GENETIC ANALYSIS OF BYDV RESISTANCE AND LOW TEMPERATURE TOLERANCE IN WINTER x SPRING BARLEY CROSSES

by

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

12/10/99

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Table of Contents

List of tables

List of figures

Abstract

The development of winter 6-row malting barley varieties is an objective of the OSU program. The program's winter germplasm is quite susceptible to Barley Yellow Dwarf Virus (BYDV). The program recently released the spring 2-row variety *Orca*, which carries the *Yd2* gene for BYDV resistance. The objective of this project was to transfer the *Yd2* gene from *Orca* to the winter varieties *Strider* and *Kold*. A molecular marker tightly linked to the *Yd2* locus was used to facilitate the introgression of this gene from the spring to the winter germplasm pool. In 1998, six-row plants were selected in F_2 populations derived from crosses of *Orca* x *Kold* and *Orca* x *Strider*. DNA was extracted from these plants for genotyping with the molecular marker *YLM*, which is tightly linked to the *Yd2* locus on the long arm of chromosome 3 (3H). In order to encourage natural infection by BYDV-infected aphids, the F_3 families tracing to selected F_2 plants were planted one month before the usual planting date in the fall of 1998. Unusually low temperatures in December 1998 led to significant winter injury, leading to the loss of approximately 70% of the F_3 families in each of the two populations. Phenotype data (visual rating of BYDV symptoms on F_2 plants and F_3 families, and visual rating of winter survival in F_3 families) and genotype data (allelic structure of each F_2 plant at the *YLM* locus) were collected to determine the genotype of the F_3 phenotypic selections and to assess the utility of the marker locus as tools for rapidly introgressing target alleles from spring to winter germplasm.

Chapter 1

Introduction

Barley yellow dwarf virus (BYDV), an aphid transmitted disease, was first described by Oswald and Houston (1951). BYDV infects all of the small grain cereals. The virus causes serious yield losses, making it of great economic concern. In the USA, losses have been estimated to be between one and three percent annually. However, under favorable conditions for the aphid to spread the virus, losses could reach up to forty percent (Burnett, 1983).

The virus can cause severe stunting of the plant, inhibition of root formation, delay or prevention of heading, and reduction in yield. In barley, a bright discoloration begins and moves rapidly down the whole leaf (Figures 1.1 and 1.2). Infected plants are found scattered throughout the crop (Burnett, 1983) (Figure 1.3).

Figure 1.1. Bright discoloration due to BYDV

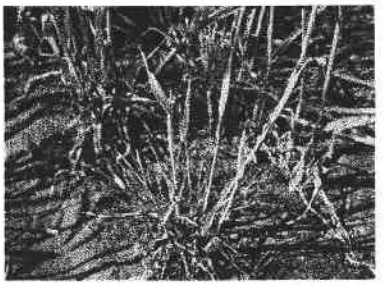

Figure 1.2. Severe stunting due to BYD

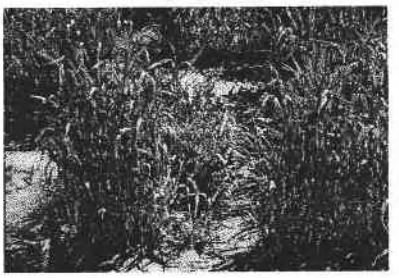

Figure 1.3. Infected plants are scattered throughout the crop.

The most economical and environmentally sound control for BYDV is genetic resistance. Resistance to BYDV was found in barleys of Ethiopian origin by Shaller et al. (1963), who determined it was conditioned by alleles at the *Yd2* locus. The gene is located in

chromosome 3 (Schaller et al. 1964), and it was mapped on the long arm, 0.5 cM from the centromere (Collins et al. 1995). A codominant PCR marker, *YLM*, was mapped 0.7 cM from the *Yd2* locus (Paltridge et al. 1998). These authors designed a pair of PCR primers which amplify the polymorphic region of the *YLM* locus in the varieties 'Proctor'and 'Shannon' giving 101 bp (susceptible) and 90 bp (resistant) products, respectively. These authors also genotyped alleles at the *Yd2* locus in 102 different barley lines to determine its suitability for marker-assisted selection. Each of the barley lines gave an amplification product of similar size to one of the two DNA fragments characterized in 'Proctor' and 'Shannon'. Resistant genotypes contained the same length variant of *YLM* as the resistant barley 'Shannon'. Eighty-five out of 93 BYDV-susceptible barleys yielded the larger fragment characteristic of 'Proctor', and 8 the fragment characteristic of 'Shannon'. Based on these results, the *YLM* marker was used to introgress the BYDV resistance allele from Orca to Kold and Strider, since phenotyping for BYDV resistance can be problematic.

Hayes et al. (1971) reported that the levels of resistance in some of the Ethiopian genotypes varied both within and between varieties and that the levels of resistance depend on the environment and their rate of growth. Other cultivars, that do not posses the *Yd2* gene, have been identified to have resistance, but very little is known about the genetic basis (Shaller 1983). Four quantitative trait loci (QTL) were recently reported for BYDV-MAV serotype resistance and three for PAV serotype resistance in the Shyri x Galena population (Toojinda et al. in press). None of these mapped to the centromeric region of chromosome 3 (3H), the site of the *Yd2* locus.

Winter hardiness is the consequence of a number of interacting characters. These include vernalization requirement, photoperiod response, and low temperature tolerance (Hayes et al. 1997). Dehydrin genes are associated with tolerance, or response, to the onset of low temperature or dehydration (Choi 1999). Large Quantitative Trait Loci (QTL) effects for cold tolerance were found on chromosome 7(5H) (Hayes et al. 1997). The *Dhn2* locus maps to this region (Pan et al. 1994). This marker could be used to select for winter survival in winter x spring progeny, if differential injury does not occur in field tests.

Plant breeding can be defined as directed evolution. Selection requires optimum measures of target phenotypes. Ideally, selection would be based on the gene (or genes) determining the target phenotype. However phenotypic selection is warranted if the genetic basis of the trait is complex, gene interactions are not completely understood, markers are not available, marker-based selection is too expensive, and/or if reliable measures of the phenotype can be obtained. The objective of this study was to use the *YLM* marker to introgress the BYDV resistance allele at the *Yd2* locus from Orca to Strider and Kold and to use phenotypic selection for winterhardiness, capitalizing on differential winter injury that occurred during the 1998-1999 field season.

Chapter 2

Materials and methods

Germplasm

Kold is a winter 6-row variety developed at Oregon State University (Hayes et al. 1995). Its yields are competitive. This cultivar is partially resistant to scald, resistant to stripe rust, and susceptible to BYDV. *Strider* is a winter 6-row variety developed at Oregon State University (Hayes et al. preparation). Its yields are competitive. It is partially resistant to scald, resistant to stripe rust, and susceptible to BYDV. *Orca* is a spring 2-row variety developed at Oregon State University (Hayes et al. in press). It has acceptable yield, a high enzyme malting quality profile, and high-test weight. This cultivar is resistant to stripe rust, and is resistant to BYDV. Resistance is determined by the *Yd2* locus (Hayes et al., 1996).

Experimental procedures and generation advance

F2 populations from the crosses of Orca/Strider and Orca/Kold were planted with a HEGE 90, single row drill, at the Hyslop Research Farm near Corvallis, Oregon in the Fall of 1997. Each population consisted of five 4.8m plots. There were six rows in each plot, and plants were spaced 20cm apart. In the spring of 1998 all six-row lines were tagged and leaf tissue was frozen for later DNA extraction and genotyping with the *YLM* PCR marker (Figure 2.1). Frozen tissue was ground and DNA was extracted with the QIAGEN DNeasy Plant Mini Kit (http://www.qiagen.com/).

Figure 2.1. Tagging and leaf tissue collection.

All six-row genotypes were genotyped for the *YLM* locus (Figure 8), following the procedures by Paltridge et al. (1998). The PCR ingredients for a 1x reaction mix included:

- 1.60µl (200mM) of a 1.25mM dNTP mix (4)
- 2.00µl (200mM) of 1xTaq polymerase buffer without $MgCl_2$
- 1.50µl (200nM) of 25mM $MgCl_2$
- 0.20µl of 20µM "F" primer
- 0.20µl of 20µM "R" primer
- 0.20µl of 5 U/µl Taq DNA polymerase (1U/reaction)
- 10.5µl of ddH_2O

17µl of the PCR mix were then distributed in each one of the wells in a PCR plate. Then a concentration of 25ng/µl of DNA (100ng/reaction), about 4 to 5µl, was added to each PCR well. Reactions were run in a MJR PCR machine, with the following program:

- 94°C for 3 minutes
- 94°C for 30 seconds
- 58°C for 30 seconds
- 72°C for 30 seconds
- Go to step 2— 40 times (cycles)
- 72°C for 5 minutes
- 4°C hold

Then 10µl of PCR product were run on a 3% agarose gel at 90V. The agarose gel was prepared by mixing 3% FMS New Sieve agarose and 1% GIBCO ultra pure agrose in 1x TBE . Then 0.5µg/ml (stock - 10µg/µl) Ethidium Bromide was added into the melted and cooled agarose mix. Gels were then observed under UV light.

In the Fall of 1998, F3 head rows derived from each six-row F2 selection were planted with a HEGE 90, single row drill, at the Hyslop Research Farm. The Orca/Kold population consisted of 24 2.4m plots, and the Orca/Strider population consisted of 20 2.4m plots. There were six rows in each population, and they were spaced 20cm apart. The head-rows were planted early in October, in order to encourage infection by BYDV infecting aphids. Head-rows were rated for BYDV reaction based on dwarfing and leaf discoloration symptoms. To confirm visual ratings, two samples of each of four genotypes were sent to the Extension Plant Pathology Laboratory at Hermiston, Oregon where they were analyzed with ELISA for the BYDV-PAV serotype. The ELISA test used followed the DAS ELISA protocol from Agdia, Elkhart, IN. (http://www.agdia.com/). A polyclonoal antibody is used for capture and a separate

polyclonal antibody is used for detection. This test recognizes and detects all known isolates of BYDV-PAV.

Winter survival was scored twice during the winter of 1998. First, plots were rated immediately after severe cold temperatures based on visual estimates of percentage survival (Figure 2.2). Plots were again rated for percent survival at maturity (Figure 2.3). Ratings were averaged from multiple observations by members of the breeding program in order to reduce bias in estimating percentage damage (Figure 2.4).

Figure 2.2. Condition of field plots immediately after severe cold temperatures.

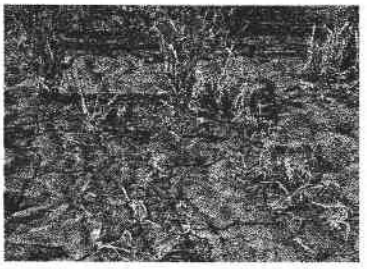

Figure 2.3. Conditions of field plots at maturity.

Figure 2.4. Assistance from members of the breeding program in rating winter survival.

Results and discussion

In a population estimated to consist of 360 plants in the Orca/Strider population, 109 six-rows were identified. In a population estimated to consist of 420 plants in the Orca/Kold population, 130 six-rows were identified. Since the two-row phenotype is dominant, all six-rows are homozygotes for the recessive allele at the *vrs1* locus on chromosome 2 (2H) (Franckowiak and Lundquvist 1997). The observed ratios fit predicted 3:1 ratios (O/S: p=0.25, O/K: p=0.5). Heads of all six-row F2 plants were retained for planting F3 head rows the following season. BYDV symptoms were severe on some plants in the F2 populations. However, the natural epidemic may have allowed escapes and the population density precluded reliable BYDV ratings of individual plants. In the Orca/Kold and Orca /Strider populations 10 six-row plants respectively, were identified that had striking BYDV symptoms. The phenotypes of these plants were related to the *YLM* genotype as described below.

Of the ten F2 plants showing severe BYDV symptoms in the Orca/Kold population none were Orca allele homozygotes, eight were heterozygotes, and two were Kold allele homozygotes. Of the ten F2 plants showing severe BYDV symptoms in the Orca/Strider population none were Orca allele homozygotes, six heterozygotes, and four were Strider allele homozygotes. One can conclude that plants homozygous for Orca alleles were resistant and heterozygotes and that plants homozygous for Strider and Kold alleles were susceptible. This suggests that resistance determined by the *Yd2* gene in this population was recessive. Hayes et al. (1971) reported that recessive alleles at the *Yd2*locus conferred resistance in the F2 generation of tolerant x susceptible crosses.

Enzyme-linked imuno sorbent assay (ELISA) techniques are a useful tool for assaying BYDV infection. Two samples of each of four genotypes were sent to the Extension Plant Pathology Laboratory at Hermiston, Oregon where they were analyzed for BYDV-PAV. The samples included the 88Ab536, Strider parent, a phenotypically resistant F3 line from the Strider x Orca population (S/O 100), and a phenotypically disease-free sample (control) from an experiment planted at the usual date (late October) and thus more likely to be free of aphid infection. We could not include a sample of Orca (the *Yd2* resistance donor) because all Orca plots had suffered complete winter injury. The results are shown in Table 2.1. The ELISA results confirmed the visual ratings for these selected samples. Strider, which showed severe infections, tested positive for the PAV serotype. 88Ab536, which showed mild infections, also tested positive for the PAV serotype. Line S/O 100, which appeared to be healthy, tested negative for the PAV serotype and homozygous for Orca alleles at the *YLM* locus). The control sample tested negative for the PAV isolate. We did not run ELISA on the full population due to time and resource restrains and concerns. Furthermore, although we planted the trial one-month early in order to encourage aphid infection and natural infection, infection was not uniform throughout the experiment. It was likely that some plants with susceptibility alleles at the *Yd2* locus would appear resistant, due to escape. Nonetheless, the limited ELISA data do suggest that plants sharing even mild BYDV symptoms were infected with the virus.

Table 2.1. ELISA tests on seven field samples

Samples	BYDV Symptom	ELISA Test (sample 1)	ELISA Test (sample 2)
88Ab536	mild (limited yellowing)	+	+
Strider	severe (yellowing stunting)	+	+
S/O 100	healthy (no yellowing or stunting)	–	–
Control	healthy (no yellowing or stunting)	–	–

We hypothesized that within the selected six-row genotypes, we would observe a genotypic ratio of 1:2:1 for the *YLM* locus, since alleles show codominant inheritance and the *YLM* locus and *Vrs1* locus show independent assortment. This ratio was tested by χ^2. We were able to successfully genotype 120 plants out of 130 alleles in the Orca/Kold population and 101 plants out of 109 in the Orca/Strider population. No amplification was observed; or bands could not be reliably scored in the remaining 18 samples. Observed ratios for the Orca/Kold population fit expected 1:2:1 genotypic ratios for alleles at the *YLM* locus (p=0.3). Forty Orca allele homozygotes; 65 heterozygotes; and 27 Kold allele homozygotes were observed. Observed ratios for the Orca/Strider

population fit expected 1:2:1 genotypic ratios for alleles at the *YLM* locus (p=0.1). Thirty-seven Orca allele homozygotes; 47 heterozygotes; and 26 Strider allele homozygotes were observed. Figure 2.5 illustrates one of the gels run with the *YLM* primers on the Orca/Kold population.

Fig. 2.5. Genotyping Orca/Kold with *YLM*

We had hypothesized that head-rows homozygous for Orca alleles at the *YLM* locus would be resistant to BYDV, that head-rows originating from heterozygous F2 plants would segregate for resistance, and that head-rows homozygous for Kold or Strider alleles would be susceptible. However, we were not able to test this hypothesis for two reasons. First, as described previously, although some BYDV symptoms were observed in the F3 head rows, infections were not uniform within each row. Furthermore other diseases were also visible, such as scald (caused by *Rhynchosporium secalis*) and stripe rust (caused by *Puccinia striiformis* f. sp. *hordei*), which complicated assessment of BYDV.

However, as shown below, a limited number of F3 rows showed no BYDV symptoms. Secondly, a low temperature event caused severe mortality, leaving only a subset of F3 head rows alive in each of the two populations.

In December 1998 unusually low temperatures led to significant winter injury. The mean survival of Orca (spring two-row) was 0%, the mean survival of Kold (winter six-row) was 62% (+/- 16 %) and the mean survival of Strider (winter six-row) was 73% (+/- 4 %). Survival of the F3 rows within the two populations ranged from 0 to 95%. Approximately 70% of the F_3 rows in each of the two populations experienced complete mortality. Figures 2.6 and 2.7 show the phenotypic frequency distributions of winter survival in the two populations, based on the second rating. These data from the second rating were chosen, due to the eventual mortality of some rows, and some plants within rows, that initially appeared to have survived the freeze stress. When F3 families with survival ratings of 0% to 20% were considered to be cold susceptible and families with 20% to 100% ratings were considered to be cold resistant, the phenotypic data follow a pattern suggesting monogenic inheritance. These data (figure 2.6 and 2.7) show that a single gene may have been a major determinant of survival. Additional variation in high surviving lines due to additional genes are also observed.

These large number of lines showing complete mortality or severe winter injury in these frequency distributions suggest that alleles at a single locus could be the primary determinants so winter survival in these two populations. The variance in the degree if winter injury in the surviving genotypes could be due to the effect of "minor" genes and experimental error. Spring habit is reported to be dominant over winter habit (Takahashi and Yasuda 1970). Hayes et al. (1997) reported that a major QTL for cold tolerance is

located on chromosome 7(5H), linked to the gene determinig winter vs. spring growing habit, as reported by Takahashi and Yasuda (1970). Therefore, survival in the Orca/Strider and Orca/Kold populations may be due to segregation of alleles at this complex of linked genes determining growth habit and winter survival. The *Dhn2* locus is linked to the complex of genes determining survival and growth habit (Hayes et al. 1997) and six-row genotypes were also to be genotyped for the *Dhn2* locus to determine the relationship of alleles at this locus with winterhardiness. However, the amplification products generated from primers provided by T. Close (personal communication) were too similar in size to allow for reliable scoring using standard protocols.

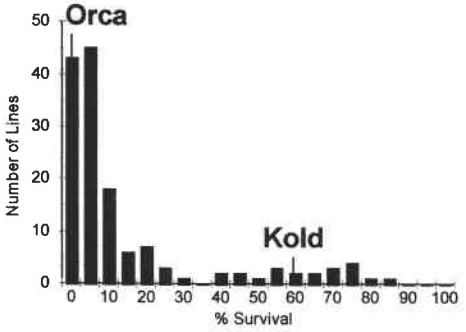

Figure 2.6. Phenotypic frequency distribution of winter survival in Orca/Kold.

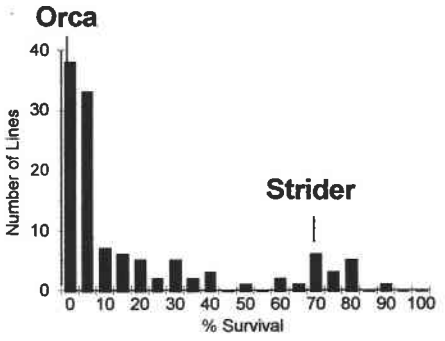

Figure 2.7. Phenotypic requency distribution of winter survival in Orca/Strider.

If winter survival and BYDV resistance were genetically independent, one would expect approximately equal percentages of the three *YLM* F2 genotypic classes (1:2:1) in the high and low surviving groups of the F3 families. The data shown in Table 2.2 and 2.3 suggest that the two traits are inherited independently. Rigorous testing of the hypothesis of independent inheritance would require larger samples and genotype/phenotype data from the same generation. However the trends shown on Table 2.2 and 2.3 support the hypothesis that BYDV resistance and winter hardiness in these populations are determined by different, unlinked genes – e.g. the *Yd2* locus is on chromosome 3 (3H) and the winterhardiness related genes are on chromosome 7 (5H). The genetic analysis of the two traits could be confounded if winter survival is affected by BYDV infection. Endo and Brown (1962) demonstrated that winter oats and barley are predisposed to winterkill, when infected with BYDV. Parry and Habgood (1986) however, showed that cv. Vixen survived well over the winter, even when infected with BYDV.

Table 2.2. Orca/Kold winter survival and BYDV resistance

F3 Survival	Orca Allele	Orca + Kold Allele	Kold Allele
0-20%	34%	47%	18%
25-95%	26%	39%	35%

Table 2.3. Orca/Strider winter survival and BYDV resistance

F3 Survival	Orca Allele	Orca + Kold Allele	Kold Allele
0-20%	32%	41%	27%
25-95%	52%	41%	8%

In terms of realizing the objective of introgressing BYDV resistance from a spring two-row (Orca) to winter six-row (Kold and Strider), the *YLM* genotyping ad winterhardiness phenotyping were effective. As shown in Table 2.4, four F3 rows were selected from the Orca/Strider population that were phenotypically resistant to BYDV and that had winter survival percentages comparable to the winter parent (Strider). All of these F3 rows were derived from F2 plants that were homozygous for Orca alleles at the *YLM* locus. Due to the tight linkage of *YLM* with the *Yd2* locus, these selections have a high probability of carrying the target resistance allele at the *Yd2* locus. Only one F3 row was selected from the Orca/Kold population that phenotypically resistant to BYDV, had high survival, and had Orca alleles at the *YLM* locus. F4 head rows from these F3 selections were planted in the Fall of 1999 at the Hyslop Research Farm for further selection and for use in the breeding program.

Table 2.4. Winter survival, BYDV resistance phenotype and *YLM* locus genotyping of F3 selections from the Orca/Strider and Orca/Kold population

F3 Line	Cross	Winter Survival	BYDV rating	*YLM* locus
S/O 30	Orca x Strider	70%	Resistant	Orca allele
S/O 50	Orca x Srider	80%	Resistant	Orca allele
S/O 100	Orca x Srider	90%	Resistant	Orca allele
S/O 104	Orca x Srider	35%	Resistant	Orca allele
S/O 109	Orca x Srider	80%	Resistant	Orca allele
K/O 38	Orca x Kold	60%	Resistant	Orca allele
K/O 90	Orca x Kold	75%	Susceptible	Kold allele

Conclusions

1) Inflorescence morphology (two-row vs. six-row) followed the hypothesized monogentic inheritance pattern. Phenotypic selection for recessive allele homozygotes was effective
2) Segregation of alleles at the *YLM* locus also followed the hypothesized monogenic inheritance pattern. The codominant PCR based marker, allowed for characterization of homozygotes and heterozygotes.
3) The relationship of *YLM* genotype in the F2 generation with BYDV resistance in the F3 generation, could not be tested due to extensive winter injury and lack of uniform natural BYDV infection.
4) The survival data suggest that segregation of alleles at a singe locus may have been primary determinant of trait expression. Additional minor genes could account for variation within the surviving lines, because spring growth habit is dominant to winter habit, and a gene determining growth habit is linked to a QTL determining winter survival on chromosome 7 (5H). Accordingly, the differential survival observed in these populations could be due to segregation of alleles at this gene complex.
5) The *Dhn*2 locus is linked to the winterhardiness gene complex on chromosome 7 (5H). The amplification products generated from primers for this marker were too similar in size to allow for reliable scoring using standard protocols.
6) The *YLM* genotype and winter survival phenotype data suggest that the two traits are independent.

7) Five F3 selections advanced in the barley breeding program had the resistance allele at the *YLM* locus, were phenotypically free of BYDV symptoms and had survival comparable to the winter parent.

8) This is the first step toward the introgression of the *Yd2* gene into a winter 6-row background.

Recommendations

- Confirm BYDV resistance in controlled experiments where plants are inoculated with viruliferous aphids.
- Confirm cold tolerance in additional field and growth chamber experiments.
- Determine if winter survival is due to the gene complex on chromosome 7 (5H) by using an alternative protocol for determining polymorphism at the *Dhn2* locus.

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