifying AFLP markers that flanked both the QTL by 4 cM it was shown that the SSR markers in the pre-existing map already flanked the QTL. The estimated relative effects of the two QTL were not altered significantly with the addition of the AFLP markers. However, the breeders now have more confidence in the flanking SSR markers as reliable tools to identify *QPst.jic.2D* and *QPst.jic.4B* in a breeding programme.

The seedling yellow rust resistance gene *Yr17* has been mapped to the short arm of chromosome 2A (Bariana and McIntosh 1993). Brigadier has been shown to carry the *Yr17-Sr37-Lr38* complex using the diagnostic SCAR marker *SC-Y15* (Robert *et al.,* 1999; Ambrozkova *et al.,* 2002). The QTL detected on chromosome 2A using a *Yr17* avirulent isolate was responsible for the majority of the seedling yellow rust resistance observed in the Alcedo × Brigadier cross and was contributed by Brigadier. It is therefore likely that the QTL detected on chromosome 2A is *Yr17*.

The seedling resistance on chromosome 2D mapped to the same location as the APR QTL *QPst.jic.2D*. While *QPst.jic.2D* gave a complete resistant phenotype at adult plant growth stages the 2D seedling QTL confered a

minor resistance effect that was stronger in the first leaf compared to the second leaf. Singh and Huerta-Espino (2003) showed that Jupateco NILs containing the adult plant leaf rust gene *Lr34* were slightly more resistant than Jupateco NILs without *Lr34* at the seedling growth stage 11, although the full resistance was not expressed until adult plant growth stages. Again it is possible that the 2D seedling QTL detected in this study is the same gene/s responsible for *QPst.jic.2D,* having an effect at the seedling growth stage. Although it cannot be ruled out that separate, linked genes confer the resistance at the two growth stages.

Lines carrying the Alcedo QTL, either together or individually are more resistant at the heading growth stage than at the seedling growth stage, confirming the adult plant nature of the Alcedo yellow rust resistance. The plant growth stage at which the 2D and 4B resistances become effective is however different. Lines with *QPst.jic.2D* show some resistance towards *P. striiformis* as early as the seedling growth stage, while lines carrying only *QPst.jic.4B* do not show any significant signs of resistance until the booting growth stage.

APR to yellow rust has been shown to be effective at different plant growth stages (Qayoum and Line 1985; Ma and Singh 1996; Boyd and Minchin 2001). The initial growth stage at which resistance is expressed has been shown to correlate with the final strength of the resistance in mature plants (Qayoum and Line, 1985; Ma and Singh, 1995). While the lines containing *QPst.jic.2D* and *QPst.jic.4B* had the same disease score at the heading growth stage in the glasshouse growth stage experiment, *QPst.jic.2D* exhibited a stronger phenotype in the field. Therefore *QPst.jic.2D,* which expressed resistance earlier than *QPst.jic.4B* would appear to confer a stronger yellow rust resistance.

In order to investigate the mechanisms involved in the resistance conferred by each Alcedo QTL a microscopy study was carried out. In Alcedo and R lines (containing both *QPst.jic.2D* and *QPst.jic.4B*) development of the fungus past the formation of the initial IH and HMC was never observed. This is in contrast to Brigadier and S lines where the

fungus was able to produce large areas of dense hyphal growth by 264 hpi and microcolonies from which sporulating uredinium developed. When *QPst.jic.2D* and *QPst.jic.4B* were present individually some hyphal growth was observed. This would usually consist of a single, unbranched RH with few HMC that did not produce any dense areas of hyphal growth.

The microphenotypes associated with *QPst.jic.2D* and *QPst.jic.4B* are different to the yellow rust APR microphenotypes reported to date in the literature. Other sources of yellow rust APR appear to involve a mechanism that acts to reduce the size of developing microcolonies (Mares and Cousen, 1977; Moldenhauer *et al.,* 2006; Melichar *et al.,* 2008).

In the lines containing both *QPst.jic.2D* and *QPst.jic.4B* the pathogen does not develop beyond the sub-stomatal cavity. In lines with only *QPst.jic.2D* or *QPst.jic.4B* more pathogen growth is observed associated with a cell death response. The Alcedo QTL therefore appear to produce a similar hypersensitive cell death reaction to that observed in race-specific seedling interactions between yellow rust and wheat (Bozkurt *et al.,* 2010).

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