



Genetic analysis of agronomic and malting quality QTLs in barley backcross breeding populations  
by Steven Richard Larson

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in  
Crop and Soil Science

Montana State University

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Abstract:

Agronomic and cereal quality quantitative trait loci (QTLs) have been identified in barley and other crops using genetic markers (RFLPs). The Steptoe x Morex doubled haploid mapping population, produced by the North American Barley Genome Mapping Project (NABGMP), exemplifies these efforts. These elite varieties have been grown in different production regions of the U.S. for three decades and have distinct utilization characteristics. Several potentially useful QTLs were identified. However, few studies have explored the reliability or breeding value of the comprehensive information gleaned from these large mapping projects. Using plant genetic materials provided by the NABGMP, efficient genetic tools (STS-PCR) were developed for genetic testing of these potentially complex QTLs. Although the final goal was to achieve practically-oriented plant breeding objectives, the focus of these experiments was to test main QTL effects and examine other possible forms of gene action including epistasis, pleiotropy, and environmental interaction.

Fifty lines were derived from a backcross of Morex and DH72 using the doubled haploid line as donor parent of the Steptoe chromosome 3. The NABGMP experiments suggested that this donor chromosome carries two yield QTLs that lack pleiotropic effects on malt quality. The fifty BC1 lines and parental checks were evaluated in five environments in Montana. Agronomic and grain quality traits were analyzed in all five experiments, and nine malting traits were analyzed using micromalted samples from one experiment. Headshattering and plant lodging QTL(s) reproduced, one of the yield QTLs, first reported by NABGMP, with major effects on yield under certain environmental conditions. The expected backcross gains for malt quality were realized.

A gene with strong effects on barley seed germination was previously identified on chromosome 7. However, the expression of this gene was evidently dependent on genetic background effects (epistasis). The effect of this seed dormancy QTL was investigated using the aPSR128 STS-PCR marker in two Steptoe and three Morex backcross populations. The aPSR128 QTL effects were similar regardless of recurrent parent and therefore lacked epistatic control. Unlike the reciprocal effects of minor QTLs, the aPSR128 QTL showed strong effects on seed germination that persisted during after-ripening.

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A thesis submitted in partial fulfillment  
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of

Doctor of Philosophy

in

Crop and Soil Science

MONTANA STATE UNIVERSITY-BOZEMAN  
Bozeman, Montana

August 1996

D378

L3282

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style and consistency, and is ready for submission to the College of Graduate Studies.

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## ACKNOWLEDGMENTS

I gratefully acknowledge the Great Western Malting Co. for micromalting analysis of the 1995 Bozeman trial of the Morex x DH72 backcross population. The Montana Agriculture Experiment Station, Pat Hensleigh and Greg Carlson in particular, were instrumental in conducting the field trials. The Montana State Seed Testing Laboratory provided useful accommodations, and suggestions, for germination tests conducted in 1994 and 1995. I also wish to acknowledge Dr. Tom Blake, my major advisor, for making these projects available to me. Finally I thank other faculty serving as graduate committee members; Dr. William Dyer, Dr. Jack Martin, Dr. Richard Smith, Dr. Richard Stout, and Dr. Luther Talbert.

## TABLE OF CONTENTS

	Page
TITLE PAGE .....	i
APPROVAL .....	ii
STATEMENT OF PERMISSION TO USE .....	iii
ACKNOWLEDGMENTS .....	iv
TABLE OF CONTENTS .....	v
LIST OF TABLES .....	viii
LIST OF FIGURES .....	x
ABSTRACT .....	xii
CHAPTER 1: INTRODUCTION .....	1
Importance of Barley .....	1
Importance of Plant Breeding .....	2
Domestication of Barley .....	3
Historical Advances in Barley Breeding .....	6
Advances in the Genetic Analysis of Complex Traits .....	9
Application of Genetic Analysis for Barley Improvement .....	11
CHAPTER 2: EVALUATION OF BARLEY CHROMOSOME 3 YIELD QTLs IN BACKCROSS BREEDING POPULATIONS .....	14
Introduction .....	14
Material and Methods .....	18
Clones and Genotypic Assay Procedure .....	18
Plant Materials .....	19
Experimental Design and Analysis .....	20
Results .....	22
STS-PCR .....	22
STS-PCR RFLP Markers .....	24
STS-PCR RPA Marker .....	27
Marker Mapping .....	29
Verification of a Barley Chromosome 3 Yield QTL in a Backcross Breeding Population .....	30
Relation of Yield, Lodging and Headshattering QTLs .....	31
Magnitude of Yield QTL x Environment Effects .....	34
Plant Height and Heading Date .....	35
Rachis Internode Length and Peduncle Angle .....	36

## TABLE OF CONTENTS -continued

Page

CHAPTER 2: EVALUATIONS OF BARLEY CHROMOSOME 3 YIELD QTLs  
IN BACKCROSS BREEDING POPULATIONS

Conclusions .....	37
The Steptoe Chromosome 3 Yield QTL with Major Effects .....	37
STS-PCR and Morphological Markers for Barley Chromosome 3 ..	39
The Second Largest Putative Yield QTL .....	40

CHAPTER 3: BACKCROSS GAINS FOR MALT QUALITY AND INTROGESSION  
OF A CHROMOSOME 3 YIELD QTL FROM FEED BARLEY .....

Introduction .....	42
Materials and Methods .....	46
Plant Materials .....	46
Grain and Malt Quality Evaluation .....	46
Experimental Design and Analysis .....	47
Results and Discussion .....	48
Kernel Weight .....	50
Kernel Plumpness .....	51
Test Weight .....	52
Grain and Malt Protein .....	53
Fine Malt Extract .....	54
Wort Color .....	55
Enzymatic Activity .....	56
Wort Clarity .....	57
Wort Viscosity .....	57
Wort $\beta$ -Glucans .....	58
Deciduous Awns in Doubled Haploid and Backcross Breeding Populations .....	59
Conclusions .....	63

## TABLE OF CONTENTS-continued

<b>CHAPTER 4: GENETIC ANALYSIS OF BARLEY CHROMOSOME 7 SEED DORMANCY QTL IN RECIPROCAL BACKCROSSES</b> .....	67
Introduction .....	67
Materials and Methods .....	70
Plant and Seed Materials .....	70
Clones and Genotypic Assay Procedures .....	71
Experimental Design and Statistical Analysis .....	73
Other Experimental Data .....	74
Results .....	75
STS-PCR RFLPs and Genetic Analysis .....	75
Analysis of Variance .....	77
Recurrent Parent Effects .....	78
QTL x Recurrent Parent x After-ripening Effects .....	79
Reexamination of Epistasis (Doubled Haploid Population) .....	80
Cytoplasmic Effects (Doubled Haploid Population) .....	81
Conclusions .....	82
<b>CHAPTER 5: DISCUSSION</b> .....	87
Six-rowed Malt Barley Breeding Outcomes for Montana .....	87
Six-rowed Feed Barley Breeding Outcomes for Montana .....	89
Application of Marker Technology to Plant Breeding .....	90
Concluding Remarks Concerning Molecular Markers .....	97
Relation of Agronomic QTLs to Genes in Wild Barley .....	98
<b>LITERATURE CITED</b> .....	100
<b>APPENDIX</b> .....	108

## LIST OF TABLES

Table	<u>Morex BC<sub>1</sub> Population (Yield and Quality)</u>	Page
1.	Chromosome 3 STS-PCR clones, primer sequences, predicted and observed products .....	23
2A.	Analysis of variance for yield using the aABG396 chromosome 3 marker. ....	30
2B.	Analysis of variance for yield of remaining 22 entries with heterozygotes excluded .....	30
3.	Percent residual variance explained for yield, headshattering, and plant lodging measured at four chromosome 3 marker loci .....	31
4.	Percent residual variance explained for yield, headshattering, and plant lodging in each of five environments measured at the aABG396 chromosome 3 marker locus. Overall trait correlations of yield with headshattering and plant lodging are shown .....	32
5.	Yield (kg/ha) x environment analysis .....	34
6.	Plant height (PH) and heading date (HD) analysis of .....	35
7.	Rachis internode length (RIL) and peduncle angle (PA) analysis .....	36
8.	Grain and malt quality analysis .....	49
9.	Analysis of variance for fine malt extract using the aABG396 chromosome 3 marker .....	54
10.	Percent residual variance explained for deciduous awns measured at six chromosome 3 markers .....	62

## LIST OF TABLES-continued

Table	<u>Step toe and Morex BC<sub>1</sub> Populations (Dormancy)</u>	Page
11.	Segregation ratios of the aPSR128 chromosome 7 marker, for a QTL with major effects on seed dormancy, in five BC <sub>1</sub> populations. Cytoplasmic and nucleoplasmic genotypes for the other seed dormancy QTLs with smaller effects are also shown . . . . .	76
12.	Analysis of variance for percent seed germination using the aPSR128 marker, recurrent parent and after-ripening as factors . . . . .	77
13.	Explanation of the seed dormancy experiments including number of homozygous progeny tested and analyzed for each cross and each generation. The number of days after-ripening is also shown . . . . .	109

## LIST OF FIGURES

Figure	<b><u>Morex BC<sub>1</sub> Population (Yield and Quality)</u></b>	Page
1.	Steptoe - Morex STS-PCR RFLPs at four chromosome 3 loci . . . . .	25
2A.	Identification of aCDO113 products from barley chromosome 3 using the wheat barley addition lines . . . . .	26
2B.	An aCDO113.2 <i>Bsa</i> MI RFLP maps to chromosome 3 by segregation analysis of Steptoe x Morex doubled haploid lines . . . . .	26
3.	A ribonuclease protection assay (RPA) used to detect and screen for a single bp sequence polymorphism between Steptoe and Morex aWG110.2 products . . . . .	28
4.	Linkage map of six STS-PCR markers for chromosome 3 and positions of the putative yield QTLs reported by Hayes et al. (1993). The position of the CDO113b RFLP used to detect the putative yield QTL with the second largest effects is also shown. . . . .	29
5.	Thousand kernel weight means, by environment, for BC <sub>1</sub> progeny fixed for Steptoe and Morex aABG377 chromosome 3 marker alleles . . . . .	50
6.	Percent kernel plumpness means, by environment, for BC <sub>1</sub> progeny fixed for Steptoe and Morex aABG377 chromosome 3 marker alleles . . . . .	52
<b><u>Steptoe x Morex Doubled Haploid Population (Quality)</u></b>		
7.	Mapmaker QTL scan of deciduous awn ranking LOD for chromosomes 2 and 3 . . . . .	61

## LIST OF FIGURES-continued

Figure	<u>Steptoe and Morex BC<sub>1</sub> Populations (Dormancy)</u>	Page
8.	Steptoe - Morex STS-PCR RFLPs at two chromosome 7 loci. ....	75
9.	Interaction plot of recurrent parent x after-ripening effects on seed germination and bar graph of recurrent parent effects .....	78
10.	Interaction plot of aPSR128 x recurrent parent x after-ripening effects on seed germination and bar graph of aPSR128 breeding values .....	79
<b><u>Steptoe x Morex Doubled Haploid Population (Dormancy)</u></b>		
11.	Interaction plot of ABG390 x PSR128 x after-ripening effects on seed germination and bar graph of aPSR128 breeding values .....	80
12.	Interaction of plot of cytoplasmic x PSR128 x ABG390 effects on seed germination .....	81

## ABSTRACT

Agronomic and cereal quality quantitative trait loci (QTLs) have been identified in barley and other crops using genetic markers (RFLPs). The Steptoe x Morex doubled haploid mapping population, produced by the North American Barley Genome Mapping Project (NABGMP), exemplifies these efforts. These elite varieties have been grown in different production regions of the U.S. for three decades and have distinct utilization characteristics. Several potentially useful QTLs were identified. However, few studies have explored the reliability or breeding value of the comprehensive information gleaned from these large mapping projects. Using plant genetic materials provided by the NABGMP, efficient genetic tools (STS-PCR) were developed for genetic testing of these potentially complex QTLs. Although the final goal was to achieve practically-oriented plant breeding objectives, the focus of these experiments was to test main QTL effects and examine other possible forms of gene action including epistasis, pleiotropy, and environmental interaction.

Fifty lines were derived from a backcross of Morex and DH72 using the doubled haploid line as donor parent of the Steptoe chromosome 3. The NABGMP experiments suggested that this donor chromosome carries two yield QTLs that lack pleiotropic effects on malt quality. The fifty BC<sub>1</sub> lines and parental checks were evaluated in five environments in Montana. Agronomic and grain quality traits were analyzed in all five experiments, and nine malting traits were analyzed using micromalted samples from one experiment. Headshattering and plant lodging QTL(s) reproduced one of the yield QTLs, first reported by NABGMP, with major effects on yield under certain environmental conditions. The expected backcross gains for malt quality were realized.

A gene with strong effects on barley seed germination was previously identified on chromosome 7. However, the expression of this gene was evidently dependent on genetic background effects (epistasis). The effect of this seed dormancy QTL was investigated using the aPSR128 STS-PCR marker in two Steptoe and three Morex backcross populations. The aPSR128 QTL effects were similar regardless of recurrent parent and therefore lacked epistatic control. Unlike the reciprocal effects of minor QTLs, the aPSR128 QTL showed strong effects on seed germination that persisted during after-ripening.

## CHAPTER 1

### INTRODUCTION

#### Importance of Barley

Barley (*Hordeum vulgare* L.) is the world's fourth most important cereal crop after wheat, maize, and rice. Barley and wheat have broad ecological adaptations that set them apart from other cereals, such as corn and rice. In climatic areas where both wheat and barley are produced, production is generally determined based on end use. Wheat is utilized for human consumption, whereas barley is primarily used for feed or malt (Poehlman, 1985). Feed and malt barley production is also very important in Montana, second only to North Dakota, in its contribution to U.S. barley production. Montana farmers planted 1.3 million acres of barley in 1995, of which malting varieties accounted for 36 percent of this acreage (Montana Agriculture Statistics, 1995). Barley accounted for 6.3 percent of the total cash marketing receipts from crop and livestock commodities sold in Montana. Most of the malt barley produced in Montana is sold to out-of-state maltsters. However, most of the barley (about 64 percent in 1995) produced in Montana is utilized as a feed supplement, which in turn contributes significantly to the livestock industry which is vital to Montana.

### **Importance of Plant Breeding**

The genetic improvement of crop plants including barley through plant breeding has made a major contribution to the production of food and fiber. Depending on breeding objectives, new cultivars may have higher yields per unit area, better utilization characteristics, expanded areas of adaptation, and reduced losses to environmental stress and other pests (e.g. competitive weeds, disease, and insects). Meeting these objectives is essential to meeting the needs of an increasing human population, and to improve the standard of living in developing countries. Future plant breeding will contribute significantly to local and national economies, and the overall welfare of humankind. Plant breeding will continue to increase food and fiber productivity, and perhaps improve the availability of natural resources such as forest products and fuel (derived from fermented grains). However, it is becoming increasingly clear that many of the expected plant breeding improvements will be the result of carefully planned breeding programs which utilize scientific advances in many fields.

### Domestication of Barley

Barley domestication is an interesting story and has relevance to this dissertation in several ways. The wild ancestor of cultivated barley is well known (Harlan and Zohary, 1966). Cultivated barley shows close affinities to a group of wild and weedy barley genotypes which are traditionally grouped in *Hordeum spontaneum* (C. Koch). However *H. spontaneum* may be classified as *Hordeum vulgare* (L.) subsp. *spontaneum* (C. Koch), a wild race or subspecies of cultivated barley (Zohary and Hopf, 1988). *Spontaneum* is the only wild *Hordeum* species that is cross-compatible and fully interfertile with cultivated barley. These wild barleys are annual, brittle, two-rowed, diploid ( $2n=14$ ), predominantly self-pollinated barley forms that show seed dormancy characteristics typical of annuals growing in Mediterranean-type or desert climates (Zohary and Hopf, 1988).

Barley was found in several pre-agricultural sites in the Near East (8000-7000 BC). Remains have been found including brittle, two-rowed barley forms that are morphologically indistinguishable from present-day *spontaneum*. The earliest record of what seems to be a wild barley harvest comes from 9000 BC Tell Abu Hureyra in Syria (Hillman, 1975). By 7000 BC brittle *spontaneum* type barley was found in contexts already showing definite signs of cultivation (Zohary and Hopf, 1988). Unmistakable remains of non-brittle barley, i.e. forms that could survive only under cultivation, appear around the 8th and 7th millennium BC.

The morphological similarity between *spontaneum* and two-rowed cultivated barley is striking. They differ mainly in their modes of seed dispersal. *Apontaneum* ears are brittle and at maturity disarticulate into individual arrow-like triplets which are highly specialized structures that ensure plant survival in the wild. During domestication, this specialization broke down because non-brittle mutants were automatically selected for in the man-made system of reaping, threshing and sowing (Zohary and Hopf, 1988). Presumably, selection of the non-brittle mutants dramatically improved the yields of cultivated barley.

The distribution of the brittle rachis genes in some of these barley progenitors have been described (Takahashi, 1955). Several of these headshattering genes including *Bt1*, *Bt2* and possibly *Bt3* have been assigned to chromosome 3 (Sogaard and von Wettstein-Knowles, 1987 and Nilan, 1964).

A second major outcome of domesticating barley is the breakdown of the wild mode of seed germination (Zohary and Hopf, 1988). Most wild grasses, including virtually all of the wild *Hordeum* species (Simpson, 1990), depend on regulation of seed germination for survival through periods of drought. This is especially true for annuals growing in Mediterranean or desert climates, where germination is delayed to avoid periods of drought (Zohary and Hopf, 1988). Seed dormancy adaptations may take several forms. Short term dormancy, over a period of several months, may be used to avoid seasonal periods of cold and/or drought. This short term dormancy probably inhibits germination in a relatively large portion of the seed population. A long term form of dormancy, over a period of one or more years, may help plants survive through

catastrophically bad years of drought. This latter form of dormancy probably occurs in a smaller fraction of the seed population. In these ways, seed dormancy can be adaptive so that germination and plant development correspond with favorable environmental conditions.

A genetic mechanism must exist that can effectively delay germination in a fraction of the seed population. Under cultivation, this wild type regulation of germination is no longer advantageous. Consequently, seed dormancy mutants have been selected automatically during the domestication of barley. Owing perhaps to the value of barley to the malting and brewing industry, seed germination has been studied more extensively in barley than the other major cereal grains. It also follows that seed germination has been a tightly controlled trait in malt barley breeding programs, although few studies make a point of this. Surprisingly however, very few efforts have been made to genetically map seed dormancy genes in any of the cereal, forage, wild, or weedy grass species.

### Historical Advances in Barley Breeding

Modern efforts to improve barley by breeding, rather than selection, are seldom mentioned in the literature. 'Horsford,' released about 1880, is the first barley cultivar known to result from planned hybridization in North America (Foster, 1987). Plant breeding efforts have gradually expanded, from the turn of the 20th century, to include large sophisticated cooperative programs (Anderson and Reinbergs, 1985). The predominant type of barley grown in North America is six-rowed, and breeding programs have been in place much longer for six-rowed than for two-rowed barley. It wasn't until about 1940 that introduced two-rowed cultivars began to increase acreage in intermountain areas of Northwestern United States (Foster, 1987) including Montana. Traditionally, specific types of barley have been grown in different regions of North America.

In both the USA and Canada, improvements in six-rowed cultivars have involved many different traits (Schwarz and Horsley, 1995). In the Midwest United States, major improvements in malting quality were achieved with the release of Morex (Rasmusson and Wilcoxson, 1979). Derivations of the Manchuria-Oderbrucker type varieties originally introduced from Germany, such as Morex, continue to be grown in these regions because they have excellent malting quality. These varietal types have six-rows, rough awns, nodding heads, and moderate size kernels.

In California, varieties of the Coast type, originally brought from Africa by the

early Spanish settlers, have predominated. These Coast type varieties, such as Steptoe (Muir and Nilan, 1973), have six-rows, rough awns, and large seeds. Steptoe has exceptional yield potential and is grown as a feed variety, the primary utilization of barley in California.

In Montana, Idaho, Washington, Colorado, and adjacent regions, two-rowed varieties first introduced from western Europe have been grown for feed and malting. Feed utilization of barley is relatively more important in these regions compared to the Midwest, where corn is a more practical feed supplement. However, two-rowed malting cultivars such as Klages (Wesenberg et al. 1974) have replaced introductions from other countries.

The two most commonly grown varieties in Montana comprised over 38 percent of the barley acreage in 1995 (Montana Agriculture Statistics, 1995). The most important widely grown of these is Harrington (Harvey and Rossnagel, 1984), a two-rowed variety recommended by the American Malt Barley Association (AMBA). A recent release from the Montana Agriculture experiment station, Chinook (Blake et al. 1996), has been approved by AMBA and has the potential to replace Harrington acreage. Following Harrington in acreage is Hector (Wells, 1973), a two-rowed feed barley that has been in production many years. Baronesse, a two-rowed feed barley recently introduced from Europe, is rapidly increasing acreage and evidently replacing Hector and Harrington. Less than 5% of Montana barley acreage is six-rowed malt barley.

Advances in the understanding and measurement of the nutritional value of barley utilized as feed will probably contribute significantly to future barley improvements.

Likewise, developing locally adapted varieties with malting characteristics adjusted for export markets is currently of keen interest to barley breeders. Approaches to testing experimental lines for traits related to cereal and forage chemistry are time consuming and expensive. In my opinion, plant breeders can benefit greatly from advances in genetic analysis and genetic testing for these cryptic traits.

### Advances in the Genetic Analysis of Complex Traits

Agronomic and cereal quality traits are often affected by one or more genes, environmental conditions, and stochastic error. Likewise, seed dormancy can be dramatically affected by conditions such as temperature, moisture and light (Simpson, 1990). Moreover, seed dormancy shows variable expression in genetically identical seeds. Consequently these complex traits, most relevant to plant improvement, have been least amenable to genetic analysis at the molecular level. Perhaps this explains why so few seed dormancy genes have been mapped in grasses. The advent of molecular genetic markers has greatly facilitated the identification of the genes controlling these quantitative traits (Botstein, 1980), referred to as quantitative trait loci (QTLs). Other advances, such as the ability to produce doubled haploid lines, significantly improve the efficiency of mapping genes with additive effects.

Low copy number RFLP markers, detected using Southern analysis, are excellent tools for generating robust linkage maps (Botstein, 1980) and the study of heterogeneous genetic traits (Lander and Botstein, 1986). Their general applicability, transferability across crosses and among related species, and their efficiency in map construction have contributed to their well-earned status as markers of choice when one wishes to construct a high-quality, medium-density linkage map. This has been illustrated in virtually all the cereal crops including barley (Graner et al., 1991, Heun et al. 1991, and Kleinhofs et al. 1993), rice (McCouch et al., 1988), and corn (Helentjaris et al. 1986). Construction of

these maps has been more difficult in the polyploid crops such as cultivated oat (O'Donoghue et al. 1995). Linkage maps in wheat, which is also hexaploid, have been developed for each chromosome on a group by group basis. For example, wheat linkage maps are available for homoeologous chromosome groups 3 (Devos et al. 1992) and 7 (Chao et al. 1989).

To facilitate genetic mapping of complex traits in barley, the North American Barley Genome Mapping Project (NABGMP) developed doubled haploid mapping populations including one derived from a  $F_1$  cross of Steptoe and Morex varieties (Kleinhofs et al. 1993). These doubled haploid mapping populations have enormous advantages for the detection of quantitative trait loci (QTLs) controlling phenotype. Moreover, analysis of these populations presumably reflects the expected nature of gene action in inbred barley varieties, the type used in commercial production. In a relatively short period of time, NABGMP was able to conduct the first comprehensive genetic analysis for barley traits of agronomic, biological and industrial interest. In part, this is because the data and findings collected from these genetically fixed lines are transferrable across breeding projects and laboratories. As previously discussed, Steptoe and Morex are commercial barley varieties that have been grown in different production regions of the U.S. for three decades. These varieties show widely different adaptation and utilization characteristics. Therefore, the first NABGMP experiment has presented some interesting insights into the genetic differences between these remarkably different and useful varieties.

### Application of Genetic Analysis for Barley Improvement

One of the NABGMP goals in constructing the Steptoe x Morex doubled haploid population was to help develop improved cultivars. At least three cultivars, Mingo and Rodeum in Canada and Gwylan in Wales have been developed using the doubled-haploid technique (Foster, 1987). After the initial hybridization in 1974, Ho and Jones (1980) released Mingo only five years later, illustrating the potential benefit of obtaining homozygous lines without repeated selfing. However, as I have discussed, any modern feed or barley breeding program will have highly specific objectives. Most breeding populations are derived from narrow crosses of elite germplasm, to avoid the infusion of many less desirable genes. However, the choice of parents used in any mapping project commonly reflects the efficient use of germplasm that is expected to show the maximum number of detectable genetic differences. From the standpoint of six-rowed malt barley breeding, Steptoe is an exotic, non-elite variety. Consequently, Steptoe would be expected to contribute many undesirable genes affecting malting characteristics. Conversely, the adaptations of the Manchurian-Oderbrucker type varieties are appropriate for the relatively humid Upper Midwest. These adaptations are not useful in the arid, sun-baked West, where bountiful yields of feed grain has been the saving grace of Steptoe. Since Steptoe and Morex have such different adaptation and utilization characteristics, it is not surprising that doubled haploid commercial varieties have not been selected directly from this hybridization. Further breeding work would be required before cultivar

improvements can be achieved based on the NABGMP Steptoe x Morex experiment.

Several of the *a priori* expectations regarding the contributions of agronomic genes from Steptoe and Morex were in fact confirmed by Hayes et al. (1993). Morex contributed favorable QTL alleles for 28 out of 31 genes identified for four malt quality traits. Steptoe contributed high alleles for 5 out of 6 yield QTLs detected, with the two largest yield QTLs reported on chromosome 3. Most fortuitously, Hayes et al. (1993) failed to detect negative pleiotropic effects on malt quality associated with the chromosome 3 yield QTL regions. Based on these new findings it was hypothesized that the agronomic value of the Steptoe chromosome 3 yield QTLs could be combined with the malt quality characteristics of Morex to produce potentially valuable genotypes (Hayes et al. 1993). Few studies have explored the reliability and nature of findings rendered from these enormous data sets combined from diverse growing environments. A number of factors such as QTL x environment or epistatic interactions could add unforeseen complexities to breeding for these putative QTLs. Indeed, Hayes et al. (1993) detected significant QTL x environment interactions for the two largest yield QTL on chromosome 3. Although the largest yield QTL effect could be explained by lodging, other locations also showed yield effects and no lodging, and some locations lacked strong yield effects altogether.

Ullrich et al. (1992) utilized the Steptoe x Morex doubled haploid population to map four QTLs affecting seed dormancy in barley, one of the few studies in the literature to do so for any of the grasses. Steptoe is unusual in that this variety may express high levels of dormancy relatively uncommon among cereals, especially barley. Most

interestingly, they reported a gene on chromosome 7 that accounted for nearly 50 percent of the phenotypic variation for this trait. Oberthur et al. (1995) reported that epistasis among these four putative QTLs was an important genetic phenomenon regulating seed dormancy in this population. Epistasis, if common, could have broad ramifications for the manipulation of potentially valuable QTLs in different breeding populations.

This study will test for the continued effects of the putative Steptoe chromosome 3 yield QTLs and the Steptoe chromosome 7 dormancy gene effects in backcross breeding populations. Several aspects of gene action including epistasis, magnitude of effects, pleiotropy, and genotype x environmental interaction will be examined.

**CHAPTER 2****EVALUATION OF BARLEY CHROMOSOME 3 YIELD QTLs IN BACKCROSS BREEDING POPULATIONS****Introduction**

Southern analysis of RFLPs was used in the North American Barley Genome Mapping Project to identify the locations of numerous genes with significant effects on yield and malting characteristics (Kleinhofs et al., 1993 and Hayes et al., 1993). Thirty-one QTLs for grain quality traits including grain protein, alpha-amylase, diastatic power and percent malt extract were detected in the analysis of a doubled haploid population derived from a cross between Steptoe and Morex varieties (Hayes et al. 1993). Morex contributed positive alleles for 28 of the 31 QTLs detected for the value-added malt quality traits. These genetic effects on grain quality appeared to be stable across diverse growing conditions of Northwestern United States. Conversely, Steptoe showed positive alleles at five of six grain yield QTLs detected while Morex contributed one positive yield QTL. However, many of these yield QTLs were environmentally conditioned (i.e. the yield QTL effects varied across environments).

The two largest QTLs with positive effects on grain yield were ascribed to the

Steptoe chromosome 3. The larger of these two yield QTLs coincided closely with clearly resolved, single-interval peaks for lodging and plant height (Hayes et al., 1993). Hayes et al. (1993) postulated that plant lodging was a causal factor of the larger yield QTL on chromosome 3. However, this yield component did not explain the beneficial value of the Steptoe allele in the Montana dryland experiment where lodging was not observed.

Neither lodging nor heading date factors could be attributed to the second largest yield QTL. Although Hayes et al. (1993) reported that the yield effects of the Steptoe chromosome 3 were consistently positive, significant QTL x environment interaction terms were reported for both QTLs on chromosome 3.

Minor negative effects for grain protein and alpha-amylase were associated with a region near the second largest yield QTL effect of the Steptoe allele. However, no other negative pleiotropic effects on value-added malting characters were apparently associated with the Steptoe chromosome 3 yield QTL. Therefore Hayes et al. (1993) postulated that the Steptoe chromosome 3 yield QTLs may have useful breeding value for developing higher yielding malting varieties. They suggested the use of an offensive breeding strategy such as marker assisted selection for the Steptoe chromosome 3 yield genes, and a defensive strategy such as backcross breeding to recover the Morex alleles at numerous other QTLs for grain quality traits.

Low copy number RFLP markers, detected using Southern analysis, are excellent tools for generating robust linkage maps. Their general applicability, transferability across crosses and among related species, and their efficiency in map construction have contributed to their well-earned status as markers of choice when one wishes to construct

a high-quality medium-density linkage map. With adequate amounts of DNA, several endonucleases, and an abundant supply of clones, sufficient polymorphic information content is generally available to construct complete RFLP linkage maps (Botstein et al. 1980) using Southern analysis (Southern, 1975). The efficiency of this technique is greatly enhanced when blots are repeatedly hybridized with different clones, each identifying one or more loci. Therefore, Southern analysis can be enormously efficient in gathering segregation data when a limited number of individuals are subjected to analysis at a large number of loci. However, this technique can be cumbersome when applied to practically-oriented plant breeding programs. Fast-paced marker-assisted selection in large populations is inefficient using Southern analysis, especially if blots are needed for only one RFLP marker.

RFLPs be detected either by Southern analysis or by following amplification by PCR (Mullis, 1986). The DNA sequences of clones mapped using Southern analysis of RFLPs can be used to design oligonucleotide primers for PCR based analysis (Saiki et al. 1985 and Mullis and Faloona, 1987). The conversion of these sequence tagged sites into polymerase chain reaction based markers (STS-PCR) has the potential to facilitate plant genome mapping experiments in a variety of ways (Tragoonrung et al. 1992 and Talbert et al. 1994). In order to make these markers more practical tools in crop improvement, techniques must be available to develop STS-PCR markers which are informative in the germplasm of interest. As useful tools, these STS-PCR markers must cosegregate with the RFLP locus from which they derive, and should also provide codominant and reliable assays.

The objective of this chapter is to demonstrate the development and use of six such STS-PCR markers. The methods used will test the hypothesis that RFLP clones which were previously mapped to barley chromosome 3 (Kleinhofs et al. 1993) can be efficiently converted to codominant PCR-based markers, allelic to the RFLP markers from which they were derived, and be used effectively for plant breeding. Following that, the second objective was to use these STS-PCR markers to evaluate genetic effects and agronomic components of the putative Steptoe chromosome 3 yield QTLs. This study will investigate and describe the magnitude of effects, genetic map resolution, and possible environmental interactions associated with the putative yield QTLs previously described for chromosome 3 (Hayes et al., 1993).

## Materials and Methods

### Clones and Genotypic Assay Procedure

The polymerase chain reaction (PCR) was conducted in 50  $\mu$ l volumes of 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 0.1 mM each dNTP, 1.5 mM MgCl<sub>2</sub>, 330 nM each primer, and 0.6 unit *Taq* polymerase. Genomic DNA samples were obtained using the protocol described by Edwards et al. (1991). For amplification, the GeneAmp PCR System 9600 (Perkin Elmer Cetus, Norwalk, CT) thermocycler was programmed for 1 cycle of a 5 minute step at 94° C, then 33 cycles of three 30 second steps of 94°, 50° and 72° C, and finally one cycle of a 5 minute step at 72° C.

The clones used to design STS-PCR primers included four American Barley Genomic (ABG) clones (Table 1) described by Kleinhofs et al. (1993). Primers were also designed using wheat genomic (WG) and oat cDNA (CDO) clones (Table 1) described by Heun et al. (1991). These clones were end sequenced, near the vector insertion points, so that primer sequences (Table 1) could be selected using OLIGO software (National Biosciences, Plymouth MN). Oligonucleotide primers were synthesized using the Model 391-PCR-MATE DNA synthesizer (Applied Biosystems, Foster City, CA). By convention, the RFLP markers, such as ABG070, are distinguished from an "amplifiable" STS-PCR marker using a small case letter "a" in front of the marker locus name (e.g. aABG070).

For all PCR primer pairs shown in Table 1, except aABG070, Steptoe and Morex

amplification products were cloned and sequenced for polymorphism analysis. Barley amplification sequences, using the aCDO113 (oat) and aWG110 (wheat) primers, were also used for designing new barley STS-PCR primers specific to chromosome 3 loci (Table 1). The PCR products were cloned using the Invitrogen TA Cloning Kit (San Diego, CA) and sequenced using the Sequenase Kit (USB, Cleveland, OH) by the dideoxy chain termination method (Sanger et al. 1977). Sequence data from Steptoe and Morex STS-PCR products were analyzed using GENEPRO software (Riverside Scientific, Bainbridge WA) to help identify restriction site polymorphisms.

Polymorphisms among parents and progeny were tested for restriction site presence/absence or ribonuclease cleavage/protection of heteroduplex PCR molecules. Endonuclease digestion of STS-PCR products were typically conducted using several-fold excess of activity units and incubated overnight at temperatures recommended by manufacturers. The Mismatch Detect kit (Ambion, Austin TX) was used to screen for a sequence polymorphism using a ribonuclease protection assay (RPA) at the WG110.2 locus, using the outer and inner nested primer sets, including T7 and SP6 phage promoter sequences overhanging the 5' ends of the inner primer sets (Table 1).

### **Plant Materials**

The 50 BC<sub>1</sub> families were derived from 50 BC<sub>1</sub>F<sub>2</sub> plants by bulking seed within families through two and three generations of self pollination. This BC<sub>1</sub> population was constructed using a cross of DH72 (Steptoe chromosome 3 donor) and Morex (recurrent parent). DH72 is a doubled haploid lined derived from a F<sub>1</sub> cross of Steptoe (Muir and

Nilan 1973) and Morex (Rasmusson and Wilcoxson 1979) as described by Kleinhofs et al. (1993). The DH72 line was selected solely because the RFLP genotype, based on the NABGMP data set, indicated that it was fixed for the Steptoe chromosome 3. Otherwise, the RFLP data and recombination analysis suggest that DH72 is 50% Morex.

### **Experimental Design and Analysis**

Irrigated and dryland field trials of  $BC_1F_4$  families were conducted during 1994 at Bozeman. Irrigated and dryland Bozeman  $BC_1F_5$  trials and a  $BC_1F_5$  dryland experiment at Havre were conducted during 1995. Each randomized complete block (RCB) experiment had two replications where blocks contained 53 entries including the 50  $BC_1$  families plus 3 checks (Steptoe, Morex, and DH72). The Bozeman plots were 1.5 m<sup>2</sup> in 1994 and 3 m<sup>2</sup> in 1995 and the plots in the 1995 Havre experiment were 4.5 m<sup>2</sup>. Straw was cut using hand sickles and grain was mechanically threshed (in 1994) or plots were harvested with small plot combines (in 1995).

Seed production from several of the  $BC_1F_3$  families was insufficient to conduct the two replicated ( $r=2$ ) experiments conducted in 1994. For example  $BC_1F_3$  family #33 produced enough seed to fill one plot in the first rep of the 1994 Bozeman irrigated experiment. Otherwise the 1994 Bozeman irrigated experiment was completely balanced. The  $BC_1F_4$  family #33 and four other lines (#05, #06, #08, and #32) were completely missing from the 1994 Bozeman dryland experiment, for lack of seed. In addition, three other lines (#09, #36, and #50) produced just enough seed, in 1993, to fill two reps in the 1994 Bozeman irrigated experiment and only one rep in the 1994 Bozeman dryland

experiment. These 14 plots were filled with 'Harrington' (Harvey and Rossnagel, 1984), a two-rowed malting variety commonly grown in Montana. The three 1995 experiments were completely balanced, each containing two reps of all 50  $BC_1F_5$  families. Therefore, the total degrees freedom, over five experiments, in Table 2 was  $(5 \times 100) - 1 - 14 = 485$ .

Headshattering and lodging traits were measured using a rating scale of 0-9, where 0 was least severe and 9 was most severe. A rating of 9 would indicate that essentially no seed remained after headshatter or that all plants were completely lodged. Lodging was not observed in the 1994 Bozeman dryland trial; therefore, lodging measurements were not taken (Table 4) for that experiment. In 1994 experiments, headshattering was determined by striking three intact heads (per plot) several times, and rating grain loss. In 1995 experiments, headshattering was rated by observation of seed loss from plants and the ground litter within each plot. All headshattering and plant lodging ratings were observed several days before harvest. Average rachis internode lengths were calculated by measuring head length and dividing by the number of internodes on the ear. Analysis of variance for average rachis internode lengths was based on plot means of five plants (ears) per plot. Peduncle angle was visually estimated as the degrees below vertical (0 degrees is erect, whereas 90 degrees is horizontally lax).

MAPMAKER (Lander et al. 1987) was used to genetically map the aCDO113 and aABG070 STS-PCR markers as described below. The ANOVA procedure of MSUSTAT (Version 5.20, Richard E. Lund, Montana State University, Bozeman, MT, 59717) was used for all statistical analyses except Table 2B. PROC GLM (SAS) was used to determine the residual genetic variation nested within marker classes for Table 2B.

## Results

### STS-PCR

The molecular sizes of STS-PCR products, determined using sequencing or PAGE, were generally close to the expected results (within the margins of estimated insert sizes). However, several non-specific products were obtained from barley using the primers designed from the wheat WG110 clone (see Table 1). However, the aWG110.2 barley product is approximately the same size as the aWG110.2 product obtained using the WG110 clone as the PCR template. Interestingly, the aWG110.2 product from barley shows 83% sequence homology to the WG110 clone. The primary aCDO113 product, from barley, was larger than the 191 product obtained using the CDO113 clone as the PCR template (Table 1 and Figure 2A). However it is not possible to predict with absolute certainty the STS-PCR product size for a cDNA clone (such as CDO113), because introns may be contained within the respective genomic DNA regions.

**Table 1.** Chromosome 3 STS-PCR clones, primer sequences, predicted and observed products.

Clones	Insert sizes (bp)	Primer sequences (5'>3')	Predicted PCR sizes (bp)	Observed PCR sizes (bp)
<b>ABG070</b>	600*	ggaccaagcaaatatctcag aacacgagtttgaatttac	578*	430*
<b>ABG057</b>	1500*	ttataagcatagactgcggt gcacgagtgagctgagagt	217	217
<b>CDO113</b>	1800*	ttcgaagctccttctctt catgggaaacagcatagc	191	186 400
<b>CDO113.2 (barley)</b>	186	cattagatcaaaatgctt aggataaggccatcgta	143	143
<b>ABG396</b>	500*	gggtcacaagacggaggag aggaaacctatgtaatcatc	388	388
<b>ABG377</b>	700*	gctgctatgaggagagaacc gtgttacagccacctattc	541*	520*
<b>WG110</b>	900*	tctgatacacctccagcg acgggatccgctccacgagc	830	888 + others
<b>WG110.2 (barley)</b>	888	atacaacaggagccactaca cgaggacatcgctgagaaga	409	409
<b>WG110.2 (barley)</b>	409	<u>gataatacgactcactataggg</u> + <u>aattttctactctc</u> ** <u>agatttaggtgacactatagga</u> + ccggccttgggttc**	413	413

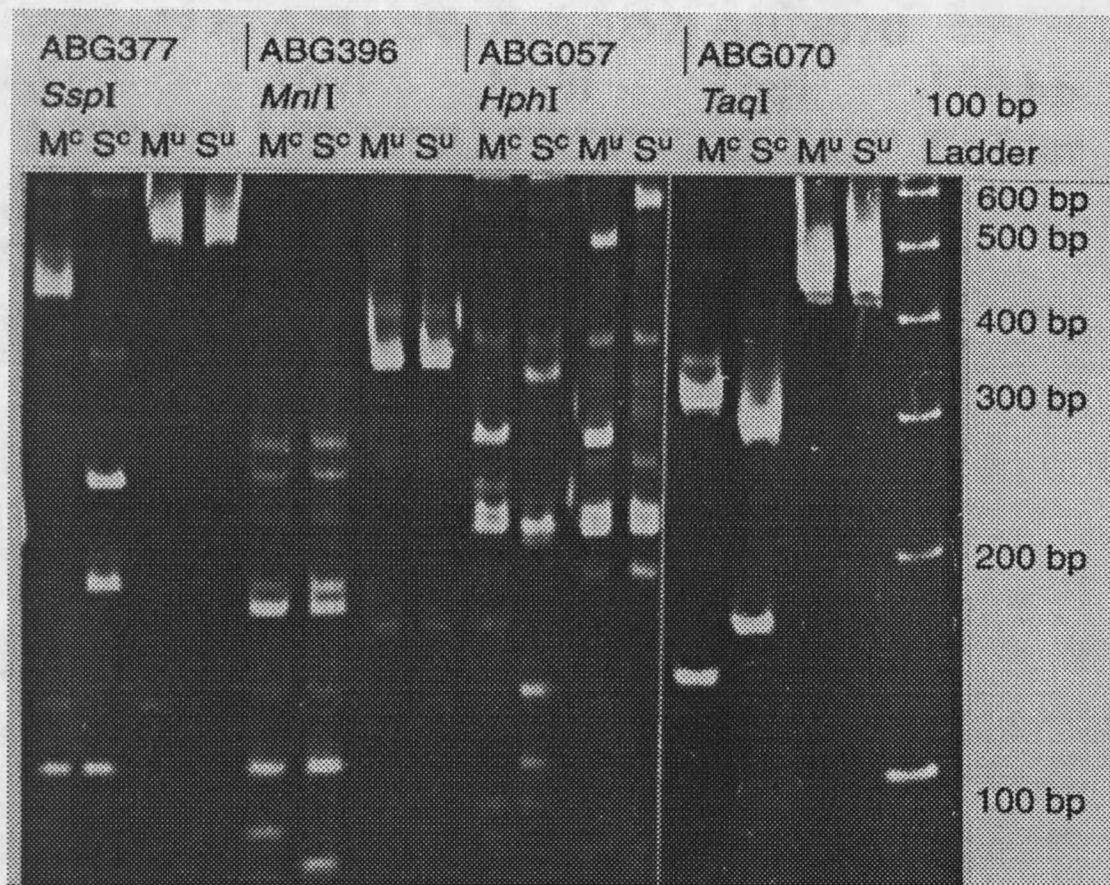
\* estimated by comparison with known size standards using PAGE

\*\* underlined sequences are phage promoter sequences (see text)

### STS-PCR RFLP Markers

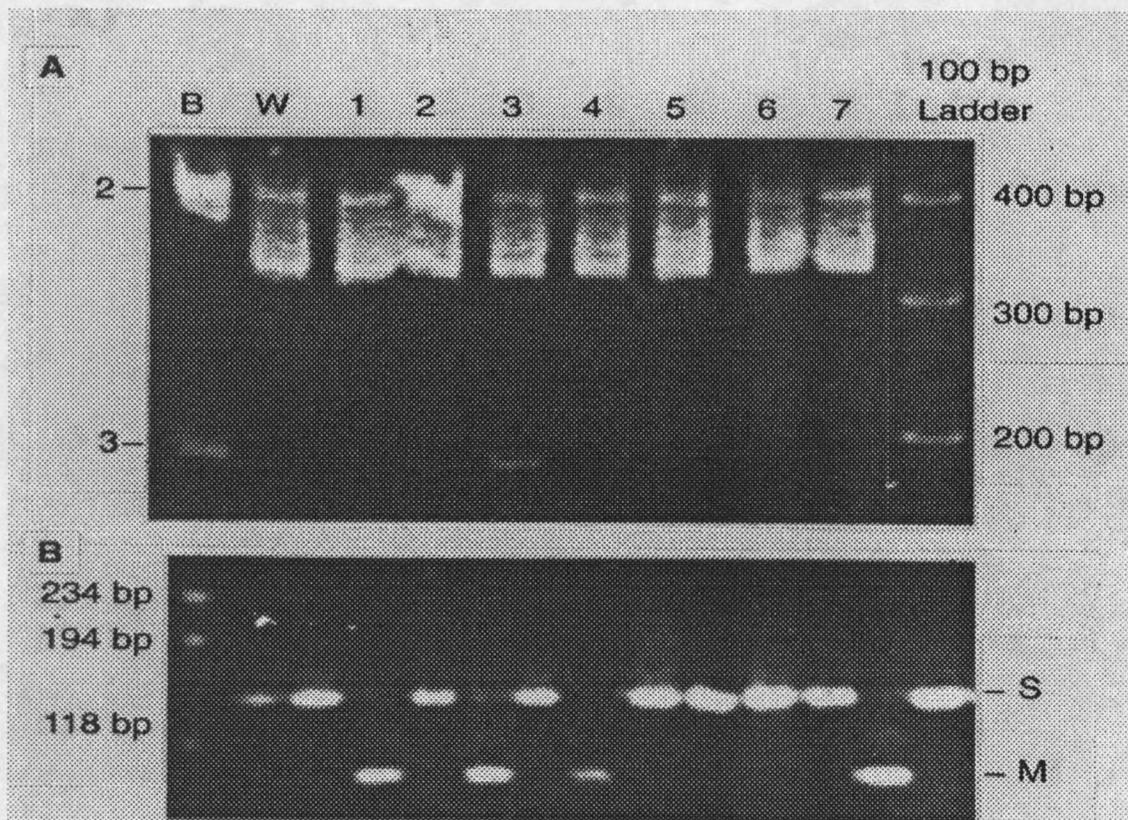
Restriction fragment length polymorphisms (RFLPs) for the STS-PCR products of the aABG070, aABG057, aABG396, and aABG377 loci are shown in Figure 1. The aABG070, aABG396 and aABG377 primers show specific products that are apparently identical in size for Steptoe and Morex. The aABG070 and aABG377 products from Steptoe and Morex show unmistakable RFLPs when digested with *TaqI* and *SspI* endonucleases, respectively. A STS-PCR RFLP, approximately 10 bp in length, was observed between two aABG396 *MnII* digestion products. However, both of these Steptoe and Morex RFLPs for aABG396 are less than 100 bp in length and may, in practice, appear as faint bands. Primers for aABG057 will reproducibly amplify a 217 bp product that contains a *HphI* endonuclease recognition sequence in Steptoe which allows its cleavage into 105 and 112 bp fragments. Depending on reaction conditions, several larger products may be observed using these aABG057 primers. Otherwise, the four STS-PCR RFLPs shown in Figure 1 are codominant. The RFLPs, predicted by sequence analysis, for aABG057 and aABG396 (Figure 1) and aCDO113 (Figure 2B) are the only confirmed restriction site polymorphisms for these Steptoe and Morex STS-PCR amplification products. In addition to the *SspI* polymorphism shown for aABG377 (Figure 1), a *HaeIII* restriction site difference also distinguishes the aABG377 products from Steptoe and Morex. The aABG70 STS-PCR amplification products were not sequenced because a screen of 26 endonucleases was sufficient to reveal seven RFLPs for seven enzymes.

**Figure 1.** Steptoe (S<sup>c</sup>) and Morex (M<sup>c</sup>) STS-PCR restriction fragment length polymorphisms (RFLPs) at four chromosome 3 loci. The products are analyzed using polyacrylamide gel electrophoresis (PAGE) stained with ethidium bromide.



The wheat-barley chromosome addition lines (Islam et al. 1981) were used to identify an aCDO113 product unique to barley chromosome 3. A faint 186 bp PCR product unique to chromosome 3 (Figure 2A) was cloned and sequenced from Steptoe and Morex so that aCDO113.2 primers could be selected. The aCDO113.2 primers amplify highly specific 143 bp products (Figure 2B). The sequence of these products shows no homology to the CDO113 clone. A one base pair sequence difference was observed between Steptoe and Morex. The Morex allele is recognized by *Bsa*MI which segregates as a codominant polymorphism among Steptoe x Morex progeny (Figure 2B).

**Figure 2 A).** Identification of aCDO113 products from barley chromosome 3 using the barley chromosome (1-7) addition lines to wheat. Betzes (B) Barley aCDO113 products are obtained from addition lines 2 and 3, but not Chinese Spring (W) wheat **B)** An aCDO113.2 *Bsa*MI RFLP segregation analysis of Steptoe x Morex doubled haploid lines.



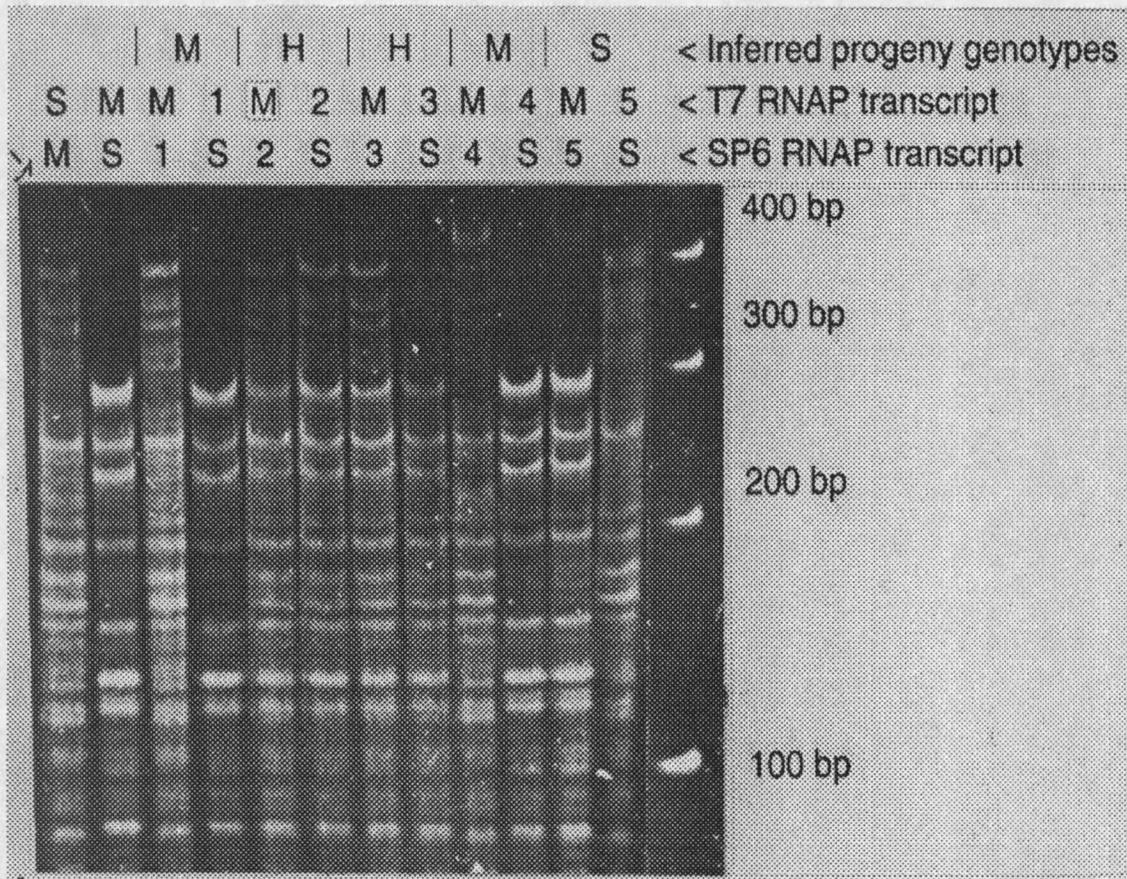
### STS-PCR RPA Marker

Wheat aWG110 primers designed from the WG110 clone (Table 1) were first tested on the wheat-barley addition lines, in order to clone and sequence Steptoe and Morex PCR products unique to chromosome 3. The approach was similar to that used for aCDO113.2 (described above). Chromosome 3 specific primers, aWG110.2, were designed from a barley aWG110 clone that evidently was specific to barley chromosome 3, based on observation of products amplified from the wheat-barley chromosome addition lines (Islam et al. 1981). The aWG110.2 primers amplified a single 409 bp product. Sequence analysis revealed a single base-pair sequence polymorphism between Steptoe and Morex that could not be detected using restriction endonucleases. A ribonuclease protection assay (Figure 3) was sensitive enough to detect a single base pair difference between the aWG110.2 STS-PCR products, using the outer and inner nested primers described in Table 1. A mismatch between the Morex T7 RNA transcript and the Steptoe SP6 transcripts is completely cleaved (second lane from the left, Figure 3). The reciprocal heteroduplex of Steptoe and Morex transcripts is not cleaved using the RNAase stock #1 (included with the Mismatch Detect I kit) as shown in Figure 3 (far left lane). However, other experiments (not illustrated here) show that both reciprocal heteroduplexes of Steptoe and Morex can be cleaved using RNAase stock #3 (Mismatch Detect II kit).

To genotype progeny, SP6 and T7 RNA transcripts from each offspring were hybridized with Morex and Steptoe T7 and SP6 transcripts respectively (see Figure 3). The SP6 transcripts of progeny homozygous for the Morex allele are protected from RNAase cleavage when hybridized to the Morex T7 transcript. The T7 transcripts of

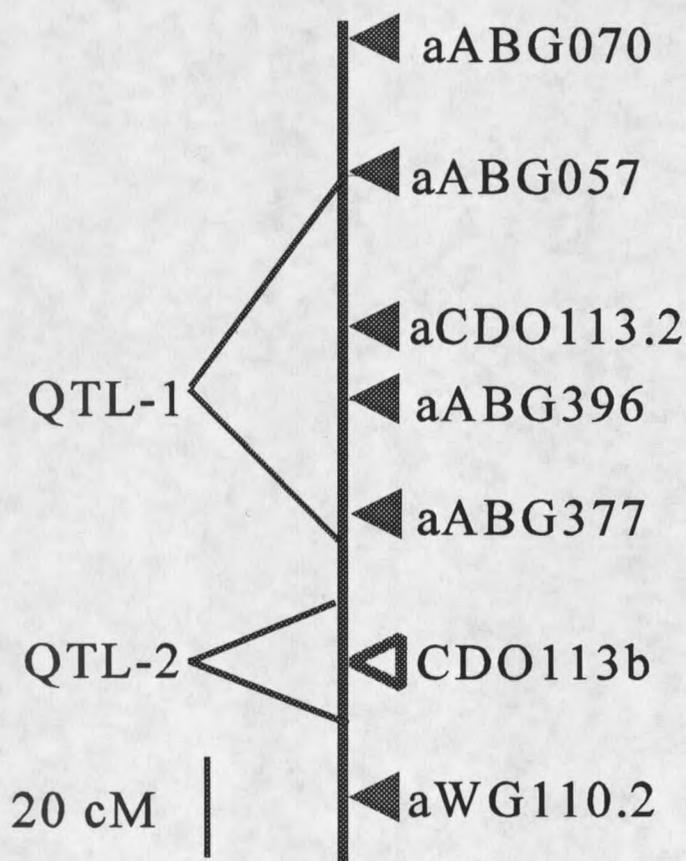
those same progeny hybridized to the Steptoe SP6 transcript are completely cleaved by RNAase. The opposite is observed for progeny homozygous for the Steptoe allele. Heterozygous progeny are partially cleaved in both hybridizations with T7 and SP6 antisense transcripts of Morex and Steptoe respectively. In this way, the aWG110.2 STS-PCR primers can also be used as a codominant marker for progeny segregating for the Steptoe and Morex chromosome 3.

**Figure 3.** A ribonuclease protection assay (RPA) used to detect and screen for a single bp sequence polymorphism between Steptoe (S) and Morex (M) aWG110.2 products. Progeny (1-5) aWG110.2 products are tested for mismatches using Morex T7 and Steptoe SP6 RNA polymerases (RNAP) to transcribe the nested products (with promoter sequence overhangs, Table 1).



### Marker Mapping

Figure 4 illustrates the approximate positions of the six STS-PCR markers when integrated with a NABGMP Steptoe x Morex data set including 434 RFLP markers. The STS-PCR markers including aABG070, aABG057, aABG396, aABG377, and aWG110.2 cosegregate with their respective chromosome 3 RFLP loci. The aABG070 marker cosegregates with barley chromosome 3 telomere sequences (A. Kilian, personal communication). The only STS-PCR marker that did not cosegregate with previous RFLP data is aCDO113.2. The second largest yield QTL was detected near the CDO113b RFLP locus (open triangle), however the aCDO113.2 mapped to a different location on chromosome 3 (Figure 4).



**Figure 4.** Linkage map of six STS-PCR markers for chromosome 3 (closed triangles). The positions of the putative yield QTLs reported by Hayes et al. (1993) are also indicated. The second largest yield effect (QTL-2) was detected using the CDO113b RFLP marker (open triangle).

### Verification of a Barley Chromosome 3 Yield QTL in a Backcross Population

The ABG396 marker on chromosome 3 showed the greatest yield effect in a previous study (Hayes et al. 1994). The analysis of variance for yield using the ABG396 marker to classify the three genotypic classes of the 50 BC<sub>1</sub> experimental lines is shown in Table 2A. In this form of RCB analysis, combined over environments, the aABG396 marker explained 8.9 percent of the residual yield variance (after fitting environments and blocks). If the non-informative, heterozygous progeny are excluded from analysis, the total S.S. drops by a factor of almost two, while the marker S.S. remains about the same (Table 2B). After one backcross, aABG396 still accounts for only half of the residual genetic variation nested within homozygous marker classes.

**Table 2 A)** Analysis of variance for yield using the aABG396 chromosome 3 marker. Results obtained from 50 Morex BC<sub>1</sub> lines evaluated over five environments.

Source	df(mis)	S.S.	M.S.	F-Value	P-Value
<b>Total</b>	485(14)	147,850			
<b><u>Env x Block Stratum</u></b>					
Environments	4	73,096	18,274	16.8	.0042
Residual	5	5,435	1,087		
<b><u>Units Stratum</u></b>					
aABG396	2	5465	2,733	20.7	<.0000
Env x aABG396	8	2293	287	2.2	.0285
Residual (error)	466(14)	61,563	132		

**B)** Analysis of variance for yield of remaining 22 entires with heterozygotes excluded.

Source	df(mis)	S.S. (Type III)	M.S.	F-Value	P-Value
<b>Model</b>	34	48,942	1,439	14.1	0.0001
<b>Error</b>	175	17,896	102		
<b>Total</b>	209	66,839			
<b>Environments</b>	4	31,398	7,849	76.8	0.0001
<b>Rep(Environments)</b>	5	2,498	500	4.9	0.0003
<b>aABG396</b>	1	4,555	4,555	44.6	0.0001
<b>Env x aABG396</b>	4	1,746	437	4.3	0.0025
<b>Entries(aABG396)</b>	20	9,121	456	4.5	0.0001

### Relation of Yield, Lodging, and Headshattering QTLs

The relation of yield, lodging, and headshattering, with regard to the chromosome 3 yield QTL was examined by the correspondance of QTL scans (Table 3) and environmental corollaries (Table 4) for these three traits. The proportion of residual variance (after fitting environments and blocks as shown in Table 2A) explained by four STS-PCR markers which showed significant marker by phenotype interaction by ANOVA are shown in Table 3. The aWG110.2 and aABG070 markers showed no significant association with the agronomic traits evaluated and are omitted from Table 3. Otherwise the percent residual variances explained (Table 3) are all significant ( $P > F$  significant at less than 0.05). The segregation ratios (homozygous Steptoe: heterozygous: homozygous Morex) of progeny for each of the markers listed in Table 3 are as follows: aABG377 (8:28:14), aABG396 (8:28:14), aCDO113.2 (4:31:15), and aABG057 (15:25:20). The segregation ratio for aCDO113.2 showed a significant deviation from the expected F2 ratio 1:2:1 using chi-square analysis. Since aABG396 showed the greatest interaction with environment for yield and is strongly associated with headshattering and plant lodging, it was selected for analysis of QTL x environment interactions and description of yield QTL breeding value as reported in the following sections (Tables 4 and 5).

**Table 3.** Percent residual variance explained for yield, headshattering, and plant lodging measured at four chromosome 3 marker loci. Results obtained from 50 Morex BC<sub>1</sub> lines evaluated over five environments.

	aABG377	aABG396	aCDO113.2	aABG057
<b>Yield</b>	6.6%	8.9%	4.7%	3.3%
<b>Shatter</b>	7.9%	13.9%	5.1%	13.7%
<b>Lodging</b>	14.1%	13.8%	5.6%	14.3%

**Table 4.** Percent residual variance explained for yield, headshattering, and plant lodging in each of five environments measured at the aABG396 chromosome 3 marker locus. Overall trait correlations of yield with headshattering and plant lodging are shown in parentheses. Results obtained from 50 Morex BC<sub>1</sub> lines.

	<b>Bozm Irr 1994</b>	<b>Bozm Irr 1995</b>	<b>Bozm Dry 1994</b>	<b>Bozm Dry 1995</b>	<b>Havre Dry 1995</b>
<b>Plant Lodging</b>	20.4% (NS)	15.4% (-.22)	NS (NS)	23.3% (NS)	NS (+.52)
<b>Headshattering</b>	6.4% (-.40)	46.5% (-.62)	12.0% (NS)	10.3% (NS)	6.1% (-.37)
<b>Grain Yield</b>	10.3%	36.5%	NS	5.4%	NS

NS = not significant

The proportion of residual variance (after fitting blocks) explained by the aABG396 marker and overall correlations of yield with headshattering and lodging (in parentheses) are reported for each trait by experiment (Table 4). The overall severity of plant lodging and headshattering was extremely variable across the five experiments. Virtually no lodging was observed in the 1994 Bozeman dryland experiment, and headshattering had to be rated using a fragility test because natural discrimination was not apparent. Conversely, ripening at the Havre location occurred so rapidly that plants appeared to be almost indiscriminately snapped, lodged, and shattered by the time plots could be harvested. Although the Steptoe marker allele at aABG396 showed reduced lodging at Havre, this putative effect was not significant (Table 4). Note however, that the putative lodging effect of the aABG396 marker in the Havre experiments was convoluted by an overall positive correlation of yield and lodging (Table 4). The strongest yield effect at the aABG396 marker was observed in the 1995 Bozeman irrigated experiment (Tables 4 and 5), where excellent discrimination of natural headshattering and

head snapping was observed. This irrigated experiment was planted 5 days later and harvested 11 days later than the 1995 Bozeman dryland experiment which showed less natural headshattering and greater overall susceptibility to a windstorm which occurred in late July.

### Magnitude of Yield QTL x Environment Effects

The breeding value of the Steptoe aABG396 marker allele in each environment and combined over environments is shown in Table 5. Breeding values are calculated as the contrast of homozygous BC<sub>1</sub> lines. Although the aABG396 marker by environment interaction term was significant ( $P > F$  significant at less than 0.05), the breeding values of the Steptoe aABG396 marker allele were consistently positive across environments. For comparison, the grain yield means of the parents are also shown in Table 5. Morex was consistently the lowest yielding parent. Steptoe was the highest yielding parent except in 1994 when Steptoe and DH72 were split across irrigated and dryland experiments.

**Table 5.** Yield (kg/ha) x environment analysis of the Morex BC<sub>1</sub> experiment.

	Means kg/ha (S.E)				Breeding Value kg /ha (S.E)
	Steptoe	Morex	DH72	BC <sub>1</sub> Pop	aABG396
<b>BozIrr94</b>	5752	4564 (507)	5962	5138 (72)	624* (212)
<b>BozDry94</b>	6569	4371 (535)	5354	4789 (75.6)	402* (239)
<b>BozIrr95</b>	7230	4817 (513)	6344	5341 (73)	1135* (199)
<b>BozDry95</b>	5629	4355 (324)	5107	4602 (46)	325 (143)
<b>Havre 95</b>	4967	2989 (391)	4704	3481 (55)	247 (168)
<b>Overall</b>	6032	4219 (205)	5494	4670 (29)	546 (88.6)

\*  $P > F$  significant at less than 0.05

### Plant Height and Heading Date

Analysis of variance for plant height and heading date showed significant effects ( $P < 0.05$ ) when tested for almost all of the chromosome 3 markers, with two exceptions indicated in Table 6. All Steptoe marker allele effects were negative, except as indicated in Table 6. The aABG396 marker explained 3.5 percent of the residual variance (within environments and blocks) for heading date. No heading date QTL marker x environmental effects were detected using analysis of variance. The aABG057 locus accounted for 18.5 percent of the residual variance (within environments and blocks) for plant height. Significant plant height QTL x environment effects were also detectable at the aABG057 locus, but not the other loci. The Steptoe aABG057 marker allele showed negative effects on plant height at each location, however the effects were more pronounced in the 1995 Bozeman experiments (about -10 cm). Note that the Steptoe allele at the aWG110.2 marker shows a positive effect on plant height (Table 6), whereas the Steptoe allele at the other marker loci show negative effects on plant height. Likewise, the Steptoe allele of the aABG070 marker showed positive effects on the Julian date of heading, however negative heading date effects were detected at the other loci.

**Table 6.** Plant height (PH) and heading date (HD) analysis of the Morex BC<sub>1</sub> experiment over five environments.

	Means (S.E.)				Breeding Values (S.E.)			
	St	Mx	DH72	BC <sub>1</sub>	aWG110.2	aABG396	aABG057	aABG070
<b>PH, cm</b>	71.9	84.9 (2.3)	82.0 (2.3)	87.9 (0.3)	2.9 (0.9)	-6.7 (0.9)	-7.1 (0.8)	NS
<b>HD, days<sup>1</sup></b>	179.4 (0.9)	181.2 (0.9)	178.0 (0.9)	178.8 (0.1)	NS	-1.3 (0.4)	-1.1 (0.3)	1.6 (0.5)

<sup>1</sup> Average Julian date given for parents

NS = not significant

### Rachis Internode Length and Peduncle Angle

Based on analysis of variance, the aABG396 chromosome 3 locus also has significant effects on peduncle angle (PA) and average rachis internode length (RIL). As shown in Table 7, the aABG396 marker explained 48 and 24 percent of the residual variance (environments and blocks) for rachis internode length and peduncle angle, respectively. The effects of the Steptoe aABG396 marker allele on rachis internode length and peduncle angle are compared to the parental means in Table 7.

**Table 7.** Rachis internode length (RIL) and peduncle angle (PA) analysis of the Morex BC<sub>1</sub> population evaluated over two and four environments for RIL and PA respectively.

	Means (S.E.)				Breeding Value (S.E.)
	Steptoe	Morex	DH72	BC <sub>1</sub> Lines	aABG396
<b>RIL (mm)</b>	3.15 (0.16)	3.46 (0.16)	2.84 (0.16)	3.20 (0.02)	-0.63 (0.07)
<b>PA<sup>o</sup></b>	1.6 (9.7)	18.1 (9.7)	2.0 (9.7)	28.3 (1.4)	-37.4 (3.9)

## Conclusions

### The Steptoe Chromosome 3 Yield QTL with Major Effects

These results confirm the positive effects of the Steptoe allele at the largest yield QTL on chromosome 3. The most probable location of this QTL is very near the aABG396 and aCDO113.2 STS-PCR loci and is flanked by two other markers, aABG057 and aABG377. The genetic map location of this yield QTL corresponds very well to the largest yield QTL effect detected in the SM NABGMP experiment. The average breeding value of the Steptoe aABG396 marker allele, over all five experiments, was 543 kg/ha. The breeding value observed in these experiments was 12.9 percent of the average yield observed for Morex. This is very similar to the previously reported values of 734 kg/ha, which was 12.8% of the average Morex yield (Hayes et al. 1993). The map position of the yield QTL is incidental with plant height, lodging and heading date QTLs, and that the yield QTL effects are strongly influenced by environment (Hayes et al. 1993).

This study demonstrates that headshattering is also a major component of the largest yield QTL. Hayes et al. (1993), reported that plant lodging was not a completely satisfactory explanation of the largest yield QTL effect associated with the Steptoe chromosome 3. Yield QTL effects were detectable for some environments where plant lodging was not observed. From Tables 4 and 5, it appears that the relative breeding value of the Steptoe chromosome 3 yield QTL is greatest when severe natural headshattering occurs. The comparison of 1995 Bozeman irrigated and dryland experiments are

especially revealing in this regard. The 1995 Bozeman growing season was cooler and wetter than usual. Consequently, other than having different planting and harvest dates (as previously discussed), only one short application of water distinguished the 1995 Bozeman irrigated and dryland experiments. Yet these differences were sufficient enough to create a more severe headshattering screen in the irrigated experiment and a more severe plant lodging screen in the dryland experiment. Based on this comparison of 1995 Bozeman experiments, it is clear that natural headshattering associated with the ABG396 marker, can have more dramatic yield effects than plant lodging. This shattering usually occurs during the latter stages of ripening and may take several forms. In some cases, the rachis internodes disarticulate or the peduncle snaps (when the Morex allele inhabits the barley chromosome 3 yield QTL). However, headshattering losses also occur when the seeds simply break away from the spike, leaving the rachis intact. In any case, these headshattering effects are associated with the chromosome 3 yield QTL first reported by Hayes et al. (1993).

Interestingly, these results also show that the major yield QTL allele on Steptoe chromosome 3 is also closely associated with a morphological gene for short rachis internode length (Table 7) and peduncle angle. This rachis internode gene may be allelic to the *erectoides* (*ert-c*) dense spike gene which maps to barley chromosome 3 (Sogaard and vonWettstein-Knowles, 1987). The headshattering effect detected in the Morex backcross population (Table 3) could also be related to this rachis internode length gene. However, it is perhaps more likely that the headshattering effect is a result of the *bt2*, *bt* genes which are separate from the *ert-c* gene (Sogaard and vonWettstein-Knowles, 1987).

If the hypothesis that *ert-c* is allelic to the rachis internode length gene is correct, then comparative mapping suggests that *bt2, bt* would be in the region of aABG396-aABG057 (Figure 4). Therefore, the putative location of the *bt2, bt* genes would seem to be consistent with the most likely map location of headshattering effects described in Table 3. The genetic map position of the lodging QTL is not clearly resolved in my experiments, and it would not be reasonable to speculate whether the putative lodging gene(s) are allelic to the genes affecting spike morphology and headshattering. It would, however, be reasonable to believe that both headshattering and plant lodging QTLs interact to produce the large yield QTL effects reported by Hayes et al. (1993) and in this study (if these QTLs are separate genes).

### **STS-PCR and Morphological Markers for Barley Chromosome 3**

This study demonstrates six codominant, reliable STS-PCR markers, five of which are allelic to the RFLP clones from which they were derived (Figure 4). Therefore, these STS-PCR primers should be robust genetic markers for barley chromosome 3.

Although the RIL breeding value effect of the Steptoe chromosome 3 is only -0.63 mm, the overall effect on spike morphology is qualitative. Introgression of the Steptoe chromosome 3 yield QTL will effectively convert the long, lax head-type of Morex into an erect, compact Steptoe-like spike (Table 7).

### The Second Largest Putative QTL

The second largest yield QTL reported by Hayes et al. (1993) was not detected in the Morex backcross experiment. This putative yield QTL was linked to the CDO113b locus between the aWG110 and aABG377 STS-PCR marker loci on Figure 1 (Hayes et al. 1993). Interestingly, this putative yield QTL coincides with a yield QTL detected in a doubled haploid population derived from a cross of European two-rowed spring barley genotypes (Thomas et al. 1995). The yield QTL reported by Thomas et al. (1995) mapped near the *denso* morphological gene, which they also mapped between the WG110 and ABG377 loci on chromosome 3 (refer to Figure 4). The yield effects that Thomas et al. (1995) observed near aWG110.2 were similar in magnitude compared to the second largest yield QTL reported by Hayes et al. (1993). Several different issues should be considered as to why this putative QTL, with the second largest yield effects (Hayes et al. 1993), was not detected in my experiments.

The BC<sub>1</sub>F<sub>2</sub> derived population used in my experiments is one third the size of the Steptoe x Morex double haploid population (including 150 lines). Approximately half of the BC<sub>1</sub> progeny are heterozygous at any given locus, therefore the effective population size of the BC<sub>1</sub> population is only about 17 percent of the doubled haploid population. This illustrates one of the advantages of using the doubled haploid technique. However, the effects of this second putative yield QTL effect should become progressively more apparent in backcross populations, compared to an equal size F<sub>2</sub> derived population. Indeed, the effects of the larger of the two putative chromosome 3 QTLs described by Hayes et al. (1993), were easy to detect in the Morex backcross experiment.

Environmental interactions may be another possible reason why I failed to detect this second putative yield QTL. Hayes et al. (1993) reported significant QTL x environmental interactions for both of the chromosome 3 yield QTLs. Therefore I may have failed to detect the second largest QTL effect because the environments tested in this study were not conducive for the expression of this gene(s). Since no other agronomic traits were associated with this putative QTL (Hayes et al. 1993 and Thomas et al. 1995), it is difficult to speculate what type of environmental conditions might favor the expression of the (unknown) underlying factors.

Recombination between the second largest putative yield QTL and my closest flanking markers, aABG377 and aWG110.2, may also have diminished my ability to detect this putative gene(s) in the BC<sub>1</sub> population. One of the only RFLP markers associated with the second largest yield QTL (Hayes et al. 1993) was the CDO113b locus (Kleinhofs et al. 1993). This locus maps in a region sparsely populated with markers, between aABG377 and aWG110.2 (Figure 4). Unfortunately, I was not able to reproduce this locus by STS-PCR, although I did obtain the aCDO113.2 marker shown in Figure 4. This result was surprising because only one chromosome 3 amplification product was observed using primers for the original CDO113 clone (Figure 2A). The only other amplification product observed clearly belonged to wheat-barley chromosome 2 (Figure 2B). Additional STS-PCR markers for the chromosome 3 minus arm would also be useful to test the validity of the second largest yield QTL reported by Hayes et al. (1993). These would certainly be of interest for breeders and geneticists working with genotypes which may carry the *denso* gene (Thomas et al., 1995).

### CHAPTER 3

## BACKCROSS GAINS FOR MALT QUALITY AND INTROGRESSION OF A CHROMOSOME 3 YIELD QTL FROM FEED BARLEY

### Introduction

Malt barley production is a valuable industry in Montana and other small grain producing states. Approximately 50% of the barley produced in Montana is utilized for commercial malts (Wilson, 1985), although only 34% of the Montana barley acreage was grown using malting-type varieties, approved by the American Malting Barley Association, in 1995 (Montana Agriculture Statistics, 1995). For historical and environmental reasons, malt barley production and utilization in Montana is distinct from that of the Upper Midwest (United States) and the central prairie provinces of Canada, which are also major barley producing regions. Most of the barley produced in Montana is the two-rowed type. In contrast, about 90 percent of barley produced in the Upper Midwest is six-rowed malting varieties (Wilson, 1985).

Unusually wet weather in many areas of United States has provided favorable conditions for proliferation of *Fusarium graminearum* especially in the wheat and barley fields of the Upper Midwest. This fungal pathogen causes scab damage or head blight and

produces the mycotoxin deoxynivalenol (DON or vomitoxin). Although DON is detectable in grain samples from many parts of the U.S., including Montana, the average level of vomitoxin in 1993 was particularly high in grain from Minnesota and North Dakota (Truckess et al. 1995). Unfortunately, infestations of mycotoxin producing *Fusarium* have persisted for several years. While steeping greatly reduces the level of naturally occurring mycotoxins, growth of mold during germination in the malting process produces several mycotoxins (Schwarz et al. 1995). The latter study clearly demonstrates the complication of *Fusarium* during the malting and brewing process. The persistence of *Fusarium* in the Midwest illustrates the potential vulnerability of any one production area.

Six-rowed malt barley is an established market class that is distinct from two-rowed malt barley (Burger et al. 1985). If scab persists in the Upper Midwest, the six-rowed malt barley market may become more accessible to barley producers in Montana, and other areas historically devoted to the two-rowed barleys. Producers in these latter regions could potentially benefit from this added value market class if properly adapted six-rowed barley varieties are available. Maltsters and brewers, in turn, could benefit from a more reliable supply of domestic six-rowed malting barley.

Malting quality characteristics in barley are strongly influenced by environment, genotype, and fertilizer management (Therrien et al. 1994). Although it might be argued that two-rowed barley is inherently more adapted to dryland production in Montana, statistics suggest that yield potentials in the major production areas of Montana are comparable to the Upper Midwest (USDA-NASS, Online). Although these data do not dispel the contention that two-rowed barley is inherently more appropriate for Montana

growing conditions, the assumption should not preclude pursuit of the six-rowed malt barley market.

Agronomic and cereal quality characteristics are often affected by one or more genes and environmental conditions. The advent of molecular genetic markers has greatly facilitated the identification of the genes controlling these quantitative traits, referred to as quantitative trait loci (QTLs). The first comprehensive study of QTLs responsible for agronomic malt quality traits was conducted by the North American Barley Genome Mapping Project (NABGMP). These QTLs are presumably equivalent to a gene or set of closely linked genes affecting one or more of these traits.

Based on a doubled haploid population derived from a cross of Steptoe and Morex in barley varieties, a medium density RFLP map (Kleinhofs et al. 1993) was used to identify 6 yield and 31 malt quality QTLs. Steptoe and Morex are widely diverged barley cultivars with different zones of adaptation and distinct end uses. Steptoe (Muir and Nilan, 1973) is a feed barley, grown primarily in the Pacific Northwest, with relatively high yield potential. Morex (Rasmusson and Wilcoxson, 1979) is the industry standard for six-rowed malt barley varieties, primarily grown in the Midwest. Morex contributed favorable QTL alleles for 28 out of 31 genes identified for four malt quality traits (grain protein, alpha-amylase, diastatic power, and malt extract). Steptoe contributed high alleles for 5 out of 6 yield QTLs detected, with the two largest yield QTL reported on chromosome 3. Most importantly, the Hayes et al. (1993) failed to detect negative pleiotropic effects on malt quality associated with the chromosome 3 yield QTL region. Pleiotropy is the term used to describe the situation where a gene affects more than one trait. Based on these

new findings it was hypothesized that the agronomic value of the Steptoe chromosome 3 yield QTL could be combined with the malt quality characteristics of Morex to produce potentially valuable genotypes (Hayes et al. 1993). The objective of this study is to test the hypothesis that the Steptoe chromosome 3 yield QTL has no negative pleiotropic effect on grain quality in Morex backcross breeding population described in Chapter 2.

## **Materials and Methods**

### **Plant Materials**

The plant materials and STS-PCR results are described in Chapter 2. The 50 backcross lines ( $BC_1F_4$  families evaluated in 1994, and  $BC_1F_5$  families evaluated in 1995), were developed by bulking seed derived from 50  $BC_1F_2$  single plants. The genotypes of these lines were assayed using six STS-PCR markers which flank the head-shattering and lodging resistance genes responsible for the positive Steptoe chromosome 3 yield effects. The chromosomal position of these markers is shown in Figure 4. The region of ABG377 to ABG057 contains the Steptoe chromosome 3 yield QTL including the head-shattering and lodging resistance QTL(s) that effectively increase yield.

### **Grain and Malt Quality Evaluation**

All grain samples were scanned for near infrared absorbance using the INFRA TEC 1225 Grain Analyzer (Perstorp Analytical, Silver Spring, MD). These scans were deposited in a database for analysis using INFRA TEC 2 Calibration Development (Version 03.10) software. This software was used, in part, to select 75 samples for grain protein calibration, by combustion method, using the FP-328 Protein/Nitrogen Analyzer (Leco Corp., St. Joseph, MI). Grain protein was expressed as percent total grain weight.

Pilot-malting was conducted using a Seeger micromalting unit. Samples from the 1995 Bozeman irrigated experiment were steeped at 12°C for eight hours and rested eight

hours in three cycles for a total of 25/24 hours. Moisture of steeped grain was adjusted to 45% by adding water. Seeds were germinated for four days at 14.5°C and 80-90% relative humidity. For kilning, temperatures were raised from 60°C to 85°C and held at 85°C for four hours. All barley and malt quality parameters were determined according to standard American Society of Brewing Chemists procedures (ASBC, 1992).

### **Experimental Design and Statistical Analysis**

Randomized complete block (RCB) experiments, with two blocks, were grown in 1994 and 1995 Bozeman irrigated and dryland sites and the 1995 Havre dryland site. The crop growth information is published in the 1994 Barley & Oat Annual Report and the 1995 Feed Crops Annual Report provided by the Montana Agriculture Experiment Station and is available from the Plant, Soil, and Environmental Science Department at Montana State University - Bozeman. The experimental mean grain yields observed in this study ranged from 5387 kg/ha, in the 1995 Bozeman irrigated field, to 3522 kg/ha, in the 1995 Havre dryland experiment.

Statistical analysis was conducted using MSUSTAT's (Version 5.20, Richard E. Lund, Montana State University, Bozeman, MT, 59717) ANOVA procedure. The analysis of variance for these RCB experiments conducted in five environments, was combined over environments. The malt quality data from the 1995 Bozeman irrigated experiment was analyzed as a single RCB design.

## Results and Discussion

Four grain quality traits including 1,000 kernel weight (1,000 KW), kernel plumpness (Kplum), test weight (TW) and grain protein (GP) were evaluated in five replicated experiments, as described above. The results for these four traits are shown in the upper portion of Table 8. Nine other malt quality traits, shown in the lower portion of Table 8, were also analyzed from micromalted samples taken from the 1995 Bozeman irrigated experiment. In addition, QTL x environmental interactions for kernel weights and plumpness are illustrated in Figures 5 and 6, respectively. The contrasts in Table 8, and QTL x environment interactions illustrated in Figure 5 and 6, are significant at  $P < 0.05$  except where noted as "not significant" (NS) in Table 8. An example of the single point, marker analyses of variance is shown in Table 9, for fine malt extract. The results for all of these grain and malt quality traits are also discussed below.

**Table 8.** Grain and malt quality analysis of the Morex BC<sub>1</sub> experiment. Results for the four grain quality traits and nine malt quality traits are shown in the upper and lower portions of the table respectively. These results were obtained from 50 Morex BC<sub>1</sub> lines evaluated over five environments for grain quality traits and one environment for malt quality traits. The standard errors are shown in parentheses. The traits listed are 1,000 kernel weight (1,000 KW), kernel plumpness (Kplum), test weight (TW), grain protein (GP), fine malt extract (FME), wort color (WCol), diastatic power (DP), alpha-amylase ( $\alpha$ -amylase), total malt protein (TMP), soluble malt protein (SMP), wort clarity (WClar), wort viscosity (WV), and wort beta-glucan content ( $\beta$ -Glu).

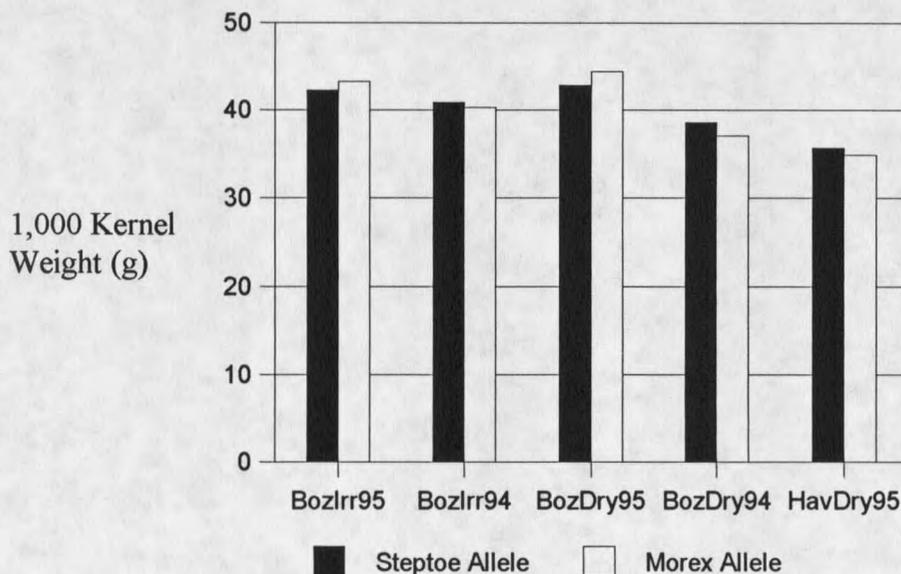
	Means (S.E.)				Breeding Value (S.E.)			
	Mx	St	DH72	BC <sub>1</sub> s	aWG110.2	aABG377	aABG396	aABG057
<b>1,000 KW, g</b>	36.7	44.7 (0.6)	42.9	40.1 (0.09)	NS	NS	NS	NS
<b>KPlum, %</b>	72.6	82.6 (2.36)	85.5	80.5 (0.33)	NS	5.77 (0.98)	5.40 (0.99)	3.45 (0.94)
<b>TW, kg m<sup>-3</sup></b>	659.8	620.6 (3.7)	636.4	646.8 (0.5)	NS	-7.2 (1.5)	-10.6 (1.5)	-8.8 (1.4)
<b>GP, %</b>	13.00	10.23 (0.169)	12.04	12.40 (0.024)	-0.36 (0.06)	-0.17 (0.08)	-0.15 (0.08)	NS
<b>FME, %</b>	79.45	76.05 (0.63)	77.20	78.29 (0.09)	NS	NS	NS	NS
<b>WCol, °ASBC</b>	2.21	1.23 (0.20)	1.59	1.91 (0.03)	NS	NS	NS	NS
<b>°DP</b>	167.5	78.0 (9.5)	113.5	132.3 (1.3)	-8.2 (3.6)	NS	NS	NS
<b><math>\alpha</math>-A, DU</b>	62.2	28.6 (2.9)	46.4	51.23 (0.4)	NS	NS	NS	NS
<b>TMP, %</b>	14.59	11.86 (0.43)	13.74	13.84 (0.06)	-0.73 (0.16)	-0.47 (0.19)	NS	NS
<b>SMP, %</b>	6.83	3.47 (0.32)	5.54	6.00 (0.05)	-0.43 (0.12)	NS	NS	NS
<b>WClar, NTU</b>	3.5	41.5 (4.1)	22.0	8.2 (0.6)	5.4 (1.5)	NS	NS	NS
<b>WV, cP</b>	1.45	1.83 (0.04)	1.50	1.50 (0.01)	NS	NS	NS	-0.03 (0.02)
<b><math>\beta</math>-Glu, mg l<sup>-1</sup></b>	236	933 (131)	443	453 (19)	NS	NS	NS	NS

### Kernel Weight

Kernel weight (1,000 KW) is a grain quality trait that has been used for selection in some breeding programs because it is a component of total grain yield (Schwarz and Horsley, 1995), and because a positive association between kernel weight and malt extract has been reported (Peterson and Foster, 1973).

Higher kernel weights were measured for Steptoe compared to Morex (Table 8). Overall, I detected no significant effect of the Steptoe chromosome 3 on kernel weights at the critical level of significance chosen in this study (i.e.  $P < 0.05$ ). However, ABG396 did show a less significant ( $P < 0.10$ ) overall kernel weight effect and both ABG396 and ABG377 showed a highly significant QTL marker x environment cross-over interaction for this trait (Figure 5). Note that the Steptoe allele showed a negative effect on kernel weight in the 1995 Bozeman experiments and a positive effect in three other experiments.

**Figure 5.** Thousand kernel weight means, by environment, for BC<sub>1</sub> progeny fixed for Steptoe and Morex aABG377 chromosome 3 marker alleles.



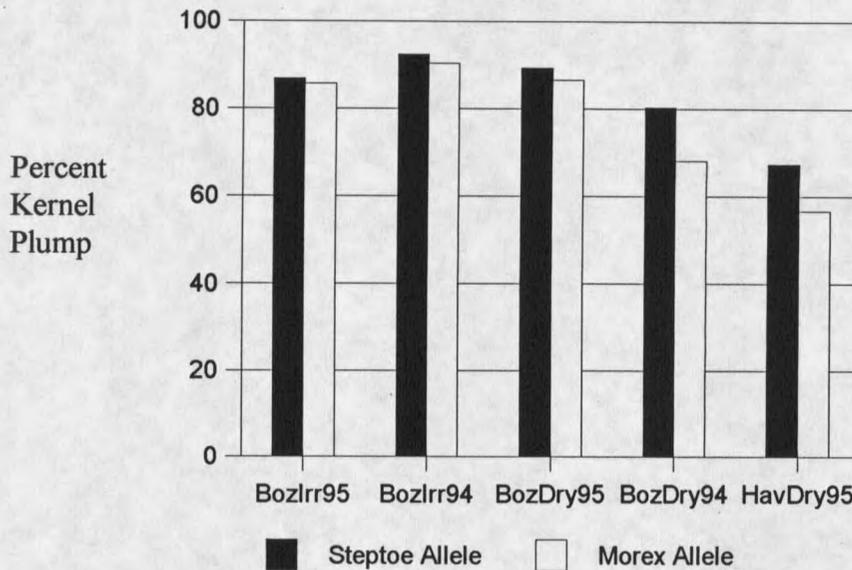
### **Kernel Plumpness**

Barley kernel size is indicative of the ratio of endosperm to total kernel size. Accordingly, other factors being equal, it bears a relation to the extract anticipated when the barley is malted and brewed (ASBC, 1992). Therefore, kernel plumpness (KPlum) is an important marketing trait for malting barley because it may be positively correlated with increased malt extract (Peterson and Foster, 1973).

We detected a strong positive effect on kernel plumpness associated with the Steptoe chromosome 3 segment spanning marker loci from ABG377 to ABG057, with the strongest effect observed at the ABG377 locus. The ABG377 locus explained 11.8 percent of the residual variance (within environments and blocks) for kernel plumpness. A significant QTL x environment interaction term was also detected for this trait. The Steptoe allele tended to show relatively higher positive breeding values in 1994 Bozeman dryland and 1995 Havre dryland experiment (Figure 6). Although these data suggest that the Steptoe chromosome 3 yield QTL may have pleiotropic effects on kernel plumpness, the effects are in a positive direction and would not appear to be detrimental.

Kernel plumpness was the only trait for which DH72, the backcross donor parent of the Steptoe chromosome 3, showed transgressive segregation (i.e. a progeny phenotypic value outside the range of parents). This suggests that Morex contributed positive kernel plumpness genes to the doubled haploid progeny DH72, in addition to the positive effects of the Steptoe chromosome 3. In theory, however, Steptoe must have contributed at least one other gene, not on chromosome 3, with positive effects on kernel plumpness to explain the loss of this transgressive effect in the backcross lines (Table 8).

**Figure 6.** Percent kernel plumpness means, by environment, for BC<sub>1</sub> progeny fixed for Steptoe and Morex aABG377 chromosome 3 marker alleles.



### Test Weight

Test weight (TW) is another grain measurement used to determine the suitability of barley for malting. It is an approximate indicator of malt extract potential. However, test weight is less accurate than kernel plumpness, as it can be influenced greatly by closeness of threshing (Peterson and Foster, 1973). Higher test weight values are obtained by skinning or hull removal which, in turn, is dependent on genotype and closeness of threshing. Test weight is essentially a density measure of weight per unit

volume (reported as  $\text{kg m}^{-3}$  in this study). The minimum test weight for U.S. No. 1 grade six-rowed malting barley is  $604.4 \text{ kg m}^{-3}$  ( $47 \text{ lb bu}^{-1}$ ).

The largest test weight effect was observed at the ABG396 marker locus which corresponds sharply with the Steptoe chromosome 3 yield QTL. This marker explained 13.2 percent of the residual variance (within environments and blocks) for test weight in the  $\text{BC}_1$  population. The effect of the Steptoe marker allele at the ABG396 locus was negative in all five experiments, and no significant test weight QTL x environmental interaction effects were detectable. The possible relationships of deciduous awns, kernel weight, kernel plumpness and grain test weight are discussed in the conclusions below.

### **Grain and Malt Protein**

Results from grain protein (GP), total malt protein (TMP), and soluble malt protein (SMP) trait measurements are reported in Table 8. In general lower protein levels are desirable in the malt barley varieties and, in fact, the American Malting Barley Association (AMBA) specifies that six-rowed barley proteins do not exceed 14%. Morex, the industry standard for six-rowed malting varieties, of course meets these criteria and breeders would not wish to introduce genes into a Morex-based breeding program that could increase protein levels.

The Steptoe chromosome 3 shows a small negative effect on grain protein and total malt protein at the ABG377 marker locus (Table 1), and all three protein traits at the aWG110.2 marker. Note that these effects are much stronger at the distal aWG110.2 marker locus. No significant grain protein QTL marker x environmental interaction term

was detected. The protein effects observed at the aWG110.2 and ABG377 loci may be the result of linkage to a single gene (QTL) affecting these three related traits, in the interval previously described (Hayes et al. 1993). These findings (Table 8) suggest that this putative gene is closer to the aWG110.2 marker locus. Therefore, the Steptoe chromosome 3 yield QTL region should dissociate by genetic recombination, away from the negative effect of the Steptoe allele at this protein QTL. Proper introgression of the Steptoe chromosome 3 yield QTL should have no pleiotropic effects on grain protein. In any case, the negative effect of Steptoe allele for this protein QTL on chromosome 3 should not be a detriment to my breeding objectives.

### Fine Malt Extract

Fine malt extract (FME) is the potential of a malt to produce wort solubles in a standard mashing regime. Wort solubles are mainly carbohydrates, but also include proteins such as  $\alpha$ -amylase and other components such as vitamins and mineral ions, etc. Fine malt extract is a valuable predictor of brewhouse yields. Consequently, this trait is of great importance to the malt barley industry and breeders. Major improvements in malt extract have been made through selective breeding (Schwarz and Horsley, 1995), exemplified by the release of Morex ("more extract", Rasmusson and Wilcoxson, 1979).

**Table 9.** Analysis of variance for fine malt extract using the aABG396 chromosome 3 marker. Results obtained from 50 Morex BC<sub>1</sub> lines evaluated in RCB design (b=2) in the 1995 Bozeman irrigated experiment.

Source	df	S.S.	M.S.	F-Value	P-Value
Total	99	80.377			
ABG396	2	1.831	0.916	1.12	.3296
Residual	96	78.275	0.815		

As expected, Morex showed significantly greater yield of fine malt extract (FME) compared to Steptoe (Table 8). The range of progeny values (79.8 to 76.7) neatly fits within the values observed between Steptoe and Morex, although some transgressive lines show values below that of their immediate parent DH72. Most importantly, no significant pleiotropic effects (positive or negative) were detected using the four chromosome 3 marker loci shown in Table 8. The analysis of variance for the ABG396 marker is shown in Table 9. This critical finding is consistent with the original study of the Steptoe x Morex doubled haploid population (Hayes et al. 1993).

### **Wort Color**

Unlike most other traits including clarity, the wort color (WCol) trait does not necessarily have a "good" or "bad" value. Wort color does, in fact, have an important characteristic effect on the final product and consumer preference. If the wort shows high clarity values, it is filtered before the color assay. Higher values indicate deeper color. High wort clarity values, on the other hand, may result in beer haze.

Although notable wort color differences are observed among Steptoe, Morex, and DH72 and in the range of BC<sub>1</sub> progeny values, no significant effects were detected at any of the chromosome 3 marker loci (Table 8).

### Enzymatic Activity

One of the main reasons why barley is used more extensively than other grains for malting is its ability to develop acceptable levels of the starch degrading, amylolytic enzymes (Loi et al. 1988). Several of the amylolytic genes including *Amy1*, *Amy2*, *Bmy1*, and *Bmy2* have been cloned, sequenced and mapped to barley chromosomes 6, 1, 4, and 2 respectively (Kleinhofs et al. 1993, Kreise et al. 1988, and Khursheed et al. 1988). Several of these loci show detectable variation for  $\alpha$ -amylase activity and diastatic power in the Steptoe x Morex population (Hayes et al. 1993). A variety of other genetic factors including modifier genes could certainly account for observed genetic variations in amylolytic activities. However, in the comparison of Steptoe and Morex, it is readily evident that a wide range of genetic variability exists even with cultivated barley varieties. Diastatic power (DP) and  $\alpha$ -amylase ( $\alpha$ -A) are two traits previously measured in the Steptoe x Morex doubled haploid population (Hayes et al. 1993) which determine the amylolytic characteristics of barley cultivars. An important hypothesis is that the Steptoe chromosome 3 yield QTL does not affect these traits.

We detected a QTL for diastatic power at the aWG110.2 marker locus that was not previously reported. However, no QTL differences between Steptoe and Morex for diastatic power or  $\alpha$ -amylase could be detected in the chromosome segment between ABG377 and ABG057 (containing the yield QTL) in this study or the previous study of this trait (Hayes et al. 1993). These findings strongly suggest that introgression of the Steptoe chromosome 3 segment, from ABG377 to ABG057, should not negatively affect the amylolytic activities of the Morex backcross breeding lines.

### **Wort Clarity**

In some cases hazy wort is thought to result in hazy beer, which may decrease consumer acceptance of brewing products. High wort clarity values also indicate the need to filter the hazy wort before color assays, as previously discussed. Wort color and clarity are distinct traits. Brewers prefer low values for wort clarity.

We detected a significant wort clarity QTL effect at the aWG110.2 locus, corresponding to QTL effects for diastatic power and protein traits. However no wort clarity effect was detected in the interval between ABG377 and ABG057 containing the Steptoe chromosome 3 yield QTL. Although Steptoe showed high wort clarity values, the BC<sub>1</sub> progeny show an average wort clarity values close to Morex (Table 8).

### **Wort Viscosity**

Excessive wort viscosity may be an indicator of incomplete cell wall modification and of subsequent problems in brewing (Schwarz and Horsely, 1995). Differences in wort viscosity among malting cultivars is typically small and no directional change has been achieved during the release of new cultivars in the past 80 years (Schwarz and Horsely, 1995). The viscosity of barley extracts is largely due to excessive residual  $\beta$ -glucan content in the barley malt (Hocket et al. 1987).

Steptoe, a feed type barley, showed a level of wort viscosity far above that observed for malting varieties (Schwarz and Horsely, 1995). DH72 showed values much closer to Morex, indicating that the backcross donor parent was already fixed for the

Morex allele for the gene(s) with major effects on wort viscosity. A small but negative wort viscosity effect was found to be associated with the ABG057 Steptoe marker allele (Table 8). The previous study (Hayes et al. 1993) had not conducted this important test. These findings suggest that the lack of complete cell wall modification observed in Steptoe will not carry through with the introgression of the Steptoe chromosome  $\beta$  yield QTL into Morex.

### Wort $\beta$ -Glucans

$\beta$ -glucans have profound effects on cell wall modification, malt extract, wort separation, and beer filtration. Mixed-linked (1-3,1-4)- $\beta$ -glucans are the major components of starchy endosperm and aleurone cell walls in barley grain (Han et al. 1995). Two (1-3,1-4)- $\beta$ -glucanases largely responsible for  $\beta$ -glucan degradation, EI and EII, have been mapped to chromosomes 5 and 1 respectively, and these can account for variation in the Steptoe x Morex cross. Other enzymes responsible for cereal  $\beta$ -glucan endo-hydrolysis are coded by the (1-3)- $\beta$ -glucanase genes which cluster near the aWG110.2 locus on the long arm of barley chromosome 3 (Li et al. 1996), and the (1-4) $\beta$ -glucanases or endo-cellulases which are not yet characterized. In addition to a myriad of  $\beta$ -glucan hydrolases, other factors such as patterns of deposition in the grain add to the complexity of malt  $\beta$ -glucan content. Therefore, no simple genetic basis for malt  $\beta$ -glucan content is expected even though much of the biochemistry is understood.

Both Steptoe and DH72 showed significantly higher  $\beta$ -glucan content remaining after the malting process. On average, the progeny showed even higher levels of

unmodified  $\beta$ -glucans remaining in the malt, compared to DH72. However, I detected no significant effects at the chromosome 3 marker loci shown in Table 8. It is not clear why backcrossing did not improve the BC<sub>1</sub> lines for wort  $\beta$ -glucan content relative to DH72. Note however, the relatively high standard errors for wort  $\beta$ -glucan content from Morex, Steptoe, and DH72 (Table 1). Note also that the wort  $\beta$ -glucan content of the BC<sub>1</sub> lines is below the midpoint of Morex and Steptoe, suggesting that backcrossing may also show gains for this trait.

The fact that wort  $\beta$ -glucan effects near the aWG110.2 marker were not detected in the Morex backcross experiment may indicate that the (1-3)- $\beta$ -glucanases (which map near aWG110.2) may not vary in the Steptoe x Morex population or that differences are too small, or too weakly linked to be detected using the aWG110.2 marker. Likewise, I failed to detect a putative malt  $\beta$ -glucan QTL in the aABG396-aABG057 interval (Han et al. 1995). These results suggest that introgression of the Steptoe chromosome 3 yield QTL into a Morex-like malting variety will not raise malt  $\beta$ -glucan levels.

### **Deciduous Awns in Doubled Haploid and Backcross Breeding Populations**

Deciduous awns describe the tendency of awns to naturally fall from the ear. The result on the grain, however, may be different from the removal of awns during threshing. When the awns naturally fall from the ear, it is often observed that the central vein portion of the kernel hull has peeled away from the endosperm, starting at the point of awn breakage. The resulting grains may imbibe water differently than grains which have been threshed free of awns (Dr. Les Wright, personal communication).

Three QTLs for deciduous awns (Figure 3) were detected in the Steptoe x Morex doubled haploid population (SM DHLs). The ABG002 and MWG503 loci on chromosome 2 explained about 25 and 10 percent of the phenotypic variation, respectively, observed in this 1995 Bozeman experiment. The breeding value of the Steptoe ABG002 marker allele was -3.3 (i.e. BC<sub>1</sub> individuals homozygous for the Steptoe allele showed less deciduous awns than homozygous Morex lines). The ABG399 locus on the SM DHL chromosome 3 map (Figure A) explained about 20 percent of the remaining phenotypic variation. The breeding value of the Steptoe ABG399 marker allele was -2.7. The ABG399 locus essentially cosegregates with the ABG396 marker.



The MAPMAKER/QTL scan for deciduous awn can be compared to results obtained in the BC<sub>1</sub> population (evaluated using visual rankings, in the two 1995 Bozeman experiments and the 1994 Bozeman dryland experiment). Table 10 shows a single point marker scan of percent residual variance explained (within environments and blocks). It is important to note that the Steptoe allele showed increased deciduous awn effects at the aWG110.2 locus, and favorable decreased deciduous awn effects at the aABG377, aABG396, aCDO113 and aABG057 loci. Evidently, the undesirable Steptoe effect at the aWG110.2 locus, detected in the BC<sub>1</sub> population, was not expressed in the 1995 Bozeman field trial of the Steptoe Morex doubled haploid population.

**Table 10.** Percent residual variance explained for deciduous awns measured at six chromosome 3 markers. Results obtained from 50 Morex BC<sub>1</sub> lines evaluated over three environments.

TRAIT	aWG110.2	aABG377	aABG396	aCDO113	aABG057	aABG070
Deciduous Awns	3.7%	1.7%	8.6%	2.2%	13.2%	NS

NS - Not Significant at P<.05

### Conclusions

It was originally suggested (Hayes et al. 1993) that the Steptoe chromosome 3 may have useful breeding value for improving the agronomics of a Morex-like variety. The previous study (Hayes et al. 1993) analyzed grain protein,  $\alpha$ -amylase, diastatic power, and malt extract. In addition to the traits which were previously analyzed (Hayes et al. 1993), several other traits including kernel weight and plumpness, test weight, wort color and clarity, wort viscosity, and wort  $\beta$ -glucan content, and total malt protein and soluble malt protein were measured in the Morex backcross experiment.

The Morex backcross experiment failed to detect significant negative pleiotropic effects associated with the Steptoe chromosome 3 yield QTL for the nine malt quality traits assayed (lower portion of Table 8). However, a grain protein QTL effect between the aABG377 and aWG110.2 markers previously reported by Hayes et al. (1993) was verified in the Morex backcross experiment. The backcross experiment also demonstrates that this grain protein effect also changes total malt protein and soluble malt protein. The coincidence of these pleiotropic QTL effects for grain protein, total malt protein, and soluble malt protein along with diastatic power and wort clarity (Table 8) suggests that all of these traits may be affected by a single common gene located on the distal portion of chromosome 3 (long arm), near the aWG110.2 locus. Although failure to detect pleiotropic effects in the yield QTL region does not refute the possibility that differences may exist, in at least several other cases (e.g. diastatic power, wort clarity, and all three

protein traits), significant QTL effects were detected at the aWG110.2 locus (unlinked to the yield QTL of interest). These effects observed at the aWG110.2 locus are near the lower end of meaningful differences in gene effect magnitudes that these experiments were designed to detect. In general, coefficients of variation near 1% (except for the wort color, wort clarity, and wort B-glucan traits where the C.V.s approached 10%) reflect the high power of the marker loci hypothesis tests in this experiment. It is acknowledged that these analyses do not accurately account for recombination between the markers and QTLs. Yet, based on the results of the Morex backcross experiments, the introgression of the Steptoe chromosome 3 yield QTL (into Morex) should not adversely affect the nine malt quality traits measured in this study, and previous studies (Han et al. 1995, and Hayes et al. 1993).

This study elucidates changes in grain quality characteristics that may occur during the introgression of the Steptoe chromosome 3 into a Morex background genotype. The Steptoe yield enhancing gene showed pleiotropic effects of increased kernel plumpness and decreased test weights (Table 8) in all five experiments, and a QTL x environment crossover interaction for kernel weights (Figure 5). The Steptoe chromosome 3 marker loci, aABG377 and aABG396, showed negative effects on kernel and test weights in the 1995 Bozeman experiments, where the positive kernel plumpness effect of the Steptoe marker allele at the aABG377 locus were almost negligible (Figure 6). Therefore, no quixotic theory is necessary to explain the lack of perfect, positive correlation among the pleiotropic effects of this QTL region in the 1995 Bozeman experiments. However, the Steptoe chromosome 3 QTL allele showed positive effects on kernel weight, and

particularly kernel plumpness, in the 1994 Bozeman dryland and 1995 Havre dryland experiments where the test weight effect was, again, negative. The negative correlation of QTL effects for test weight compared to kernel weight and plumpness, obtained in the Havre and 1994 Bozeman dryland experiments, are contradictory. A positive correlation between these traits is generally anticipated. Evidently this chromosome contains a gene or several genes with complex effects on kernel development and morphology. Perhaps the headshattering resistance and reduced deciduous awn effects, associated with the Steptoe chromosome 3, also confer reduced skinning and hull removal. Resistance to skinning or hull removal during threshing could significantly decrease test weights (Peterson and Foster, 1973). These findings lend support to the hypothesis that greater hull and awn retention cause lower test weights associated with the Steptoe chromosome 3 yield QTL. Generally speaking, the increased kernel plumpness observed with the introduction of the Steptoe chromosome 3 should be of positive value and a better predictor of malt extract yield than the corresponding negative test weight effects associated with this Steptoe chromosome region. The increased kernel weight and plumpness effects, observed in the Havre and 1994 Bozeman dryland experiments but not in the 1995 Bozeman experiments, may indicate that the expression of this positive Steptoe QTL effect is related to moisture limiting growth conditions.

The higher kernel weights and kernel plumpness observed for Steptoe may actually confer a yield advantage over Morex. However, the inverse patterns of QTL x environmental interaction observed for kernel weights (Figure 5) vs. grain yield traits (Chapter 2) suggest that kernel weight is not an important yield determinant of the Steptoe

chromosome 3 yield QTL. For example, the Steptoe chromosome 3 yield QTL showed its strongest effects under high yield conditions such as the 1995 Bozeman irrigated experiment (Chapter 2), whereas this QTL allele showed negative effects on kernel weights in the same experiment.

For all traits except kernel plumpness, DH72 showed trait values in between its parents. This result was not surprising, since DH72 was selected as the backcross donor parent, solely based on the fact that its RFLP genotype (Kleinhofs et al. 1993) indicated that it carried the entire Steptoe chromosome 3. Likewise, the BC<sub>1</sub> lines showed average values, for all traits except  $\beta$ -Glucan, between their parents, Morex and DH72 (see Table 8). In general, this is anticipated since no selection (genotypic or phenotypic) was practiced before these progeny were evaluated. For virtually every trait, individual BC<sub>1</sub> segregants showed transgressive phenotypic values (outside the range) relative to their parents, Morex and DH72. Therefore, effective recovery of most the grain and malt quality characteristics (with the possible exception of test weight) of Morex will be achieved with continued backcrossing and marker assisted selection for the Steptoe chromosome 3 yield QTL. Under certain environmental conditions, the pleiotropic effects of the Steptoe chromosome 3 on kernel plumpness may even have favorable effects.

Molecular markers greatly enhance our ability to genetically dissect complex agronomic and cereal quality traits and increase our understanding of these important characteristics. For the breeding objective outlined in this project, significant breeding gains may already have been achieved using a combination of backcrossing and marker assisted selection for the Steptoe chromosome 3 yield QTL, as discussed in Chapter 5.

## CHAPTER 4

**GENETIC ANALYSIS OF BARLEY A CHROMOSOME 7 SEED DORMANCY  
QTL IN RECIPROCAL BACKCROSS POPULATIONS****Introduction**

Dormancy is an adaptive trait that optimizes the distribution of germination over time within a population of seeds. At least part of this delay in germination is genetically controlled (Simpson, 1990). Early genetic investigations (Burass and Skinnes, 1984) revealed that seed dormancy in Scandinavian barleys was governed by several recessive, nucleoplasmic loci with high heritability. More recent genetic analyses of  $F_1$ -derived doubled haploids (SM DHLs) from a cross of Steptoe and Morex barley varieties identified four QTLs with significant effects on dormancy (Oberthur et al. 1994). Morex is the U.S. industry standard for 6-rowed barley malting varieties, and is therefore characteristic of varieties which exhibit no dormancy and readily germinate in uniform fashion. Under humid conditions, delayed harvest of these varieties may lead to pre-harvest sprouting which can ruin grain quality and value. The Steptoe variety, conversely, is a feed type barley which presumably was bred for high yield potential and can exhibit high levels of seed dormancy. Therefore the SM DHL population offered a nice system to

study of barley seed dormancy.

One of the QTLs identified by Ullrich et al. (1992) and Oberthur et al. (1995), near the PSR128 marker locus, accounted for approximately 50% of the phenotypic variation for dormancy in replicated trials of the SM DHL population. This locus showed major effects on seed germination of progeny class means, especially when the Steptoe allele inhabited the dormancy QTL near the ABG390 marker (Oberthur et al. 1995). Based on the observation that the PSR128 seed dormancy QTL showed significant interaction with other QTLs, Oberthur et al. (1995) suggested that epistasis is a significant genetic component of seed dormancy in barley. A model for epistasis was presented, stating that the PSR128 dormancy QTL regulated the expression of genes near Amy2 and ABG390 on chromosomes 1 and 7, respectively. If indeed epistasis is occurring among these QTLs, the results of seed dormancy analysis in the SM DHLs (Oberthur et al. 1995) suggest that the PSR128 dormancy QTL will have less effect in a Morex BC population than in a Steptoe BC population. This hypothesis could have broad ramifications for using QTLs in plant breeding, since the effect of economic QTLs may be germplasm-dependent for reasons unrelated to the putative QTLs *per se*. Oberthur et al. (1995) also reported a "dramatic" interaction of the PSR128 seed dormancy QTL with postharvest after-ripening treatments. Another recent study (Han et al. 1996) verified the dormancy QTLs near the PSR128 and ABG390 loci, and reported evidence of epistasis and cytoplasmic effects for barley seed dormancy. The Steptoe cytoplasm had positive effects on seed germination in one reciprocal cross and negative effects in another reciprocal cross.

A major objective of this study was to verify the breeding value of a major

dormancy QTL using STS-PCR markers for the PSR128 locus on chromosome 7 in backcross breeding populations. The markers identified in this study should be of practical value for the modification of seed germination characteristics in barley. Use of these markers also enabled us to empirically test the hypothesis that complex epistatic interactions are important genetic effects related to seed dormancy in barley. If epistatic gene action occurs among these putative QTLs, then the PSR128 locus should have less effect in a Morex BC than in the reciprocal Steptoe BC. That is, the breeding value of the PSR128 locus may be dependent on interactions with other nuclear and possibly cytoplasmic genes. Since the putative cytoplasmic effects reported by Han et al. (1996) could significantly affect the results of my experiments, it was also appropriate to fully explore their hypothesis using data from the original SM DHL seed dormancy experiment (Ullrich et al. 1992). Another objective of this study was to closely examine and describe the nature of the QTL x after-ripening treatment interaction (Oberthur et al. 1995) and investigate the hypothesis that the PSR128 QTL is somehow directly involved with extended dormancy and after-ripening in seed populations.

## Materials and Methods

### Plant and Seed Materials

The Steptoe backcross (BC) populations were constructed using DH72 and DH177 as donor parents of the Morex PSR128 dormancy QTL. The reciprocal Morex backcross populations were constructed using DH167, DH70 and DH181 as donor parents of the Steptoe PSR128 dormancy QTL. A genetic description of these populations with regard to the four dormancy QTLs was reported by Ullrich et al. (1992) and Oberthur et al. (1995), and their cytoplasmic genotypes (kindly provided by Dr. Patrick Hayes), is provided in Table 11. The donor parents are  $F_1$  derived doubled haploid lines from a cross of Steptoe and Morex, described by Kleinhofs et al. (1993). The registered barley varieties, Steptoe (Muir and Nilan, 1973) and Morex (Rasmusson and Wilcoxson, 1979), were used as recurrent female parents.

Experimental lines including 50  $BC_1F_3$  plant families from each of the five BC populations (Table 11), were grown at Bozeman in 1995 (dryland site). The 250  $BC_1F_3$  families were planted (May 18, 1995) as single-rowed plots derived from individual  $BC_1F_2$  plants grown during the summer of 1994. Three of the five populations were also advanced one generation, during the winter of 1994-1995 so that 150  $BC_1F_4$  families were also planted on May 18, 1995 with the  $BC_1F_3$ s. The  $BC_1F_4$  families were constructed from bulked seeds taken from seven  $BC_1F_3$  plants derived from seven seeds sampled from each  $BC_1F_2$  plant for the three, advanced  $BC_1F_4$  populations identified in

Table 11. The BC<sub>1</sub>F<sub>4</sub> and BC<sub>1</sub>F<sub>5</sub> seed was harvested August 30 using a hand sickle and immediately threshed and frozen at -20° C the same day of harvesting. The seed samples were stored at -20° C until germination tests were conducted. The number of days of postharvest after-ripening were counted from the date that seed samples were removed from the freezer. Germination tests were then started using sub-samples of 100 kernels immediately (0 days after-ripening) and at approximately 20 and 50 days after-ripening (as detailed in Table 13).

### Clones and Genotypic Assay Procedures

The clones used to design STS-PCR primers include the American Barley Genomic clone ABG391 (Kleinhofs et al. 1993) and the wheat cDNA clone PSR128 (Chao et al. 1989). These clones were sequenced and STS-CPR primer sequences were selected:

aABG391) catcaactcaatgcaagtg and cggatgaattccgtgcatt,

aPSR128) aactttaagattacaacaca and tacgtccatcctcttca.

The small letter "a" in front of the locus name, designates an "amplifiable" STS-PCR marker. Graingenes (<http://probe.nalusda.gov:8300/cgi-bin/dbrun/graingenes>), shows primer sequences for both of these loci. However the aPSR128 primer sequences employed in this study are designed from a different sequence of the same clone.

Polymerase chain reactions (PCR) were conducted in 50 ul volumes of 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 0.1 mM each dNTP, 1.5 mM MgCl<sub>2</sub>, 330 nM each primer, and 0.6 unit *Taq* polymerase. PCR assays were conducted as follows:

primary denaturing step at 94° C for 5 minutes, then 33 cycles of 94° C, 50° C and 72° C steps of 30 seconds each, and a final elongation step of 72° C for 5 minutes. Aliquots of 10 to 20  $\mu$ l PCR product were typically digested with 3 units of restriction enzyme overnight to assure complete digestion. PCR products and restriction digests were analyzed on 6% polyacrylamide gels stained with ethidium bromide.

The progeny genotypes were assayed using a bulk of leaf tissue collected from not less than seven BC<sub>1</sub>F<sub>3</sub> plants for each unique BC<sub>1</sub>F<sub>2</sub> derived family, following the protocol of Edwards et al. (1991). Therefore, these composite genotypes have a high probability of recreating the BC<sub>1</sub>F<sub>2</sub> single plant genotypes, especially using PCR analysis. Because these BC<sub>1</sub>F<sub>2</sub> plants were derived from F<sub>1</sub> crosses of doubled haploid donor parents and appropriate recurrent parents, the expected segregation ratio of three possible genotypes (homozygous Steptoe allele, heterozygous, or homozygous Morex) is 1:2:1. At the stage of phenotypic evaluation, families classified as heterozygous are expected to segregate 1:2:1 (i.e. within BC<sub>1</sub>F<sub>3</sub> families derived from heterozygous F<sub>2</sub> plants) or 3:2:3 (i.e. within BC<sub>1</sub>F<sub>4</sub> families derived from heterozygous F<sub>2</sub> plants). The mixture of genotypes in families classified as heterozygous greatly reduces my ability to detect meaningful genotype x phenotype interactions (i.e. dominance deviations, of the truly heterozygous plants, are diminished by the average effect of the two homozygous genotypes present in these families). Therefore the families classified as heterozygous were excluded from experimental analysis.

### Experimental Design and Statistical Analysis

Germination tests were conducted on 100 seeds seated between four disposable, absorbent towels saturated with tap water. Four stacks of two 100 kernel samples were placed on a sheet of wax paper, and incubated at 20° C under a 12-hour day length (AOSAP, 1970). After 7 days, the number of dormant seeds was counted in each sample of 100 kernels so that percent germination values could be calculated by subtraction. Seeds were classified as nondormant if the coleoptile emerged through the kernel hull.

The actual experimental design was somewhat complex, however Table 1 and Table 13 (in the appendix) will help explain the form of ANOVA (Table 12). The number of progeny families analyzed (Tables 12 and 13) was first determined by the elimination of heterozygous  $BC_1F_2$  genotypes for the aPSR128 dormancy QTL marker. The 1995 field grow outs included a  $BC_1F_3$  generation for all populations, and a  $BC_1F_4$  generation for a subset of three populations, as shown in Table 13. Germination tests were conducted at two levels of after-ripening for all populations and three levels for only three populations, also detailed in Table 13. Although 500 germination tests were initiated (Table 13), 28 tests were interrupted by human mistakes which left 471 total degrees freedom (Table 12). The analyses of variance and calculations of progeny class means were computed using the GLMODEL procedure of MSUSTAT (Version 5.20, Richard E. Lund, Montana State University, Bozeman, MT, 59717) and PROC GLM of SAS (for Microsoft Windows, release 6.10). Although replication of lines was not conducted, the aPSR128 treatments (the parameter of interest) were effectively replicated by progeny nested within homozygous PSR128 genotypic classes.

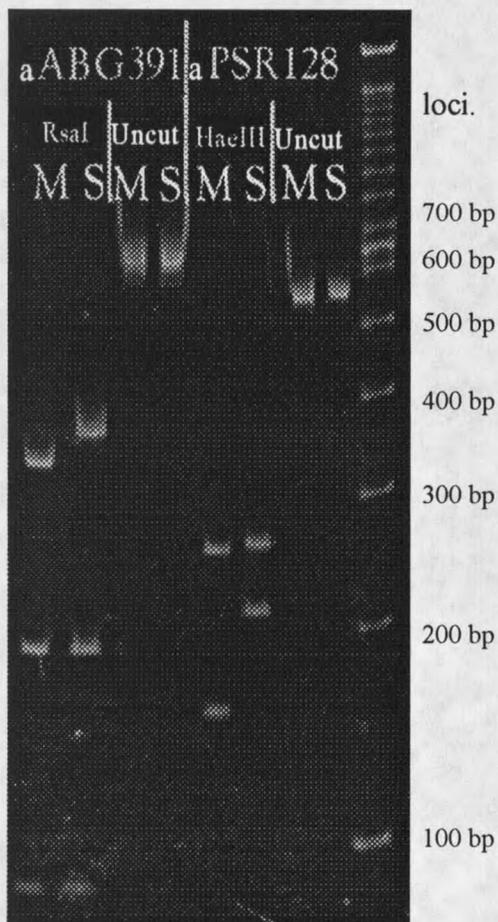
**Other experimental data**

To compare results of the reciprocal backcross experiments to previous studies (Oberthur et al. 1995 and Han et al. 1996) genotypic and phenotypic data from the Steptoe x Morex doubled haploid population was utilized. The results of seed germination tests (Ullrich et al. 1992 and Oberthur et al. 1995) were kindly provided by Dr. Laura Oberthur of Montana State University. The genotypic data (Kleinhofs et al. 1993) from the original Steptoe x Morex doubled haploid population was obtained from Dr. Tom Blake (Montana State University). For reexamination of the cytoplasmic effects reported by Han et al. (1996), cytoplasmic classifications of the Steptoe x Morex doubled haploid progeny was kindly provided by Dr. Pat Hayes of Oregon State University.

## Results

### STS-PCR RFLPs and Genetic Analysis

Two codominant STS-PCR markers for two seed dormancy QTLs on barley chromosome 7 (Ullrich et al. 1992) are shown in Figure 8. Both of these markers cosegregate with their respective RFLPs in the SM DHL population. The aPSR128 marker is closely linked to the QTL with the largest effects (Ullrich et al. 1992).



**Figure 8.** Steptoe (S) and Morex (M) STS-PCR RFLPs at two chromosome 7

loci.

700 bp

600 bp

500 bp

400 bp

300 bp

200 bp

100 bp

The results of the genetic analysis using STS-PCR RFLPs (Figure 8) are shown in Table 11. Cytoplasmic and nucleoplasmic genotypes, as determined by female recurrent parent and RFLP genotypes of the doubled haploid donor parents, for other reported seed dormancy QTLs with smaller effects are also shown. Approximately half of the 250 BC<sub>1</sub>F<sub>2</sub> progeny were heterozygous at the aPSR128 locus, as expected based on the expected 1:2:1 segregation ratio. When the heterozygous progeny were excluded from further analyses, the remaining aPSR128 segregation ratio was 46:80 (Morex: Steptoe), indicating that significantly more progeny were fixed for the Steptoe allele (Table 11). The segregation ratios in the two Steptoe and three Morex reciprocal backcross populations were 14:33 and 32:47, respectively, which favors the Steptoe allele in both cases with significant overall bias in the two Steptoe backcross populations.

**Table 11.** Segregation ratios of the aPSR128 chromosome 7 marker, for a QTL with major effects on seed dormancy, in five BC<sub>1</sub> populations. Cytoplasmic and nucleoplasmic genotypes for the other seed dormancy QTLs with smaller effects are also shown.

Population	Cytoplasm	Amy2	BCD402	aPSR128 <sup>1</sup>	aABG391 <sup>1</sup>
Steptoe//DH72	Steptoe	Steptoe	ND <sup>2</sup>	9:15	Steptoe
Steptoe//DH177	Steptoe	Steptoe	Steptoe	5:18*	Steptoe
Morex//DH167	Morex	Morex	Morex	6:15*	Morex
Morex//DH70	Morex	ND <sup>2</sup>	Morex	22:15	15:10
Morex//DH181	Morex	ND <sup>2</sup>	Morex	4:17*	Morex

<sup>1</sup> Ratio of progeny fixed for Steptoe: Morex alleles

<sup>2</sup> Segregation not determined

\* Significant deviation from 1:1 using chi-square analysis.

### Analysis of variance

Based on analysis of variance, Table 12 indicates that main effects for recurrent parent, aPSR128, and after-ripening treatments are all highly significant when analyzed over the five backcross populations. Interactions of recurrent parent with after-ripening treatments and PSR128 with after-ripening were both significant (Table 12).

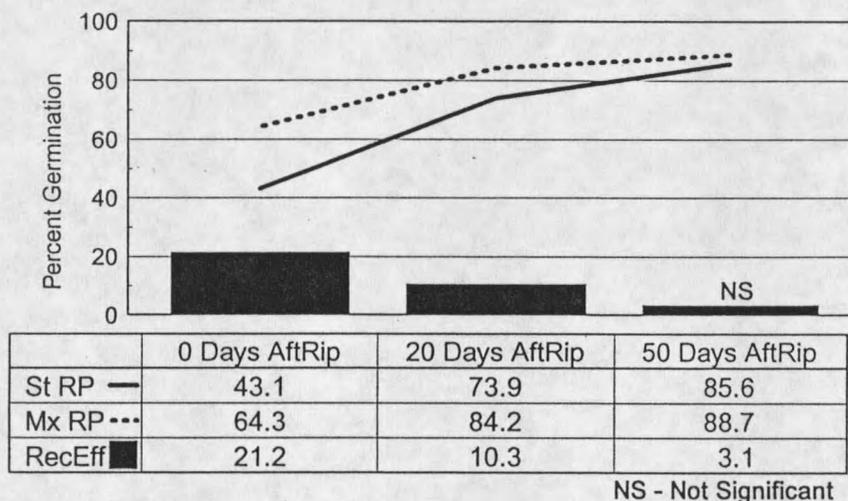
**Table 12.** Analysis of variance for percent seed germination using the aPSR128 marker, recurrent parent and after-ripening treatments as factors. Results obtained from five backcross populations (n=208) evaluated as shown in Table 13.

Source	df (mis)	S.S. (Type III)	M.S.	P-Value
<b>Total</b>	471 (28)	294,620		
<b>Recurrent parent</b>	1	9,796	9,796	<0.0001
<b>aPSR128</b>	1	28,078	28,078	<0.0001
<b>After-ripening</b>	2	68,384	34192	<0.0001
<b>Recurrent parent x aPSR128</b>	1	15	15	0.8112
<b>Recurrent parent x After-ripening</b>	2	4,162	2,081	0.0003
<b>aPSR128 x After-ripening</b>	2	2,965	1,482	0.0033
<b>aPSR128 x After-ripening x Recurrent Parent</b>	2	640	320	0.2880
<b>Error</b>	460 (28)	117,880	256	

### Recurrent Parent Effects

Figure 9 illustrates the genetic x environmental interaction of recurrent parent genotype (e.g. Steptoe or Morex) effects with after-ripening treatment which is supported by the analysis of variance (Table 12). At zero days after-ripening, Morex BC progeny showed a 21.2% higher level of germination than Steptoe BC progeny. However, this reciprocal effect (measured by the bar graph) declines as percent germination increases during after-ripening. After 50 days storage at room temperature, the recurrent parent effect is not statistically significant. The average recurrent parent effect, over three levels of after-ripening, was 11.5%. The 50 day germination tests were conducted for only three of the 8 populations tested at 0 and 20 days afterripening (Table 13).

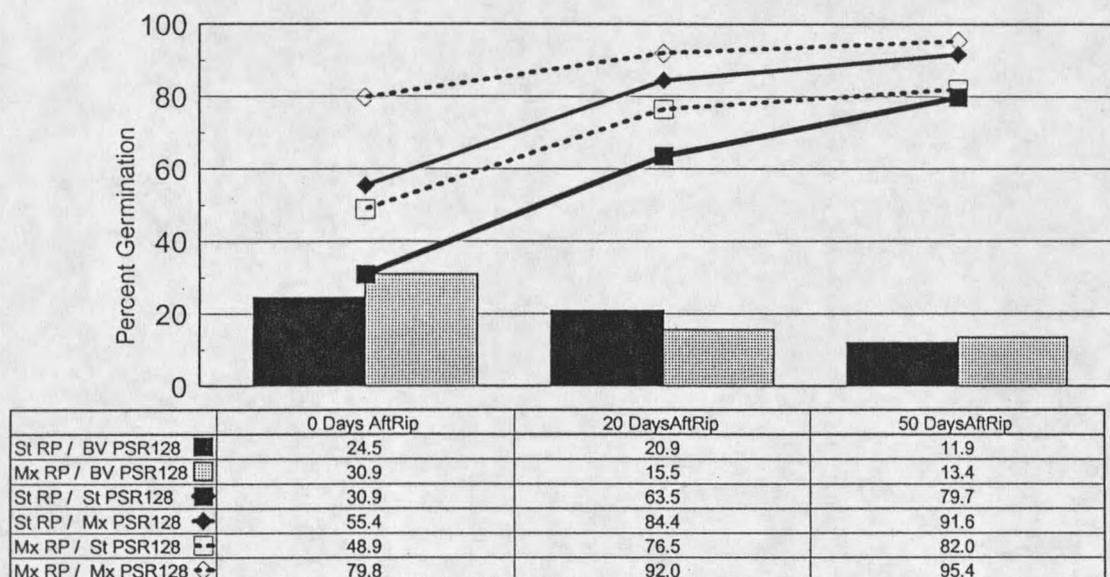
**Figure 9.** Interaction plot of recurrent parent (RP) x after-ripening (AftRip) effects on seed germination and bar graph of recurrent parent effects (RecEff). Results obtained from two Steptoe and three Morex backcross populations.



### QTL x Recurrent Parent x After-ripening Effects

Another genetic x environmental interaction is evident by the decline in aPSR128 effects as percent germination increases during seed after-ripening (Figure 10). This second G x E term is supported by analysis of variance (Table 12). Based on Figure 10, it can be deduced that the average aPSR128 QTL effect on percent seed germination, over three levels of after-ripening, was 19.1 and 19.9 in Steptoe and Morex BC populations, respectively. No significant interaction of aPSR128 with recurrent parent was detected (Table 12). Therefore, the aPSR128 seed dormancy QTL effect was not significantly different in Steptoe BC populations compared to Morex BC populations. The overall germination rate was 69.5 percent.

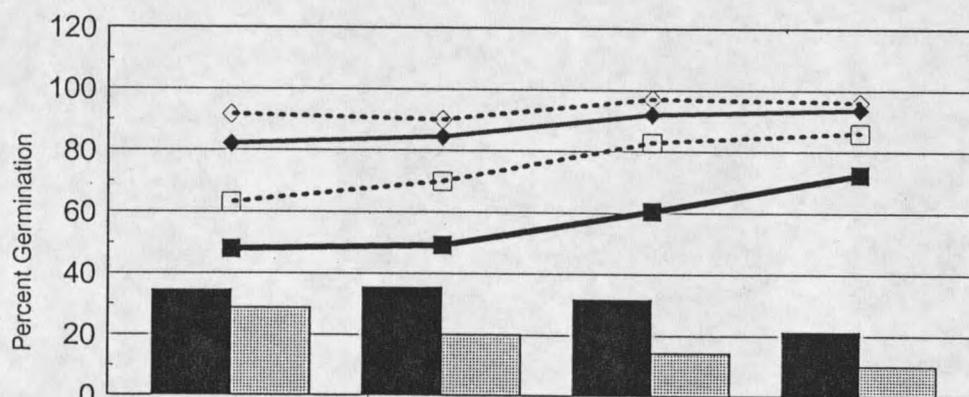
**Figure 10.** Interaction plot of aPSR128 with recurrent parent (RP) with after-ripening (AftRip) effects on seed germination and bar graph of aPSR128 breeding values (BV). Results obtained from two Steptoe (St) and three Morex (Mx) backcross populations.



### Reexamination of Epistasis (Doubled Haploid Population)

Epistatic and environmental interactions (Oberthur et al. 1995) for two seed dormancy QTLs are reexamined in Figure 10. The interaction of PSR128 and after-ripening is evident by the decline in the PSR128 effects as percent germination increases during after-ripening. The epistatic interaction of the PSR128 and ABG390 was most evident at 14 days after-ripening. At this level of after-ripening, progeny containing the Morex PSR128 allele showed nearly 100 percent seed germination (diamond symbols), regardless of ABG390 genotype. Progeny containing the Steptoe PSR128 allele (square symbols) showed approximately 60 and 80 percent germination depending on the ABG390 genotype. Therefore, at 14 day after-ripening the PSR128 marker showed about 20% less effect when the Morex allele inhabited the ABG390 locus compared to the ABG390 Steptoe allele (bar graph). The overall germination rate was 78.5 %.

**Figure 11.** Interaction plot of ABG390 x PSR128 x after-ripening (AftRip) period on seed germination and bar graph of aPSR128 breeding values (BV). Results obtained from the Steptoe (St) x Morex (Mx) population (Ullrich et al. 1992, Oberthur et al. 1995).

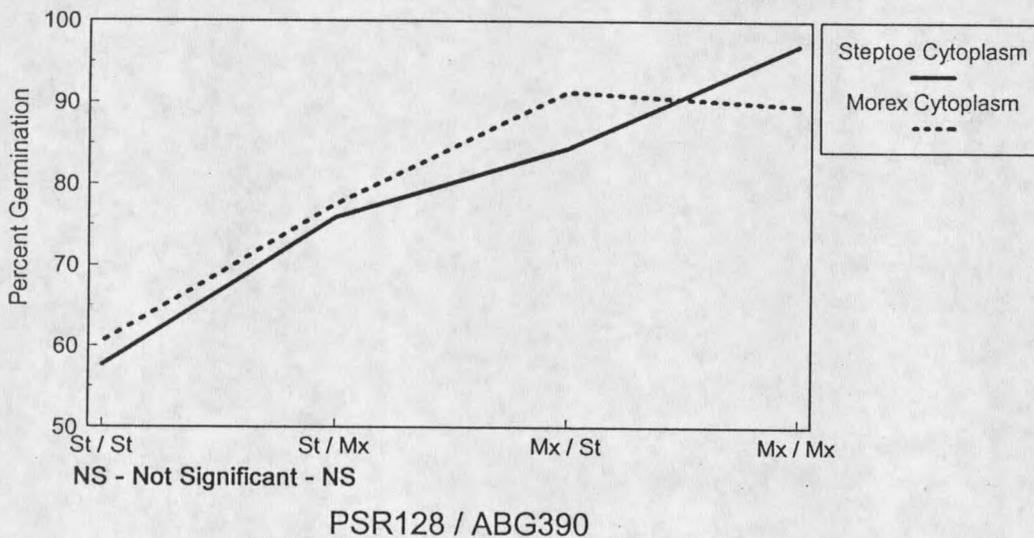


	0 Days AftRip	7 Days AftRip	14 Days AftRip	20 Days AftRip
St ABG390 / BV PSR128	34.3	35.3	31.5	21.1
Mx ABG390 / BV PSR128	28.6	20.0	14.2	10.0
St ABG390 / St PSR128	47.8	49.2	60.5	72.5
St ABG390 / Mx PSR128	82.1	84.5	92.0	93.6
Mx ABG390 / St PSR128	63.1	70.0	82.9	86.1
Mx ABG390 / Mx PSR128	91.7	90.0	97.1	96.1

### Cytoplasmic Effects (Doubled Haploid Population)

In order to investigate the putative cytoplasmic effects (Han et al., 1996), the seed germination data (Ullrich et al. 1992) was reexamined using cytoplasmic genotype classifications. A separate ANOVAs was conducted at fixed genotypic levels of PSR128 and ABG390 marker loci (chromosome 7). No significant cytoplasmic effects were detected when the Steptoe allele inhabited the PSR128 locus. However, highly significant cytoplasmic effects were observed when PSR128 was fixed for the Morex allele (Figure 12). However, these cytoplasmic effects showed a significant crossover interaction with the ABG391 Steptoe and Morex alleles, when the Morex allele inhabited the PSR128 locus.

**Figure 12.** Interaction plot of cytoplasmic x PSR128 x ABG390 effects on seed germination. Results obtained from the Steptoe (St) x Morex (Mx) doubled haploid population (Ullrich et al. 1992 and Oberthur et al. 1995).



### Conclusions

This study demonstrates reliable and codominant STS-PCR markers for barley chromosome 7. The aPSR128 marker was used to examine the gene action of a barley seed dormancy QTL previously reported by Ullrich et al. (1992) in reciprocal backcross populations. These markers could also be useful to test the hypothesis that orthologous seed dormancy QTLs are present in other related cereals, forage grass species, and perhaps some more distantly related weedy grass species. The reciprocal backcross seed dormancy QTL experiments shed new light on the complexity of seed dormancy gene effects.

The average percent germination difference between Steptoe and Morex aPSR128 marker alleles was 19.1 and 19.9 in the Steptoe and Morex BC populations, respectively. The average percent germination difference between the Steptoe and Morex BC populations (recurrent parent effect) was 11.5. These results confirm that the aPSR128 chromosome 7 seed dormancy QTL explains most of the genetic differences for seed dormancy observed between Steptoe and Morex, as suggested by Ullrich et al. (1992). Both recurrent parent and aPSR128 genetic effects are dependent on the after-ripening treatment (Table 12), as suggested by Oberthur et al. (1995). However, unless the Steptoe recurrent parent effect actually kills about 21.2% of the seeds, this interaction term is inevitable as percent germination approaches 100% during seed after-ripening (Figure 9). Likewise the interaction of aPSR128 and after-ripening occurs because the

QTL effect decline as percent germination approaches 100% during seed after-ripening.

The reciprocal backcross seed dormancy QTL experiment, presented here, were also designed to test the hypothesis that the aPSR128 QTL would have less effect in the Morex backcross compared to the Steptoe backcross. This hypothesis was based on epistatic interactions observed among seed dormancy QTLs in the Steptoe x Morex doubled haploid population (Oberthur et al. 1995) as illustrated in Figure 11. One relevant difference between this and the previous study is the overall level of percent germination. The reciprocal backcross experiments happened to show about 10% overall lower seed germination rates than the previous study conducted using the doubled haploid progeny (Ullrich et al. 1992 and Oberthur et al. 1995). The percent germination class means, in the reciprocal backcross experiments, show less tendency to cluster near 100% (compare Figures 10 and 11). The epistatic interaction of PSR128 and ABG390 occurred, in the doubled haploid studies, occurred when the PSR128 effect declined as percent germination increased during seed after-ripening--which occurred especially fast when the Morex allele inhabited the ABG390 locus.

These findings suggest that the epistatic interactions (Oberthur et al. 1995) and the QTL x after-ripening interactions (confirmed in this study) may be an artifact of using percentage data. In other words, this hypothesis states that epistasis (in a strict biochemical or molecular context) is not real and that after-ripening may affect the range of possible effects (especially near 100% germination) but that after-ripening treatments do not really affect the gene action (in a strict biochemical or molecular context) of Steptoe and Morex seed dormancy QTL alleles any differently. In order to test this

hypothesis, these putative QTL x TL and QTL x after-ripening interactions need to be tested in the overall range of germination between about 30 and 70%.

While it is possible that many of the epistatic and genetic x environmental interactions observed for seed dormancy are a result of limitations imposed by percentage data, it is interesting that the recurrent parent effects seem to fade away faster than aPSR128 effects during seed after-ripening (compare Figures 9 and 10). The reciprocal dormancy effects have faded completely away after 50 days of after-ripening. Presumably these reciprocal effects of the recurrent parents are largely a result of the ABG390 dormancy QTL reported by Ullrich et al. (1992). However, the aPSR128 dormancy effect was still apparent in these same populations even after 50 days of after-ripening. Based on these observations (comparing Figures 9 and 10) it is tempting to speculate why the seed dormancy effect of the aPSR128 marker might persist more than other genetic effects during after-ripening. If seed dormancy differences between Steptoe and Morex are representative of seed dormancy as it occurs in many of the wild grasses, then perhaps allelic differences for the PSR128 seed dormancy QTL assure that some wild grass seeds remain dormant for extended periods while other seeds germinate as soon as possible. Such genetic differences seem to have evolved in order to help assure the survival of plants.

Han et al. (1996) suggested that Steptoe x Morex doubled haploid lines may show seed dormancy differences explained by cytoplasmic origin. Some of the doubled haploid lines were derived from F<sub>1</sub> lines of a cross of Steptoe x Morex, while other doubled haploid lines were derived from F<sub>1</sub> cross of Morex x Steptoe. This hypothesis was more

fully explored, as shown in Figure 12. Based on this figure there appears to be a significant epistatic interaction between cytoplasmic and nuclear genes affecting seed dormancy in the SM DHL population. The cytoplasmic effects were significant only when the Morex allele inhabited PSR128 and showed a crossover interaction with the ABG390 nuclear QTL effect. However, the crossover interaction shown in Figure 12 is essentially the opposite of the crossover of cytoplasmic and ABG390 genotypes observed by Han et al. (1996). In any case, it is apparent that cytoplasmic differences between Steptoe and Morex may affect seed dormancy and germination. Therefore, these putative cytoplasmic differences should be taken into consideration with regard to the recurrent parent effects observed in my reciprocal BC experiment.

It is interesting to note that the dormancy QTL region near ABG390 on barley chromosome 7L is positionally similar to a gene on wheat 5AL which conditions drought-induced ABA production in maturing wheat seeds (Quarrie et al., 1994) and a gene on wheat 5DL for resistance to preharvest sprouting (Anderson et al. 1993). It could be postulated that the chromosome 7 QTLs identified in barley are homologous to genes in wheat which determine resistance to preharvest sprouting and/or ABA production during seed development. If this hypothesis is true, then we might speculate that ABA production during seed development conditions other gene effects such as the putative cytoplasmic effects illustrated in Figure 12. The underlying basis of the larger chromosome 7 QTL effect, near PSR128, is largely unknown. One study (Schuurink et al. 1992) suggests that dormancy of barley grain is correlated with gibberellic acid responsiveness of the isolated aleurone layer. GA and ABA are thought to be perceived

by plasma membrane receptors of aleurone cells (Gilroy and Jones, 1994), however they have not been cloned. Moreover, a number of other cis and trans genetic factors involved in the signal transduction of GA perception could vary between Steptoe and Morex. Nevertheless, it might be informative to test the hypothesis that the PSR128 dormancy QTL is correlated to GA perception by aleurone protoplasts. Like PSR128 and the reciprocal genetic effects of the other dormancy QTLs, the correlation of dormancy and GA responsiveness shows complex interactions with environmental parameters including after-ripening.

Han et al. (1996) agreed with Oberthur et al. (1995) that epistasis plays a significant part in the action of nuclear dormancy genes. However, both the cytoplasmic and epistatic effects reported by Han et al. (1996) were essentially opposite in nature to those reported in the SM DHL study (Oberthur et al. 1995). Therefore, no reasonable consensus can be made with regard to epistasis in these three independent studies (including Chapter 4 of this dissertation). Again, it is interesting to note that the overall germination levels reported by Han et al. (1996) were much lower than those in this study or in Oberthur et al. (1995), perhaps because their germination tests were conducted at 30°C.

## CHAPTER 5

### DISCUSSION

#### Six-rowed Malt Barley Breeding Outcomes for Montana

One of the primary objectives of my projects was to develop improved six-rowed barley varieties. Several important steps have been made in this regard. Chapter 2 clearly demonstrated the breeding value of the Steptoe chromosome 3 yield QTL under growing conditions in Montana. This QTL showed a benefit of 624 and 1135 kg/ha yield in the 1995 Bozeman dryland and irrigated experiments, respectively. Therefore, under certain conditions, this QTL can improve production of Morex by nearly 23 percent. Although failure to detect subtle (and potentially negative) pleiotropic effects does not prove the lack thereof, Chapter 3 presents convincing evidence that the Steptoe chromosome 3 yield QTL may be useful in a malting variety as first proposed by Hayes et al. (1993).

Substantial gains for yield and malt quality, demonstrated in Chapters 2 and 3, were made from the DH72 donor parent in only one backcross. The backcross progeny showed vast improvements over Steptoe for malt quality characteristics. The malt quality values of these experimental lines are approaching Morex after only one backcross. Approximately 25 BC<sub>2</sub> lines fixed for the Steptoe chromosome 3 yield QTL are in the 1996 preliminary replicated yield trials at Bozeman and Huntley. These experimental lines show much

better uniformity than selected BC<sub>1</sub> lines that were also advanced in the 1996 experiment. The chromosome 3 STS-PCR markers were used to genotype approximately two-hundred BC<sub>2</sub> lines, as F<sub>2</sub> plants grown in the greenhouse, before these lines could be planted as head-rows in the 1995 field. This allowed the conscientious selection of BC<sub>2</sub>F<sub>3</sub> progeny as improved donor parents, for the Steptoe chromosome 3 yield QTL, in breeding crosses with other elite Midwestern cultivars. It will be very interesting to see if the Steptoe chromosome 3 shows useful breeding value in these populations.

Although six-rowed malt barley is not yet an important component of the Montana agriculture commodities, there may be real opportunities to develop this crop. Barley is clearly an adapted crop in Montana and growers have been exporting two-rowed malt barley out-of-state for many years. If the Midwest growers continue to have problems associated with *Fusarium*, the Midwestern six-rowed barley markets could be a valuable market for barley growers in Montana. A primary job for plant breeders is to anticipate the future needs and opportunities for growers. Plant breeding is a time consuming process that invariably takes over five or ten years for the development of useful and novel cultivars.

### Six-rowed Feed Barley Breeding Outcomes for Montana

Fortuitously, the dormancy experiments may have more breeding value than might have been expected. Dr. Tom Blake (my advisor) and Dr. Jan Bowman (Animal and Range Science Dept., MSU) have identified a feed efficiency QTL associated with the Morex chromosome five, near a gene with known proteinase inhibitory effects. From the dormancy project, I have hundreds of BC<sub>2</sub>F<sub>2</sub> seeds derived from two backcross populations to Steptoe. As luck would have it, these seeds should segregate for Morex QTL alleles on chromosomes 4 and 7, with potentially useful breeding values for feed efficiency and dormancy respectively. As was discussed in the introduction, most barley grown in Montana is utilized as feed and contributes significantly to the livestock industry which is so important to the Montana economy. Steptoe is already an important feed barley in many Western States. Two genes have now been identified which may help improve the agronomic and feed quality of this variety. Codominant STS-PCR markers for the chromosome 7 QTL with large effects on dormancy (aPSR128) and the feed efficiency gene on chromosome 4 (TB19,20; Tagroonrung et al. 1992) will facilitate the improvement of Steptoe through marker-assisted breeding.

### The Application of Marker Technology to Plant Breeding

As I postulated in the beginning of my dissertation, it is becoming increasingly clear that many of the expected plant breeding improvements will be the result of carefully planned breeding programs which utilize scientific advances in many fields. Molecular markers are one of the relatively new technologies that have gained widespread popularity among plant breeders in the last decade. Despite this, there are few examples where molecular marker techniques have led to the creation of new crop varieties enhanced for one or more quantitative traits. Tanksley and Nelson (1996) postulated that two factors may be contributing to the less-than-expected impact of marker-based QTL analysis on the development of varieties with enhanced quantitative traits.

*1) QTL discovery and variety development are currently separate processes*

*2) Most breeding-related QTL studies are targeted toward manipulating quantitative trait variation existing within elite germplasm*

In the context of introgressing genes from unadapted germplasm (e.g. wild relatives), they propose a marker assisted breeding method, AB-QTL, where QTL analysis is delayed until advance backcross lines are developed. The authors postulate that this approach may overcome the lack of current success in using molecular marker technologies and the vast reservoir of potentially useful QTLs in unadapted germplasm. The method is based on previous studies demonstrating that advanced backcross populations can be used to increase the probability of successful selections (Bliss, 1981), and avoid the infusion of undesirable genes associated with breeding populations balanced with unadapted

germplasm. I think it is informative to compare this hypothesis and other speculations they make to the results of my breeding studies.

I think the most important facet of this dissertation is demonstrating that predictive power of comprehensive QTL analysis in balanced populations and the efficient use of molecular makers (STS-PCR) for subsequently introgressing genes for complex agronomic traits. Comprehensive QTL analysis allows breeders to determine which genomic regions have added value (yield or quality) without having negative pleiotropic effects. Following that, a variety of efficient molecular techniques, including STS-PCR, are now available to accelerate the introgression of these QTLs (as discussed below).

Traditionally, backcrossing has been a useful breeding procedure when easily identified characters controlled by one or two genes need to be added to an existing cultivar. A short list of examples suitable for backcrossing include earliness, short stature, absence of proanthocyanidin, and aleurone color (Foster, 1987). An example of a cultivar that was developed by backcrossing is Erbet (Hockett and Eslick, 1972). Erbet was derived by transferring a gene for earliness, into a widely used cultivar Betzes. Six Betzes backcross populations were made after the original cross was made in 1958. The final release of the backcross derived line, Erbet, was made in 1971 after three years of state and regional trials of the backcross-derived line. However, backcrossing is an "inherently" flawed method for breeding quantitatively inherited traits, since many potentially useful QTLs are lost after the first generation, in the process of selecting one or two donor parents. This is especially true since the effective heritability of many potentially valuable QTLs is presumably low.

Bliss (1981) acknowledged the difficulties associated with the use of unadapted germplasm to improve quantitatively inherited traits, but states that this could be a particularly productive area. Several promising areas are cited including the horizontal transfer of resistances, incorporation of higher yielding ability from unadapted germplasm, and the improvement of quality. The simultaneous improvement of seed protein and yield in common bean, *Phaseolus vulgaris*, is an example of using exotic germplasm as a source of useful genetic variability for quantitative traits (Bliss, 1981). To avoid the difficulties associated with the low heritability of QTLs and the infusion of too many undesirable genes, Bliss (1981) proposed a modified backcross approach for quantitative traits. However, to avoid the loss of potentially useful QTLs, Bliss (1981) proposed that one or *several successive* backcross populations should be made to produce the desired number of individuals or families.

Tanksley and Nelson (1996) identified three shortcomings of breeding populations balanced with unadapted germplasm (e.g. F<sub>2</sub>, BC<sub>1</sub>, RI). From the standpoint of malt barley breeding programs, I think the Morex x Steptoe doubled haploid population and the Morex x DH72 BC<sub>1</sub> population (Chapters 2 and 3) qualify as so-called balanced population.

*A) Undesirable QTL alleles from the unadapted parent occur in high frequency and can seriously reduce (or eliminate altogether) the ability to collect meaningful data on yield and other field performance traits. For example, the presence of shattering and/or sterility genes would make it difficult to measure yield in balanced populations derived from crosses of cultivated wheat or barley with one of their wild relatives.*

*B) Epistatic interactions are statistically difficult to detect, yet are likely to occur in balanced populations where donor alleles occur in a high frequency. For breeding purposes it is desirable to identify QTLs not requiring epistatic interactions among donor alleles - a goal which is more difficult to achieve in balanced populations*

*C) Subtle (and often negative) pleiotropic effects may go unnoticed in balanced populations due to the segregation of donor alleles in high frequency. These pleiotropic effects may not become obvious until the QTL has been backcrossed into an elite line and the genetic variance reduced.*

The objective of this discussion is not to take issue with the most widely respected plant geneticists in practice today, Dr. Tanksley. The points made by Tanksley and Nelson (1996) will be extremely useful for many objectives. However, the NABGMP and related studies (including these in Chapters 2,3, and 4) demonstrate that plant breeders may need to carefully consider the context of their breeding objectives and materials before the most efficient breeding method can be achieved. Considering the time and effort given to many breeding projects, choosing an efficient breeding method is paramount.

The NABGMP Steptoe x Morex has probably been the most comprehensive QTL analysis conducted in barley or wheat. It is true that few plant breeders would have expected any of the doubled haploid lines to be released as commercial varieties. However, the construction of such a diverse array of doubled haploid materials, from a cross of very different but elite varieties, has justified the interest of the plant breeders across America and beyond. A major objective of this dissertation is to help demonstrate why the interests of the NABGMP, for example, are justified. With regard to point A made by Tanksley and Nelson (1996), Chapters 2 and 3 demonstrate that positive yield

QTLs, such as the chromosome three headshattering and lodging resistance genes, can be introgressed across from a feed barley into widely diverged malt barley germplasm. Ironically though, these same headshattering genes may be the same genes (see below) which could, in fact, confound the detection of more meaningful yield QTLs in crosses between *H. spontaneum* into cultivated barley, or between two- and six-rowed barleys. As Tanksley and Nelson (1996) point out, dramatic headshattering effects could obscure the more subtle yield benefits obtained from drought tolerance genes, for example. In the case of the Morex x DH72 malt barley breeding project, the adapted recurrent parent shows moderate headshattering losses under certain environments. In the cross of cultivated barley and *H. spontaneum*, the unadapted parent is contributing a gene which may cause essentially complete seed disarticulation in half of the progeny, reducing the experimental efficiency by at least 50 percent.

It is ironic that the reciprocal backcross dormancy experiments (Chapter 4) showed strong and nearly identical effects of the PSR128 dormancy QTL, despite the epistasis detected in the Steptoe x Morex doubled haploid population (Oberthur et al. 1995). Using computer simulations, Tanksley and Nelson (1996) showed that it is unlikely that QTLs requiring epistatic interactions will be detected in backcross populations. Interestingly, Tanksley and Nelson (1996), also suggested that it would be difficult to detect epistatic interactions in balanced populations (see point B). Chapter 4 suggests that the breeding value of main effects such as the dormancy QTLs on chromosome 7 and the major yield QTL on chromosome 3 can be reliably predicted based on main effects observed in a balanced population using relatively unadapted parents.

Finally, Tanksley and Nelson (1996) state that subtle (and often negative) pleiotropic effects of otherwise valuable QTLs may go unnoticed in a balanced population, containing a diverse array of genetic effects. They go on to say that these effects may then become obvious in an advanced backcross breeding population. I disagree with this statement for several reasons.

Firstly, I think one of the great advantages of balanced QTL mapping analysis (e.g. Hayes et al. 1993) is that these studies are typically comprehensive. If the negative pleiotropic effects are so large as to become obvious, then there is no reason to expect that these effects cannot be detected in these balanced populations, if proper experimental procedures are used. Indeed, Hayes et al. (1993) speculated that the Steptoe chromosome 3 may have useful breeding value since they failed to detect negative pleiotropic effects for this chromosome, despite extreme variation between Steptoe and Morex (Table 8, for example). These expectations were borne out of a balanced experimental population, and appear to be holding true during the introgression of the Steptoe chromosome into Morex, as demonstrated in Chapter 3. Any reasonable yield QTL study, balanced or unbalanced, should conduct routine grain quality evaluations such as test weight, moisture (in corn), grain protein, or whatever other criteria are important. Moreover, based on observation of the parental germplasm and the progeny, an astute plant breeder should be on the lookout for possible problems.

Secondly, the development of the AB-QTL plant materials, as described by Tanksley and Nelson (1996), is not a trivial task. There is no advantage to developing QTL-NILs (near isogenic lines), as they describe, only to discover the gene has "obvious"

negative pleiotropic effects. In either case there are situations where cryptic variation may exist for otherwise useful gene effects. One prominent example in the literature was the Southern corn leaf blight which destroyed a large portion of the U.S. corn crop in 1970, due to the widespread use of cytoplasmic male sterility (Ullstrup, 1978). Other genes with negative pleiotropic effects may be so potentially useful that correction measures have been attempted. In the early 1960's, the discovery of the certain maize mutants such as *opaque 2* promised great improvements in corn breeding (Mertz et al. 1964). However, by the late 1980's, the best *o2* genotypes were still showing 10 percent reduction in yield, (Glover and Mertz, 1987) and poor grain quality characteristics. Beginning with early attempts to modify the soft-kernel problems associated with *o2* (Paez et al., 1995), it has taken years to correct for the negative pleiotropic effects of this gene (Gevers and Lake, 1992). Ultimately, mapping and cloning the modifier genes may facilitate our understanding and use of the *o2* genotypes via marker-assisted breeding or transformation (Burnett et al. 1996).

### Concluding Remarks Concerning Molecular Markers

In comparison to phenotypic evaluations (on a line by line basis) selections based on molecular techniques (on a QTL by QTL basis) have the added advantage of being insensitive to environmental parameters and stochastic error, which are especially troublesome for many agronomic and quality traits. Moreover, molecular markers can be a cost effective alternative to phenotypic evaluations (for traits such as malt quality) which may also be labor intensive and expensive. By taking advantage of comparative genome mapping (Van Deynze, 1995) and with map-based cloning becoming a reality (Tanksley, 1995), I think genetic analysis using molecular markers will gain even more credence both as a breeding method (Tanksley and Nelson, 1996) and a tool for biological studies (Van Deynze, 1995 and Tanksley 1995).

### Relation of Agronomic QTLs to Genes in Wild Barley

The distribution of the brittle rachis genes in some of the barley progenitors has been described (Takahashi, 1955). Several of these genes including *Bt1*, *Bt2* and possibly *Bt3* have been assigned to chromosome 3 (Sogaard and von Wettstein-Knowles, 1987 and Nilan, 1964), located near the a headshattering QTL described in Chapter 2 of this dissertation. This suggests that the *Bt* gene(s) and the headshattering QTL on barley chromosome 3 may be the same gene. Patterson et al. (1995) identify QTLs that affect seed dispersal which show correspondence across anciently diverged taxa (e.g. 65 million years). Yet several QTLs were identified in maize but not in rice or sorghum. The barley chromosome 3 (Nilan, 1964 and Larson, 1996) is homologous to maize chromosomes 3 and 8 (Van Deynze, 1995). Although QTLs affecting seed dispersal were detected in a cross of teosinte (*Zea mays* ssp. *parviglumis*) and corn (*Z. mays* race Reventador), corresponding QTLs were not detected in rice or sorghum (Patterson, 1995). Although maize chromosomes 3 and 8 homologous to barley chromosome 3, the correspondence of seed dispersal QTLs is weak.

The PSR128 QTL effect is reminiscent of some very interesting biological adaptations observed in nature. The inhibitory effect of the Steptoe allele for this gene rapidly fades for most seeds, but seems to show unusual persistence in a smaller fraction of the seed population. The phenomenon of seed after-ripening is very unusual in that genetically identical seeds (at least in barley) which developed under nearly identical environmental conditions, can show markedly different periods of dormancy. In nature, a seed population may be programmed to germinate over a period of several years, helping

ensure the continued survival of the species. In part, it is obvious that a number of different genes and different alleles can help assure that germination proceeds in such a fashion. However, the unusual gene action of the PSR128 seed dormancy QTL suggest that other mechanisms may have evolved. Moreover, this putative PSR128 gene action, which distributes germination of genetically identical seeds, is very difficult to explain at the molecular level. Therefore, I argue that the molecular mechanism which presumably underlies this phenomenon, probably has not evolved more than once--independently. The QTL near PSR128 may represent one of the interesting candidates for positional cloning experiments in the future.

**LITERATURE CITED**

- Anderson, J.A., M.E. Sorrells, and S.D. Tanksley. 1993. RFLP Analysis of Genomic Regions Associated with Resistance to Preharvest Sprouting in Wheat. *Crop Sci.* 33:453-459.
- Anderson M.K. and E. Reinbergs. 1985. Barley breeding. In *Barley*. D. C. Rasmusson, Ed. American Society of Agronomy, Madison, WI. pp. 231-268.
- AOSAP. 1970. Association of official seed analysts rules for testing seeds. *Assoc. Off. Seed Anal. Proc.* 60:1-116.
- ASBC. 1992. *Methods of Analysis*, 8th ed. Malt-4 Extract, Malt-6 Diastatic Power, Malt-7  $\alpha$ -Amylase Standard Reference Method, Wort-9 Color, Wort Clarity, Barley-2C Assortment, Barley-2B Bushel Weight, Barley-7B Barley Protein, Wort-18  $\beta$ -Gulcan determination by fluorescence. Barley 2D 1,000 KW. The Society, St. Paul, MN.
- Bonierbale M.W., R.L. Plaisted, S.D. Tanksley. 1988. RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. *Genetics.* 120:1095-1103.
- Blake, T.K., P.F. Hensleigh, E.A. Hockett, H.F. Bowman, G.A. Carlson, L.E. Oberthur, G. Kushnak, D. Wichman, J. Eckhoff, and K.M. Gilbertson. 1996. Registration of 'Chinook' barley. *Crop Sci.* 36:809-810.
- Bliss, F.A. 1981. Utilization of vegetable germplasm. *HortScience.* 16(2):129-132.
- Botstein D., R.L. White, M. Skolnick, and R.W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32:314-331.
- Buraas, T., and Skinnes. 1984. Genetic investigations of seed dormancy in barley. *Hereditas.* 101:235-244.
- Burger. W.C., and D.E. Laberge. 1985. Malting and brewing quality. In *Barley*. D. C. Rasmusson, Ed. American Society of Agronomy, Madison, WI. pp. 367-401.
- Burnett, R.J., L.M. Gloverson, and B.A. Larkins. 1996. Development of Quality Protein Maize. *Probe.* 7(2):9-11.
- Chao S., P.J. Sharp, A.J. Worland, E.J. Warham, R.M.D. Koebner, and M.D. Gale. 1989. RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. *Theor. Appl. Genet.* 78:495-504.

- Devos, K.M., M.D. Atkinson, C.N. Chinoy C.J. Liu, M.D. Gale. 1992. RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye. *Theor. Appl. Genet.* 83:931-939.
- Dyer, W.E. 1993. Dormancy-associated embryonic mRNAs and proteins in imbibing *Avena fatua* L. caryopses. *Physiologia Plantarum.* 88(2):201-211.
- Edwards, C. Johnstone, and C. Thompson. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Res.* 19(6):1349.
- Foster, A.E. 1987. Barley. In *Principals of Cultivar Improvement*. W.R. Fehr, Ed. Macmillian Publishing Company. pp. 83-125. ISBN 0-02-949181-9.
- Gevers, H.O., and J.K. Lake. 1992. Development of modified *opaque 2* maize in South Africa. In: Mertz, E.T., ed. *Quality Protein in Maize*. The American Society of Cereal Chemists, St. Paul, MN, pp. 49-78.
- Gilroy, S., and R.L. Jones. 1994. Perception of gibberellin and abscisic acid at the external face of the plasma membrane of barley (*Hordeum vulgare* L.) aleurone protoplasts. *Plant Physiol.* 104:1185-1192.
- Glover, D.V., and E.T. Mertz. 1987. Corn. In: Olson, R.A. and K.J. Frey, eds., *Nutritional Quality of Cereal Grains, Genetic and Agronomic Improvement*, ASAS-CSSA-SSSA, Madison, WI, pp. 184-336.
- Graner, A., A. Jahoor, J. Schondelmaier, H. Siedler, K. Pillen, G. Fischbeck, G. Wenzel, R.G. Herrman. 1991. Construction of an RFLP map of barley. *Theor. Appl. Genet.* 83:250-256.
- Han, F., Ullrich, S.E., Chirat, S., Menteur S., Jestin L., Sarrafi A., Hayes, P.M., Jones B.L., Blake, T.K. Wesenberg, D.M. Kleinhoffs, A., and Kilian, A. 1995. Mapping of  $\beta$ -Glucan content and  $\beta$ -Glucanase activity loci in barley grain and malt. *Theor. Appl. Genet.* 91(6-7):921-927.
- Han F., Ullrich S.E., J.A. Clancy, V. Jitkov, A. Kilian, and I. Romagosa. 1996. Verification of barley seed dormancy loci via linked molecular markers. *Theor. Appl. Genet.* 92:87-91.
- Harlan, J.R., and D. Zohary. 1966. Distribution of wild wheats and barley. *Science.* 153:1074-80.

- Harvey, B.L., and B.G. Rossnagel. 1984. Harrington barley. *Can. J. Plant Sci.* 64:193-194.
- Hayes P.M., B.H. Liu, S.J. Knapp, F. Chen, B. Jones, T.K. Blake, J. Franckowiak, D. Rasmusson, M. Sorrells, S.E. Ullrich, D. Wesenberg, and A. Kleinhofs. 1993. Quantitative trait locus effects and environmental interaction in a sample of North American barley (*Hordeum vulgare*) germplasm. *Theor. and Appl. Genet.* 87:392-401.
- Helentjaris, T.D., M. Slocum, S. Wright, A. Schaefer, and J. Nienhuis. 1986. Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. *Genetics.* 118:356-363.
- Heun M., A.E. Kennedy, J.A. Anderson, N.L.V. Lapitan, M.E. Sorrells, and S.D. Tanksley. 1991. Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). *Genome.* 34:437-447.
- Hillman, G. 1975. The plant remains from Tell Abu Hureyra: A preliminary report. *Proc. Prehist. Soc.* 41:70-73.
- Ho, K.M., and G.E. Jones. 1980. Mingo barley. *Can. J. Plant Sci.* 60:279-280.
- Hockett, E.A., and R.F. Eslick. 1972. Registration of Erbet barley. *Crop Sci.* 12:712-713.
- Hockett, E.A., C.F. McGuire, C.W. Newman, and N. Prentice. 1987. The relationship of barley  $\beta$ -Glucan content to agronomic and quality characteristics. *Barley Genet. New.* 5:851-860, 1987.
- Islam A.K.R.M., K.W. Shepard, and D.H.B. Sparrow. 1981. Isolation and characterization of euplasmic wheat-barley chromosome addition lines. *Heredity.* 46:161-174.
- Khursheed, B., and J.C. Rogers. 1988. Barley  $\alpha$ -Amylase Genes. *J. Biol. Chem.* 263(35):18593-18960.
- Kleinhofs A., A. Kilian, M.A. Saghai Maroof, R.M. Biyashev, P. Hayes, F.Q. Chen, N. Lapitan, A. Fenwick, T.K. Blake, V. Kanazin, E. Ananiev, L. Dahleen, D. Kudrna, J. Bollinger, S.J. Knapp, B. Liu, M. Sorrells, M. Heun, J.D. Franckowiak, D. Hoffman, F. Skadsen, and B.J. Steffenson. 1993. A molecular, isozyme, and morphological map of the barley genome. *Theor. and Appl. Genet.* 86:705-712.

- Kreis, M.K., M.S. Williamson, P.R. Shewry, and M. Gale. 1988. Identification of a second locus encoding  $\beta$ -amylase on chromosome 2 of barley. *Genet. Res. Camb.* 51:13-16.
- Lander, E.S., and D. Botstein. 1986. Strategies for studying heterogeneous genetic traits in humans by using a linkage map of restriction fragment length polymorphisms. *Proc. Natl. Acad. Sci. USA* 83:7353-7357.
- Lander E.S., P. Green, P. Abrahamson, J. Barlow, A. Daly, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics*. 1:174-181.
- Li, C.D., P. Langridge, R.C.M. Lance, P. Xu, G.B. Fincher. 1996. Seven members of the (1-3)- $\beta$ -glucanase gene family in barley (*Hordeum vulgare*) are clustered on the long arm of chromosome 3 (3HL). *Theor. and Appl. Genet* 92(7):791-796
- Loi, L., B. Ahluwalia, and G.B. Fincher, G.B. 1988. Chromosomal location of genes encoding barley (1-3, 1-4)- $\beta$ -Glucan 4-glucanohydrolases. *Plant Physiol.* 87:300-302.
- McCouch, S.R., G. Kochert, Z.H. Yu, Z.Y. Wang, G.S. Khush, W.R. Cofman, S.D. Tanksley. 1988. Molecular mapping of rice chromosomes. *Theor. Appl. Genet.* 76:815-829.
- Mertz, E.T., L.S. Bates, and O.E. Nelson. 1964. Mutant gene that changes the protein composition and increases the lysine content of maize endosperm. *Science*. 145:279-280.
- Montana Agriculture Statistics. 1995. Volume XXXII. Montana Agriculture Statistics Service.
- Muir, C.E., and R.A. Nilan. 1973. Registration of Steptoe barley. *Crop Sci.* 13:770.
- Mullis, K.B., and F.A. Faloona. 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods Enzymol.* 155:335-350.
- Nilan, R.A. 1964. The cytology and genetics of barley. Washington State University.
- Oberthur, L.E., W. Dyer, S.E. Ullrich, and T.K. Blake. 1995. Genetic analysis of seed dormancy in barley (*Hordeum vulgare* L.) *J Quantitative Trait Loci* [Online]. Available: World Wide Web: <http://probe.nalusda.gov:8000/otherdocs/jqtl/>.

- O'Donoghue, L.S., S.F. Kianian, P.J. Ryapati, G.A. Penner, M.E. Sorrells, S.D. Tanksley, R.L. Phillips, H.W. Rines, M. Lee, G. Fedak, S.J. Molnar, D. Hoffman, C.A. Salas, B. Wu, E. Autrique, A. Van Deynze. 1995. A molecular map of cultivated oat. *Genome*. 38:368-380.
- Paez, A.V., J.P. Ussary, J.L. Helm, and M.S. Zuber. 1969. Survey of maize strains for lysine content. *Agron. J.* 61:896-899.
- Paterson, A.H., Y.R. Lin, Z. Li, K.F. Schertz, J.F. Doebley, S.R.M. Pinson, S.C. Liu, J.W. Stansel, and J.E. Irvine JE. 1995. Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. *Science* 269:1714-1718.
- Peterson, G.A., and Foster, A.E. 1973. Malting barley in the United States. *Adv. Agron.* 25:327-378
- Poehlman, J.M. 1985. Adaptation and distribution. In *Barley*. D. C. Rasumuson, Ed. American Society of Agronomy, Madison, WI. pp. 1-17.
- Quarrie, S.A., M. Gulli, C. Calestani, A. Steed, and N. Marmioli. 1994. Location of a gene regulating drought-induced abscisic acid production on the long arm of chromosome 5A of wheat. *Theor. Appl. Genet.* 89:794-800.
- Rasmusson, D.C., and R.D. Wilcoxson RD. 1979. Registration of 'Morex' barley. *Crop Sci.* 19:293.
- Saiki, R.K., S. Scarf, F. Faloona, K.B. Mullis, G.T. Horn, H.A. Erlich, N. Arnheim. 1985. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle-cell anemia. *Science* 230:1350-1354
- Sanger, F., S. Niklen, A.R. Coulsen. 1977. DNA sequencing with chain terminating inhibitors. *Proc Natl Acad Sci USA* 74:5463-5467.
- Schwarz, P.B., H.H. Casper, and S. Beattie. 1995. Fate and Development of Naturally Occurring Fusarium Mycotoxins During Malting and Brewing. *J. Am. Soc. Brew. Chem.* 53(3):121-127, 1995.
- Shwarz, P. B., and R.D. Horsley. 1995. Malt Quality Improvement in North American Six-Rowed Barley Cultivars Since 1910. *J. Am. Soc. Brew. Chem.* 53(1):14-18.
- Schuurink, R.C., N.J. Sedee, and M. Wang. 1992. Dormancy of the barley grain is correlated with gibberellic acid responsiveness of the isolated aleurone layer. *Plant Physiol.* 100:1834-1839.

- Simpson, G. 1990. Seed dormancy in grasses. Cambridge University Press. ISBN 0-521-37288-7.
- Sogaard, B., and P. vonWettstein-Knowles. 1987. Barley: genes and chromosomes. *Carlsberg Res. Commun.* 52:123-196.
- Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503-517.
- Talbert, L.E., N.K. Blake, P.W. Chee, T.K. Blake, G.M. Magyar. 1994. Evaluation of "sequence-tagged-site" PCR products as molecular markers in wheat. *Theor. Appl. Genet.* 87:789-794.
- Tanksley, S.D., M.W. Ganal, and G.B. Martin. 1995. Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes. *Trends Genet.* 11:63-68.
- Tanksley, S.D., and J.C. Nelson. 1996. Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theor. Appl. Genet.* 92:191-203.
- Thomas, W.T.B., W. Powell, R. Waugh, K.J. Chalmers, U.M. Barua, P. Jack, V. Lea, B.P. Forster, J.S. Swanston, R.P. Ellis, P.R. Hanson, R.C.M. Lance. 1995. Detection of quantitative trait loci for agronomic, yield, grain and disease characters in spring barley (*Hordeum vulgare* L.). *Theor. and Appl. Genet.* 91:1037-1047.
- Therrien, M. C., C.A. Carmichail, J.S. Noll, and C.A. Grant. 1994. Effect of fertilizer management, genotype, and environmental factors on some malting quality characteristics in barley. *Can. J. Plant Sci.* 74: 545-547.
- Tragoonrung, S., V. Kanazin, P.M. Hayes, T.K. Blake. 1992. Sequence-tagged-site-facilitated PCR for barley genome mapping. *Theor. Appl. Genet.* 84:1002-1008.
- Trucksess, M. W., F. Thomas, K. Young, M.E. Stack, W.J. Fulgueras, and S.W. Page. 1995. Survey of Deoxynivalenol in U.S. 1993 Wheat and Barley Crops by Enzyme-Linked Immunosorbent Assay. *Journal of AOAC International* 78(3):631-636.
- Ullrich, S., P.M. Hayes, W.E. Dyer, T.K. Blake and J.A. Clancy. 1992. Quantitative trait locus analysis of seed dormancy in Steptoe barley. In MK Walker-Simmons and JL Reid (eds). *Pre-Harvest Sprouting in Cereals*. Westview Press, Boulder, CO.

USDA-NASS (United States Department of Agriculture - National Agricultural Statistics Service), [Online]. Available: World Wide Web:  
<http://www.usda.gov/nass/aggraphsp>.

Van Deynze, A.E., J.C. Nelson, E.S. Yglesias, S.E. Harrington, D.P. Braga, S.R. McCouch, M.E. Sorrells. 1995. Comparative mapping in grasses. Wheat relationships. *Mol. Gen. Genet.* 248:744-754.

Wells, S.A. 1973. Hector barley. *Can. J. Plant Sci.* 53:497-498.

Wilson, W. W. 1985. Production and Marketing in the United States and Canada. In *Barley*. D. C. Rasumuson, Ed. American Society of Agronomy, Madison, WI. pp. 483-510.

Zohary, D. and M. Hopf. 1988. Domestication of plants in the old world: the origin and spread of cultivated plants in West Asia, Europe, and the Nile Valley. Oxford University Press. ISBN 0-19-854198-8.

**APPENDIX**

**Table 13.** Explanation of the seed dormancy experiments including number of homozygous progeny tested and analyzed for each cross and each generation. The number of days after-ripening is also shown in parentheses.

Population	Germ Test 1	Germ Test 2	Germ Test 3	Total (lines within pops over germ tests)
Steptoe//DH72 BC <sub>1</sub> F <sub>3</sub>	24 (0)	24 (20)	24 (55)	72
Steptoe//DH72 BC <sub>1</sub> F <sub>4</sub>	24 (0)	24 (13)		48
Steptoe//DH177 BC <sub>1</sub> F <sub>3</sub>	23 (0)	23 (20)	23 (52)	69
Morex//DH167 BC <sub>1</sub> F <sub>3</sub>	21 (0)	21 (8)		42
Morex//DH167 BC <sub>1</sub> F <sub>4</sub>	21 (0)	21 (19)		42
Morex//DH70 BC <sub>1</sub> F <sub>3</sub>	37 (0)	37 (17)	37 (52)	111
Morex//DH70 BC <sub>1</sub> F <sub>4</sub>	37 (0)	37 (13)		74
Morex//DH181 BC <sub>1</sub> F <sub>3</sub>	21 (0)	21 (19)		42
<b>Total (lines over pops. within germ tests)</b>	208	208	84	<b>500 (lines over pops over germ tests)</b>

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