Genetics of Russian wheat aphid resistance in barley
by Rosa Maria Nieto Lopez

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
Montana State University
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Abstract:
Russian wheat aphid (RWA), Diuraphis noxia (Mordvilko), is an important pest in small grain cereals in several areas of the world. This research was conducted to determine the inheritance of resistance and identify molecular markers linked to resistance genes in barley (Hordeum vulgare L.) lines PI366444 and PI366453. Artificial infestation was performed in the field and in growth chamber conditions. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis, Southern blotting, and polymerase chain reaction (PCR) techniques were used to genotype plants in the laboratory. PI366444 and PI366453 were intercrossed and each was. also crossed with the susceptible cultivars Stark and Bearpaw. F2 progeny from the cross between resistant lines showed that they shared common or linked resistant genes. F2 segregation ratios from crosses among resistant and susceptible plants measured in F3 progeny indicated that there were at least two genes involved in resistance in both of the PI lines. Two different regions in the barley genome were determined to be associated with RWA resistance genes. The Sequence-tagged-site-PCR (STS-PCR) markers B Hordein and D14 on the short arm of chromosome 5 were found to be associated with variation for both chlorosis and rolling in F2 plants from crosses with both resistant lines. Tlie B Hordein marker accounted for 29% -42% of the total variance depending on the specific cross. The chromosome 2 STS-PCR marker ABG8 was found to be associated with rolling only in one cross. Knowledge of the inheritance of resistance and linkage of markers to resistance genes will be helpful in barley breeding programs.

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# RESISTANCE IN BARLEY

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in Agronomy

MONTANA STATE UNIVERSITY
Bozeman, Montana

March 1993

n378
n558



**APPROVAL**

of a thesis submitted by

Rosa Maria Nieto Lopez

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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I would like to dedicate this thesis to my beloved family: Amelia, Felix, Maria Jose, Felix Jr, Luis Carlos, Luis, Maria Jesus, Sonsoles, Sonsoles Jr, Cristina and Marta.

## ACKNOWLEDGMENTS

I would like to express gratitude to:

The Spanish Ministry of Agriculture for giving me the opportunity of working on this thesis.

Dr. Tom Blake for his guidance and encouragement during my graduate program.

The members of my committee Dr. John Sherwood and Dr. J.M. Martin for their support and assistance.

All the people working in the laboratory, for their companionship and tolerance, especially Vladimir Kanazin for his endless patience.

My family and my friends for their encouragement and moral support.

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## ABSTRACT

Russian wheat aphid (RWA), Diuraphis noxia (Mordvilko), is an important pest in small grain cereals in several areas of the world. This research was conducted to determine the inheritance of resistance and identify molecular markers linked to resistance genes in barley (Hordeum vulgare L.) lines PI366444 and PI366453. Artificial infestation was performed in the field and in growth chamber conditions. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis, Southern blotting, and polymerase chain reaction (PCR) techniques were used to genotype plants in the laboratory. PI366444 and PI366453 were intercrossed and each was also crossed with the susceptible cultivars Stark and Bearpaw. $F_2$ progeny from the cross between resistant lines showed that they shared common or linked resistant genes. $F_2$ segregation ratios from crosses among resistant and susceptible plants measured in $F_3$ progeny indicated that there were at least two genes involved in resistance in both of the PI lines. Two different regions in the barley genome were determined to be associated with RWA resistance genes. The Sequence-tagged-site-PCR (STS-PCR) markers B Hordein and D14 on the short arm of chromosome 5 were found to be associated with variation for both chlorosis and rolling in $F_2$ plants from crosses with both resistant lines. The B Hordein marker accounted for 29% -42% of the total variance depending on the specific cross. The chromosome 2 STS-PCR marker ABG8 was found to be associated with rolling only in one cross. Knowledge of the inheritance of resistance and linkage of markers to resistance genes will be helpful in barley breeding programs.

# INTRODUCTION

Russian wheat aphid (RWA), Diuraphis noxia (Mordvilko), is a cosmopolitan pest in small grain cereals in several areas of the world (Archer and Bynum, 1992). RWA was first collected and identified in the USA in 1986 (Stoetzel, 1987). Between 1986 and 1990 economic losses due to lower production and costs for insecticide use in the USA were estimated to be more than $660,000,000.00 (Quick, 1992). Sources of resistance in barley have been reported in H. vulgare and other wild Hordeum species (Kindler and Springer, 1991; Robinson et al., 1991; Webster et al., 1991). Resistance in the genotypes S12 (ASE/2CM//B76BB) and S13 (Gloria/Come) was reported to be controlled by a single dominant gene and this gene was the same in both genotypes (Robinson, 1992). Resistance in PI366450 from $F_1$ and $F_2$ segregation studies was reported to be due to the action of a single partially dominant gene for leaf rolling and to show multiple additive gene action for leave chlorosis (Mornhinweg, 1992). The inheritance of RWA resistance in PI366444 and PI366453, two resistant lines from Afghanistan (Webster et al, 1991), has not yet been determined.

Molecular markers (proteins and restriction fragments length polymorphisms, RFLPs) have been proven to be important tools in breeding programs which provide indirect selection techniques for characters with low heritability (Lande and

Thompson, 1990, Young and Tanksley, 1989; Lande, 1991). In many cases the use of molecular techniques is more expensive and tedious than direct selection of traits. New techniques based on polymerase chain reaction (PCR) where a particular segment of DNA can be specifically replicated, are being developed to produce efficient screening procedures. Sequence-tagged sites (STS) are short unique sequences amplified by PCR that identify known locations on a linkage map (Olson et al., 1989). Approaches to obtains STSs from a random genomic library used in the North American Barley Genome Mapping Project (NABGMP) for barley genome mapping have been previously reported (Tragoonrung et al., 1992).

The objective of this study was to determine the inheritance of resistance in lines PI366453 and PI366444 and identify molecular markers associated with RWA resistance genes.

## BIBLIOGRAPHICAL REVIEW

### Improving Plant Resistance to Insects

Host plant susceptibility often occurs with the introduction of crops to parts of the world where they are not native or with the avoidance of quarantine and introduction of pests in new areas (Gatehouse, 1991). The Hessian fly was introduced in the USA during the latter half of the eighteen century (Russell, 1978). Plant breeders occasionally also eliminate factors important in host plant resistance. Lines of cotton were developed which lacked gossypol, a compound toxic to mammals. These were found to be susceptible to attack by various insects including the cotton budworm (Gatehouse, 1991).

Several steps are necessary to develop new resistant varieties such as finding sources of resistance, evaluating resistance in the plant and studying the factors that affect the expression of resistance. It is also useful to know the mechanisms and the genetics of resistance for durability and effectiveness of the resistance. Hessian fly and greenbug have been the most studied pests in small grains in USA (Smith, 1989).

Sources of resistance can be found in varieties of the crop in question, wild genotypes of the same species or close relatives (Gatehouse 1991) . Resistance to biotype A of greenbug in wheat was first reported from a durum wheat

germplasm, Dickinson 485, and was transferred to hexaploid wheats (Painter and Peters, 1956). Resistance to biotype A,B and C was transferred to common wheat from "Isave FA" rye, Secale cereale L. (Sebesta and Wood, 1978) and resistance to biotype C and E was derived from Triticum tauschii (Coss) (Joppa et al., 1980).

Plants evolved together with insects through time and have developed physical mechanisms (lignified tissues, cuticular waxes, spines, hairs) and chemical mechanisms (insecticidal components and antifeedants) to defend themselves against insects (Gatehouse 1991). Resistance in wheat cultivar "Vel" to Hessian fly is due to its pubescence (Roberts et al., 1979).

The resistance mechanisms that affect insect behavior are measured in terms of antixenosis and antibiosis. Antixenosis is related to the inability of a plant to serve as a host to an insect (Kogan and Ortman, 1978). Antibiosis is related to the negative effects of a resistant plant on the biology of the insect. A third component of resistance, tolerance, refers only to the plant and is determined by the ability of the plant to outgrow an insect infestation (Smith, 1989).

Techniques to measure insect activity and plant resistance are different, depending upon the particular combination of pest insect and host plant. A model was developed for evaluating greenbug resistance in the laboratory based on the antixenosis, antibiosis and tolerance components

of resistance and combined these three indexes to yield a unified Plant Resistance Index (Inayatullah et al., 1990). This model has been used to evaluate Russian wheat aphid resistance (Webster et al., 1991)

Variations of resistance can be due to the plant, to the insect, to the environment or to interactions among them (Smith, 1989). As an example, greenbugs selected barley plants towards the direction of the sun (Webster and Inayatullah, 1988). In Hessian fly on wheat, high temperatures diminished expression of resistance (Sosa and Foster, 1976).

Knowledge of the genetics of resistance of a plant to a specific insect pest is helpful to develop new cultivars with broad bases of resistance (horizontal resistance). When resistance relies only in one major gene (vertical resistance), it is occasionally easily overcome by new insect biotypes (Maxwell, 1980). Dickinson selection 28A and CI9058 of wheat carried an identical single recessive gene pair conditioning resistance to greenbug, $F_1$ hybrids of Dickinson selection 28A by susceptible varieties of wheat showed susceptibility to be incompletely dominant (Curtis et al., 1960). Different genes responsible for resistance in wheat to different biotypes of greenbug were given unique names. The recessive gene in Dickinson selection 28A resistant to biotypes A was named Gb1. The single dominant gene in the Amigo variety resistant to biotypes A, B and C was named Gb2. The single dominant gene in the Largo variety resistant to

biotypes C and E is Gb3, and Gb4 is the name of a gene similar to Gb3. Gb5 is a single dominant gene which confers resistance to biotype E in CI17882 (Tyler et al., 1987). Polygenic sources of resistance have been found in sorghum to biotype E of greenbug. General Combining Ability, Specific Combining Ability and maternal effects of seven sources of resistance and three susceptible lines were studied using diallel analysis (Dixon et al., 1990). The seven sources of resistance when crossed with the susceptible lines produced positive transgressive segregations in the $F_2$ generation, apparently due to useful alleles contributed by both parents in each cross. Recurrent selection was recommended to accumulate different useful genes from various sources of resistance (Dixon et al., 1991).

Hessian fly is an example of a gene for gene interaction. For each avirulence gene in the pathogen there is a resistance gene in the plant (Hatchett and Gallun, 1970). More than 20 resistance genes have been identified in <u>Triticum</u> species for use in breeding resistant cultivars. Some of these genes have been mapped in wheat with the use of monosomic and disomic addition lines. The resistance genes H3, H6, and H9 were assigned to chromosome 5A (Stebbins et al., 1982), H13 to chromosome 6D (Gill et al., 1987) and H20 to chromosome 2B (Amri et al., 1990). The H3 resistance gene was found in Illinois No 1 selection W38 and provided resistance to biotypes A and C of Hessian fly (Caldwell et al., 1946), H6 in

Lathop's conferred resistance to biotypes A and B (Stebbins et al., 1980), and H5 in Ribeiro gave resistance to biotypes A, B, C and D (Shands and Cartwright, 1953). The gene H9 in Elva conferred resistance to biotypes B, C, and D, H11 in PI94587 provided resistance to GP,B,C and D (Stebbins et al., 1983), H12 in Luso gave resistance to biotypes B and D (Oellermann et al., 1983), and H20 in Jori provided resistance to biotype D (Amri et al., 1990).

Genetic diversity exists within insect species and when the insect population is under selection pressure variants in the population that are able to survive form populations of a new biotype. Aphids produce new biotypes with high frequency because of their relatively short life cycle and their parthenogenetic habit of reproduction (Gallun et al., 1975). Greenbug biotypes A and B were able to attack small grains but not sorghum (Wood, 1961). Biotype C was able to attack sorghum (Harvey and Hackerott, 1969). Biotype D resisted organophosphate insecticide applications (Teetes et al., 1975). Biotype E had twice the reproduction rate than biotype D (Porter et al., 1982). Biotype F was able to feed on Canada bluegrass (Kindler and Spomer, 1986). Biotype G did not damage "Wintermalt", a barley variety susceptible to all previous biotypes of greenbug, and biotype D was avirulent on all sorghum (Puterka et al., 1988).

By 1971 seven races of Hessian fly had been reported. These included GP (Great Plains), A, B, C, D, E, and F (Gallun

and Reitz, 1971). The F and G biotypes were developed in the greenhouse from crosses of GP and C before they were found in the field (Woottipreecha, 1971). Biotype L was also selected in the greenhouse (Sosa, 1978).

The further exploitation of the resistance modality antixenosis and the use of molecular biological techniques and new technologies in computer sciences constitute some new trends in breeding for resistance (Ponti and Mollema, 1992). More research is being done in the antixenosis component of resistance because a reduction in attractiveness keeps the insects away from its food source and reproduction site and the contact with the plant is smaller and chance of adaptation is expected to be smaller (Ponti and Mollema, 1992).

Some primary gene products that are very toxic to insects can be obtained from different organisms including bacteria, plants and animals. These genes can be transferred by molecular biology techniques to susceptible plants. Transgenic tobacco plants that expressed the Bt2 toxin gene from Bacillus thuringiensis were protected from feeding damage by larvae of the tobacco hornworm (Vaeck et al., 1987). Resistance in insects to toxins from B. thuringiensis has been reported and the mechanisms of insect resistance to the microbial insecticide are being studied (Rie et al.,1990).

New technologies in computer science have contributed to the building of insect population growth models. It was calculated that a simultaneous release of two resistance genes

to the Hessian fly in wheat would result in a better durability than a sequential release (Gould, 1986).

## Russian Wheat Aphid

Diuraphis Noxia, the Russian wheat aphid, is a small (<2.3 mm), spindle shaped aphid with short antennae that may be distinguished from other aphids that commonly occur in wheat and barley by the absence of prominent cornicles and appearance of a "double tail" when viewed from one side (Stoetzel, 1987). The Russian wheat aphid is a cosmopolitan pest in small grain cereals in several areas in the world. Although historically known in Asia, Africa, Middle East and Europe, it has most recently spread to North and South America (Archer and Bynum, 1992). The aphid was first collected and identified in USA in Bailey County, Texas in 1986 (Stoetzel, 1987). Since then this pest has spread to the Great Plains and was detected in Canada in 1988 (Jones et al., 1989). It is believed that wind currents were responsible for moving D. noxia and dispersing it to other states (Stoetzel, 1987). Winged aphids can only fly short distances but are capable of riding long distances on prevailing winds. From April to July 1988 Russian wheat aphid spread from Big Horn county to Liberty county, Montana a distance of several hundred miles (Johnson, 1989). Each adult female of Russian wheat aphid can produce 40 to 50 nymphs during her life of 40 days. No males have been found in the USA (Johnson, 1989). The Russian wheat

aphid is more cold tolerant than greenbug and can overwinter in areas such as Colorado, Nebraska, Montana and Wyoming (Massey et al., 1992). The Russian wheat aphid can survive in oversummering hosts. It survived in 47 cold-season grasses and 18 warm season grasses (Kindler and Springer, 1989). Yield loses of 35% and 65% have been recorded in South African wheat field tests (Webster et al., 1987). Yield reductions of 80% and 60% in 1987 and 1988 respectively on dryland winter wheat in the western United States and Canada were reported (Archer and Bynum, 1992). Oats and rye are less susceptible than wheat, barley and triticale (Worral and Scott, 1991). Infested leaves typically show a whitish or yellowish longitudinal streaking caused by a toxin injected during feeding. Lengthwise rolling on leaves forms tubes within which the aphids feed on the upper leaf surface and are protected (Jones et al., 1989). Economic losses due to lower production and costs for insecticide use in the USA have been estimated to be more than $ 660 million during 1986-1990 (Quick, 1992).

The wild _Hordeum_ species _H_. _bulbosum_ L. and _H_. _brevisubulatum_ have high levels of resistance (Kindler and Springer 1991). The genotypes of _H_. _vulgare_ S12 (ASE/ 2CM//B.7.6.B.B.) and S13 (Gloria/Come) with high-yield and multiple disease resistance background were selected as a source of resistance to _D_. _noxia_ in Mexico (Robinson et al., 1991). Barley lines PI366444, PI366447, PI366449, PI366450, PI366453 (Afghanistan); CI1412, PI430140, PI430142 (Iran); and

PI447219 (Spain) exhibited different levels of resistance to the Russian wheat aphid. When these barley lines from Afghanistan, Iran and Spain were tested for antibiosis, antixenosis, and tolerance, they showed different values for each test, suggesting that either these lines had different mechanisms of resistance (Webster et al., 1991) or that background genotype had an effect on the expression of resistance. Putative hybrid plants from the cross Elymus trachycaulus by cultivated barley were resistant to Russian wheat aphid but they did not shed viable pollen (Aung, 1991). Resistance in the genotypes S12 (ASE/ 2CM//B76BB) and S13 (Gloria/Come) was estimated to be controlled by a single dominant gene and this gene is thought to be the same in both genotypes (Robinson et al., 1992). Resistance in PI366450 from $F_1$ and $F_2$ segregation studies was reported to be due to a single partially dominant gene for leaf rolling and to show additive gene action for leaf chlorosis (Mornhinweg and Porter, 1992).

Expression of resistance to Russian wheat aphid in barley varies with the growth stage and with the growth cycle of the plant (Calhoun, 1991a). Ratings based on foliar Russian wheat aphid symptoms (chlorosis and leaf rolling) identified barley genotypes that showed minimal yield reduction under Russian wheat aphid infestation in field experiments (Calhoun, 1991b).

The inheritance of resistance in Triticum aestivum L. to D. noxia has been reported to be governed by a single dominant

gene in lines PI 137739, PI 262660 and PI 372129, and the genes from two of them were independently inherited (Du Toit, 1989; Nkongolo et al., 1991). PI372129 is the source of resistance in Corwal, a hard red winter wheat released by Colorado Agricultural Experiment Station (Quick, 1992). The transfer of resistance from Triticum monococcum resistant accessions to common wheat provided limited protection at the hexaploid level (Potgieter et al., 1991). The transfer of resistance from triticale to common wheat was not successful (Kkongolo, 1992b). Resistance to Russian wheat aphid in triticale lines PI386148, PI386149 and PI386156 was determined to be controlled by a single dominant gene in each line (Nkongolo, 1992a).

Using Molecular Markers for Introduction of Resistance Genes

A marker locus serves to identify the chromosomal segment in its vicinity and enables that region to be followed in inheritance studies (Stuber et al., 1987). Molecular markers are suitable for breeding programs because of their absence of pleiotropic effects, lack of dominance and multiple allelic forms (Beckman and Soller, 1983).

Isozymes are multiple molecular forms of an enzyme with the same catalytic activity (Markert and Moller, 1959). Restriction fragment length polymorphisms (RFLPs) result from specific differences in DNA sequence that alter the size of the fragments obtained after digestion of genomic DNA with

restriction endonucleases (Hulbert and Michelmore, 1987). Hordeins are alcohol-soluble storage proteins of barley that can be divided into three major groups which differ in their mobility in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and in their composition. The genes encoding the B hordeins (Hor-2) and C hordeins (Hor-1) are closely linked to each other and also to the mildew resistance loci Ml-a and Ml-k on barley chromosome 5, and the Hor-3 locus encoding the D hordeins is more loosely linked on the long arm of barley chromosome 5.

Molecular markers are located on chromosomes using classical linkage methods. A backcross or intercross may be performed between two strains differing in a trait of interest. Progeny can then be scored both for the trait and for the markers. An interaction is then sought between the trait of interest and the inheritance pattern of one or more markers. When a significant interaction is identified the marker can be used to track the presence of the gene modifying the trait of interest (Lander and Botstein, 1989).

In the case of quantitative traits the use of molecular markers may substantially increase the rate of improvement by artificial selection, especially in traits of low heritability. The efficiency of marker assisted selection depends on the heritability of the character, the proportion of the additive genetic variance associated with the marker loci and the selection scheme (Lande and Thompson, 1990).

Soluble solids content in tomato is a quantitative trait with high environmental variation and low heritability. Increasing the soluble solid content in raw tomatoes is of great interest because tomatoes are often priced according to this trait. RFLP markers were found which were closely linked to soluble solid content in tomato and have since been applied in plant breeding programs (Osborn et al., 1987).

One of the most useful molecular markers for a monogenic trait was the acid phosphatase Aps-1 locus in tomato linked to the Mi gene conferring nematode resistance (Weeden, 1991). Direct screening required maintaining live nematodes and since resistance is dominant, it was necessary to identify homozygous lines by progeny testing. Indirect selection for Mi gene provided a reliable indicator of the presence of the resistance gene (Messeguer et al., 1991). Adh-1 is a marker for pea enation mosaic virus resistance and is used in New Zealand, where this virus does not exist, to screen breeding material to introgress the resistant gene without repeated screens at foreign locations (Weeden, 1991).

Molecular markers may help to breed plants with more than one gene for resistance to a disease. They may help to distinguish among plants containing different resistance genes, especially in resistance to pests where resistance based on only one gene can be easily overcome by the pathogen (Weeden, 1991).

Genes introduced into cultivated plants by backcross breeding programs are flanked by introgressed segments of DNA derived from the donor parents. It was proposed during the analysis of the size of chromosomal segments retained around the Tm-2 locus of tomato during backcross breeding, that monitoring recombination around genes of interest with linked RFLP markers greatly reduces the amount of time necessary to eliminate drag associated with introgression (Young and Tanksley, 1989). Undesirable traits from *L. chmielewskii* were closely linked with elevated soluble solid concentration and a high density map was developed around this important agronomic factor to be able to select with RFLP markers for only the desirable traits (Paterson et al., 1990).

A high resolution RFLP map around the root knot nematode resistance gene (Mi) in tomato helped to identify resistance in varieties which carry less DNA from the donor of resistant *L. peruvianum* and lack the Aps-1 allele. In selection with the newly generated markers, it might be possible to separate some negative effects (fruit cracking) that were associated with resistant tomatoes carriying the Asp-1 gene (Messeguer et al., 1991).

In many cases the use of molecular techniques is more expensive and tedious than direct selection of traits. New techniques based on polymerase chain reaction, where a particular segment of DNA can be specifically replicated, are being developed to produce efficient screening procedures.

Sequence-tagged sites (STS) are short unique sequences amplified by polymerase chain reaction that identify known locations on a linkage map (Olson et al., 1989). To develop STSs it is necessary to have well-characterized DNA sequences (Cole et al., 1991). Approaches to obtain STSs from a random genomic library used in the North American Barley Genome Mapping Project for barley genome mapping were previously reported (Tragoonrung et al., 1992).

RAPD, Random Amplified Polymorphic DNA, markers are based on the amplification by the polymerase chain reaction using single primers of arbitrary nucleotide sequence (Williams et al., 1990), this technique does not need any previous characterized DNA sequence. Pto Pseudomonas resistance gene was reported linked to three RAPD markers (Martin et al., 1991) and another RAPD marker in tomato was reported tightly linked to the nematode resistance gen Mi (Klein-Lankhorst et al., 1991).

# MATERIALS AND METHODS

## Plant Material

Russian wheat aphid resistant barley lines PI366444 and PI366453 were crossed with the susceptible cultivars Stark and Bearpaw (Hockett et al., 1990). $F_1$, $F_2$ and $F_3$ seeds were produced in the greenhouse. Fifty $F_2$ plants from the crosses Stark/PI366453, Stark/PI366444, Bearpaw/PI366444 and Bearpaw/PI366453 were grown in the greenhouse. Two leaves were excised from each individual $F_2$ plant for DNA extraction and seeds were collected from these plants for RWA resistance analysis.

## Experimental Design

Twelve plants from the cross Stark/PI366444 and sixteen from the cross Stark/PI366453 were selected for RWA resistance in the $F_2$ and $F_3$ generations. Six seeds from each individual $F_3$ plant were planted in the greenhouse in pots 15 cm in diameter. DNA was extracted from plants from each pot. Molecular marker segregation studies were done with the DNA of this $F_4$ population. Plants regrew and seeds from plants from each pot were collected to form the $F_5$ population to assay for RWA segregation. The rest of the seeds from each individual $F_3$ plant ($F_4$ population) were used for a hill-plot experiment in the field, at Bozeman, MT in 1992. Hills were planted 0.5 m apart with approximately 6 seeds planted/hill. Twenty eight

genotypes (12 from Stark/PI366444 and 16 from Stark/PI366453) were arranged in a randomized complete block with 2 replications. Hills were infested at the 3 leaf stage with approximately 30 aphids/hill. Data were recorded the second and fourth weeks after infestation.

## RWA Infestation

To evaluate RWA reaction seeds were planted in small containers 2.5 cm in diameter. Each rack contained 200 containers. Ten plants from each parent were randomly placed in the rack to check uniformity of infestation. Ten $F_3$ plants were tested from each $F_2$ family and ten plants from each genotype in the $F_5$ population.

Seedlings were grown in the greenhouse until the 2 leaf stage and then moved to a growth chamber, GC, (16 hours light, 8 dark; 16/22°C day/night). Each plant was infested with 5 adult aphids using a paint brush. Aphids were provided by the Entomology department of Montana State University and reared on the barley cultivar Klages.

After 20 days infested plants were scored using a scale of 1-10 for leaf chlorosis and 1-3 for leaf rolling. Plants were considered susceptible for values higher than 4 for chlorosis or 2 for rolling.

## DNA Isolation

Plant DNA was extracted as described by Dellaporta et al., 1983, modified as follow: 10 grams of fresh tissue was ground with liquid nitrogen and incubated two hours at 55°C in 40 ml of extraction buffer (100mM Tris-HCl pH 8, 100mM EDTA, 250 mM NaCl, 100$\mu$g/ml of proteinase K, 1% N-lauroyl-sarcosine). The lysate was extracted with 40 ml phenol:chloroform solution (72% chloroform, 25% phenol and 3% isoamyl alcohol) followed by ethanol precipitation.

A rapid DNA extraction method was also used as described by Edwards et al., 1991.

## SDS-PAGE

Hordeins were extracted and separated by SDS-PAGE according to the method of Doll and Andersen, 1981 as modified by Blake et al., 1982.

## Marker Selection

DNA markers were selected based on their location in the barley linkage map (Kleinhofs et al., in press) and based on the criteria of spacing (20-25 cM apart) and polymorphism between the crosses tested. Morphological markers were rough/smooth awn and sterile/fertile glumes. Molecular markers were hordeins, STS-PCR markers and RFLPs. Most of the primers used for PCR reactions were developed by the laboratories of Dr. Blake and Dr. Luther Talbert at Montana State University.

Clones for RFLPs were supplied by the North American Barley Genome Mapping Project (NABGMP) (Kleinhofs et al., in press), by Cornell University (Heun et al., 1991), and by the Institute for Resistance Genetics, Grunbach, Germany (Graner et al., 1991).

PCR Amplification

PCR amplifications were performed in a 100 $\mu$l reaction volumes containing 2.5 units of Taq polymerase (Perkin-Elmer), 1x PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3), 1.5 mM $MgCl_2$, 100 mM of each dNTP, 0.2 $\mu$g of each primer and 50 ng of barley DNA as a template. For primers KV27-KV28 the $MgCl_2$ concentration was modified to 2 mM to improve product yield. PCR conditions were, one cycle of 4 min at 95°C and 30 cycles of one min at 94°C, one min at 55°C and one min at 72°C. For primers of D14 annealing temperature was 45°C. Primers KV1-KV2 required one cycle of 4 min at 95°C and 30 cycles of 50 seconds at 94°C and 5 seconds at 55°C (Kanazin et al., in press). Fragments from PCR amplification were electrophoresed on 0.8% agarose gels. Fragments that showed no polymorphism in agarose gels were digested with restriction endonucleases, *Hha*I, *Hae*III, *Hinf*I, *Alu*I, *Taq*I, *Msp*I and *Rsa*I (Promega) and electrophoresed in 6% polyacrylamide gels at 250 V for 2 hours.

## Southern Blots

Fifteen μg of barley genomic DNA was digested with the restriction endonucleases DraI, EcoRV and XbaI, separated on 0.8% agarose gels and transferred to Hybond-N+ nylon membranes following the protocol described by the manufacturer (Amersham). DNA inserts were amplified from their vectors with the PCR reaction. Fragments were precipitated with 95% ethanol, washed with 75% ethanol and dissolved in sterile water. Hybridization was done following Maniatis et al., 1982 and inserts were labeled with $^{32}P$ by random priming (Feinberg and Vogelstein, 1983). Filters were washed at 65°C, 30 min each wash and a final stringency of 0.2 x SSC + 1% SDS and exposed to X-ray film at -70°C in cassettes containing intensifying screens.

## Statistical Analysis

The program MREGRESS was used to calculate regression coefficients. Chi-square for segregation ratios were calculated with the program CSQ2. AVMF program was used to find associations between leaf chlorosis and leaf rolling with each of the markers using genotype of the markers as a classifying factor (Osborn et al., 1987). Variation explained by markers was described by using $r^2$ value which is the proportion of the total variance among the $F_2$ plants explained by the marker genotype-classes (Keim et al., 1990). Contrast of means among classes determined by genotype of the marker

were done to determine additive and dominant effects for each marker with AVMF (1/2(A1A1+A2A2) v A1A2 and A1A1 v A2A2). MREGRESS, AVMF and CSQ2 are in MSUSTAT, Statistical Package (Lund, 1986). Distances between markers and percentage of variation attributable to each marker were calculated with MAP-MAKER QTL (Lander and Botstein, 1989).

**RESULTS**

## Genetics of RWA Resistance

In growth chamber conditions, Stark and Bearpaw were susceptible cultivars with high average values for chlorosis (7.0 - 7.1) and rolling (3.0 - 2.9). PI366444 and PI366453 were resistant lines with low average values for chlorosis (2.6 and 3.2) and rolling (1.2) (Table 1). A positive significant (0.01 level) correlation between leaf chlorosis and leaf rolling was shown in segregating $F_3$ generations for all crosses except for $F_5$ Stark/PI366444 (Table 2). To observe the possibility of different mechanisms of resistance for rolling and chlorosis, these traits were analyzed separately in this study.

The field experiment was performed to observe whether or not genotypes selected for resistance to Russian wheat aphid in growth chamber conditions were resistant in field conditions. Average values for damage were higher in controlled environments than in the field (Table 1). However, there was still a significant positive correlation between both populations ($r$=0.51 $p<0.01$) for chlorosis. Rolling had a lower correlation value ($r$=0.35 $p>0.01$).

All $F_2$ plants from the cross between the resistant varieties PI366444 and PI366453 were resistant. The $F_1$ plants were resistant in all crosses but their means were slightly higher than the corresponding resistant parent. Differences in

means between parents and $F_1$ depended on the specific cross (Table 1).

$F_3$ populations fit a one gene model in the crosses Bearpaw/PI366444 and Stark/PI366453 for chlorosis and a two gene model in the cross Bearpaw/PI366444 for rolling. In the remaining cases the ratio of resistant to susceptible individuals did not fit either a one gene or a two gene hypothesis (Table 3). Phenotypic ratios for $F_2$ families fit a 2 gene model (15 resistant and segregating to 1 susceptible) for both chlorosis and rolling (Table 3).

Molecular Marker Screening.

A total of 130 clones scattered over the seven chromosomes of the barley genome were tested for the presence of RFLPs. In the lines Stark, PI366453, and PI366444 64 of these clones showed polymorphism between Stark and at least one of the two PI lines (Table 4) . Twenty five STS-PCR markers were assayed and twelve showed polymorphism between Stark and the two PI lines (Table 5). Hordeins B,C and D also showed polymorphism between resistant and susceptible lines (Fig. 1).

The $F_4$ and $F_5$ populations of Stark/PI366444 and Stark/PI366453 worked as a small model to test putative linked markers to RWA resistance. Marker associations with chlorosis and rolling were calculated using genotypes of the markers in the $F_4$ population and average damage values for rolling and

chlorosis in $F_5$ population. B-hordein and the RFLP marker BCD175 with P-value < 0.05 were selected.

Verification of Linkage between Markers and Phenotype

RFLP marker BCD175 was reported to be located on the short arm of chromosome 2 (Heun et al., 1991; Kleinhofs et al., in press). Three polymorphic STS-PCR markers were studied in that area of chromosome 2 in $F_2$ populations. PCR products amplified with ABG8 primers showed polymorphism among resistant and susceptible lines when electrophoresed in 0.8% agarose gels (Fig. 2). PCR products amplified with ABC358 showed polymorphism between Stark and the two PI lines when electrophoresed in 6% acrylamide gels and fragments amplified with BCD175 primers showed polymorphism among resistant and susceptible lines when digested with _Hae_III and electrophoresed in 6% acrylamide gels (Table 5). Recombination ratios were calculated in the $F_2$ Stark/PI366444 (n=40) and the order and distances of the markers in chromosome two are shown in Fig. 3.

Segregation for B-hordeins in $F_2$ populations were studied by the use of the primers KV1-KV9 and KV1-KV2 (Kanazin et al., in press). An additional STS-PCR marker, D14, located in the short arm of chromosome 1 in wheat (homoeologous to chromosome 5 in barley) showed polymorphism among resistant and susceptible lines. PCR products from the primers KV1-KV9 were separated in 0.8% agarose gels in the cross Bearpaw/PI366453

and in 6% acrylamide gels in the cross Bearpaw/PI366444 (Fig. 4) In the case of Stark/PI366444 PCR products were digested with RsaI and separated in 6% acrylamide gels. Fragments amplified with primers from the clone D14 were digested with HhaI for segregation analysis in the Stark/PI366444 $F_2$ population and with HinfI in the Bearpaw/PI366444 $F_2$ population (Fig. 5). From these two crosses (75 plants) the calculated distance between B-hordein and D14 was 6 cM. The two possible orientations for D14 are shown in Fig. 6.

All markers were tested for aberrant segregation ratios in the $F_2$ generation (Table 6). ABG8 showed skewed segregation with a higher frequency of alleles from the resistant parent than expected in the two crosses assayed which had Bearpaw as a parent.

In all crosses studied the B-hordein and D14 markers were associated with RWA resistance (P-values < 0.01). The portion of variation attributable to the B-hordein marker measured as $r^2$ varied depending on the specific cross and character that was considered ($r^2$ = 0.49 - 0.29, Table 7). The markers located on chromosome 2 were only important for leaf rolling in the cross Stark/PI366444 ($r^2$ =0.14 - 0.17, Table 7). The percentage of variability explained by each marker obtained with the QTL analysis (Fig. 3 and Fig. 6) were similar to the ones obtained with the regression analysis and $r^2$ values (Table 7).

Multiple regression analysis was performed with leaf rolling using the B-hordein and ABG8 markers in the Stark/PI366444 $F_2$ population. The effect of both markers was additive. The proportion of variance accounted for adding both variables was approximately 50%.

## DISCUSSION

RWA is a new pest in North America for the small grains. It spread from Mexico, where it was first reported in 1984, to the USA and Canada where it was detected in 1988 (Jones et al., 1989). Cultivated varieties are not resistant to this aphid and yield reductions of 60%-80% may be observed if weather conditions are favorable for the reproduction of the aphid and no insecticide is applied (Archer and Bynum, 1992). Resistant barley lines PI366453 and PI366444 are being used as sources of resistance genes to develop commercially acceptable resistant varieties. Determination of the mode of inheritance of resistance genes and identification of molecular markers linked to resistance genes can make more effective use of available resistant germplasm.

Crosses from resistant lines PI366444 and PI366453 with two susceptible cultivars were evaluated for chlorosis and rolling. These two measures of resistance were reported as having different genetic mechanisms in the resistant line PI3666450 (Mornhinweg and Porter, 1992). The r=0.5 value for $F_3$ Stark/PI366444 decreased to r=0.27 in the $F_5$ population where selection was performed. The possibility of different mechanisms of resistance was observed and chlorosis and rolling were studied independently.

The fact that there was significant correlation for chlorosis in GC and in the field but not for rolling suggests

that rolling is more affected by environmental factors than chlorosis.

The $F_2$ plants from the cross between the two resistant lines PI366444 and PI366453 were all resistant indicating that they have common or linked resistance genes.

None of the $F_1$ plants from any of the crosses tested was completely susceptible. Differences in resistance in different crosses could not be clearly characterized due to the small number of individuals assayed in some of the crosses. From the data from $F_1$ plants, resistance appears to be due to incomplete dominance.

Segregation in the $F_2$ families was consistent for all crosses and for both chlorosis and rolling. At least two major genes were segregating. The fact that segregation ratios for individual $F_3$ plants fit a two or a one gene model depending on the measure evaluated and the specific cross suggested that the importance of the effect of the two genes is different in each cross and that other modifier genes may cause small variations in the phenotypes depending on the cross and specific measure of chlorosis or rolling.

Use of the pre-selected markers in $F_4$ and $F_5$ populations was of great help to locate putative linked markers to resistance genes. B-hordein on chromosome 5 was the best marker. It showed normal segregation in the $F_2$ populations and explained an important part of the variation for resistance (42% in the most favorable case) in the three $F_2$ crosses

studied. When data from all $F_2$ crosses was merged (120 plants) the marker explained 37% of the variation for chlorosis and 32% for rolling. B-hordein is an easy marker to use for screening by SDS-PAGE in seeds or from a small amount of fresh tissue using PCR with primers such us KV1-KV2 and KV1-KV9.

The ABG8 marker only explained a portion of the variance for rolling in the cross Stark/PI366444. It was possible to map BCD175 and ABC358 to ABG8 but they did not give more information about resistance and ABG8 has the advantage of detecting polymorphism between amplified DNA fragments with a 0.8% agarose gel. The Stark/PI366444 $F_2$ population analyzed for ABG8 consisted of only 42 plants, and when segregation for the marker over all crosses was performed (120 plants) a skewed segregation towards the resistant parent was observed. A larger population is necessary to confirm the effects of this marker.

## SUMMARY

This study provides some insights into the genetics of resistance in the barley lines PI366444 and PI366453 from Afghanistan that are being used in the USA barley breeding programs as sources of resistance to Russian wheat aphid.

PI366444 and PI366453 share common or linked resistance genes. Resistance is incompletely dominant. There are at least two genes involved in resistance. The two measures of resistance, chlorosis and rolling, have different genetic mechanisms.

The B-hordein marker in chromosome 5 is associated with Russian wheat aphid resistance genes in both resistance lines explaining 29%-42% of the total variability depending upon the measure (chlorosis or rolling) and the specific cross. The ABG8 marker in chromosome 2 explains 19% of the variability but only for rolling in the cross Stark/PI366444.

The STSs PCR markers were of great help to screen and genotype plants.

These results can help in selection for commercial barley plants resistant to Russian wheat aphid.

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# APPENDICES

# APPENDIX A

TABLE 1. Means of Russian wheat aphid damage for chlorosis and rolling for parental barleys, $F_1$, $F_3$, $F_4$, $F_5$ of crosses among resistant and susceptible lines and $F_2$ of the cross between resistant lines.

| Parents and progeny | Chlorosis* | Rolling** | N |
|---|---|---|---|
| Stark (GC) | 7.1 | 3.0 | 42 |
| Bearpaw (GC) | 7.0 | 2.9 | 39 |
| PI366444 (GC) | 2.6 | 1.2 | 37 |
| PI366453 (GC) | 3.2 | 1.2 | 37 |
| $F_1$ Bearpaw/PI366453 (GC) | 4.0 | 1.5 | 37 |
| $F_1$ Bearpaw/PI366444 (GC) | 3.7 | 1.5 | 20 |
| $F_1$ Stark/PI366453 (GC) | 3.2 | 1.4 | 4 |
| $F_1$ Stark/PI366444 (GC) | 3.2 | 1.5 | 11 |
| $F_2$ PI366444/PI366453 (GC) | 2.5 | 1.1 | 133 |
| $F_3$ Bearpaw/PI366453 (GC) | 4.9 | 1.9 | 330 |
| $F_3$ Bearpaw/PI366444 (GC) | 4.6 | 1.7 | 358 |
| $F_3$ Stark/PI366453 (GC) | 5.1 | 2.0 | 439 |
| $F_3$ Stark/PI366444 (GC) | 4.8 | 1.9 | 374 |
| $F_5$ Stark/PI366453 (GC) | 3.9 | 1.7 | 126 |
| $F_5$ Stark/PI366444 (GC) | 4.3 | 1.9 | 84 |
| Stark (Field) | 6.0 | 3.0 | 12 |
| PI366453 (Field) | 3.0 | 1.0 | 12 |
| $F_4$ Stark/PI366453 (Field) | 3.5 | 1.3 | 192 |
| $F_4$ Stark/PI366444 (Field) | 3.3 | 1.2 | 144 |

* Scored on 1 to 10 scale
** Scored from 1 to 3 scale
N Number of plants
GC Growth Chamber

TABLE 2. Correlation (r) between chlorosis and rolling in $F_3$ and $F_5$ populations.

| Population | Chlorosis/rolling | |
|---|---|---|
| | r | N |
| $F_3$ Stark/PI366453 | 0.61 ** | 439 |
| $F_3$ Stark/PI366444 | 0.50 ** | 374 |
| $F_3$ Bearpaw/PI366453 | 0.62 ** | 330 |
| $F_3$ Bearpaw/PI366444 | 0.63 ** | 358 |
| $F_5$ Stark/PI366453 | 0.53 ** | 126 |
| $F_5$ Stark/PI366444 | 0.27 * | 84 |

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

N Number of plants

TABLE 3. Russian wheat aphid damage segregation ratios for resistance in $F_3$ plants and $F_2$ families.

| Progeny | Expected | Chlorosis Observed | Chlorosis $X^2$ | Chlorosis P | Rolling Observed | Rolling $X^2$ | Rolling P |
|---|---|---|---|---|---|---|---|
| $F_3$ Bearpaw/PI366453 | 205R:123S (1 gene) | 187:141 | 4.21 | 0.04 | 249:79 | 25 | 0.00 |
| | 282R:46S (2 genes) | | 228 | 0.00 | | 28 | 0.00 |
| $F_3$ Bearpaw/PI366444 | 223R:133S (1 gene) | 230:126 | 0.76 | 0.40 | 308:48 | 86 | 0.00 |
| | 306R:50S (2 genes) | | 134 | 0.00 | | 0.09 | 0.76 |
| $F_3$ Stark/PI366453 | 274R:165S (1 gene) | 280:159 | 0.28 | 0.58 | 315:124 | 16 | 0.00 |
| | 378R:61S (2 genes) | | 183 | 0.00 | | 75 | 0.00 |
| $F_3$ Stark/PI366444 | 231R:139S (1 gene) | 263:107 | 11 | 0.00 | 251:119 | 4.61 | 0.03 |
| | 318R:52S (2 genes) | | 68 | 0.00 | | 100 | 0.00 |
| $F_2$ Bearpaw/PI366453 | 32R:2S (2 genes) | 30:4 | 1.82 | 0.32 | 33:1 | 0.62 | 0.44 |
| $F_2$ Bearpaw/PI366444 | 36R:2S (2 genes) | 34:4 | 1.14 | 0.29 | 32:2 | 0.43 | 0.48 |
| $F_2$ Stark/PI366453 | 45R:3S (2 genes) | 45:3 | 0.00 | 1.00 | 44:4 | 0.35 | 0.58 |
| $F_2$ Stark/PI366444 | 37.5R:2.5S (2 genes) | 36:4 | 0.96 | 0.33 | 37:3 | 0.10 | 0.75 |

S (chlorosis, $F_3$) values >5.
S (rolling, $F_3$) values >2.
S (chlorosis, $F_2$) values >5 for all plants of that family.
S (rolling, $F_2$) values >2 for all plants of that family.

TABLE 4 . RFLPs polymorphic markers for Stark and the PI resistant lines.

| Marker | Reference | Restriction enzyme |
|---|---|---|
| Chromosome 1 | | |
| Glx | Kleinhofs | DraI, XbaI |
| WG789 | Heun | DraI, EcoRV |
| ABG380 | Kleinhofs | DraI, EcoRV |
| pKSU44 | Shin | DraI, EcoRV |
| ABC465 | Kleinhofs | DraI, EcoRV |
| CDO36 | Heun | EcoRV, XbaI |
| CDO464 | Heun | XbaI |
| ABC455 | Kleinhofs | DraI, EcoRV |
| CDO673 | Heun | EcoRV |
| WG420 | Heun | DraI |
| MWG18 | Graner | DraI |
| MWG66 | Graner | DraI, EcoRV |
| MWG70 | Graner | EcoRV |
| Chromosome 2 | | |
| BCD175 | Heun | XbaI |
| ABC454 | Kleinhofs | XbaI |
| CDO588 | Heun | DraI |
| CDO366 | Heun | DraI |
| ABC451 | Kleinhofs | DraI |
| ABG14 | Kleinhofs | DraI, EcoRV, XbaI |
| BCD266 | Heun | XbaI |
| CDO373 | Heun | DraI |
| WG645 | Heun | XbaI |
| ABC311 | Kleinhofs | EcoRV |
| BG123 | Kleinhofs | XbaI (only PI366453) |
| ABG19 | Kleinhofs | DraI (only PI366444) |
| MWG503 | Graner | EcoRV |
| MWG83 | Graner | DraI |
| MWG996 | Graner | DraI (only PI366444) |
| MWG64 | Graner | EcoRV, XbaI |
| Chromosome 3 | | |
| CDO395 | Heun | EcoRV |
| ABG460 | Kleinhofs | DraI, EcoRV |
| ABG10 | Kleinhofs | DraI, EcoRV, XbaI |
| pKSU22 | Shin | DraI, EcoRV, XbaI |
| ABG4 | Kleinhofs | EcoRV |
| CDO105 | Heun | XbaI |
| CDO474 | Heun | XbaI |
| cMWG680 | Graner | EcoRV |
| MWG630 | Graner | XbaI |

TABLE 4. CONTINUED

| | | |
|---|---|---|
| <u>Chromosome 4</u> | | |
| BCD402 | Heun | DraI |
| ABG3 | Kleinhofs | DraI, EcoRV, XbaI |
| ABG366 | Kleinhofs | DraI |
| CDO795 | Heun | DraI, EcoRV, XbaI |
| MWG616 | Graner | DraI, EcoRV |
| <u>Chromosome 5</u> | | |
| Ica1 | Kleinhofs | DraI |
| pMSU51 | Shin | EcoRV |
| ABG452 | Kleinhofs | DraI |
| WG241 | Heun | DraI (only PI366444) |
| MWG78 | Graner | EcoRV |
| <u>Chromosome 6</u> | | |
| ABG458 | Kleinhofs | EcoRV |
| ABG1 | Kleinhofs | EcoRV |
| BCD269 | Heun | XbaI |
| cMWG652 | Graner | DraI, XbaI |
| <u>Chromosome 7</u> | | |
| pKSU26 | Shin | XbaI (only PI366453) |
| Ipa | Kleinhofs | DraI, EcoRV, XbaI |
| WG564 | Heun | EcoRV |
| WG364 | Heun | EcoRV |
| pKSU24 | Shin | DraI, EcoRV |
| pMSU73 | Shin | EcoRV |
| CDO113 | Heun | XbaI |
| CDO484 | Heun | EcoRV |
| ABG463 | Kleinhofs | DraI |
| CDO506 | Heun | XbaI |
| cMWG650 | Graner | DraI, EcoRV |
| MWG596 | Graner | DraI, EcoRV |

TABLE 5. PCR markers that identify variation between the resistant lines PI366453 and PI366444 and susceptible lines Stark and Bearpaw.

| Marker | Primers | Chromosome | Restriction enzyme | Susceptible line |
|---|---|---|---|---|
| BCD175 | L-R | 2 | HaeIII | Stark, Bearpaw |
| ABG8 | KV27-KV28 | 2 | ** | Stark, Bearpaw |
| ABG358 | L-R | 2 | ** | Stark |
| ABA5 | L-R | 2 | RsaI | Stark |
| pMSU21* | TB59-TB61 | 2 | ** | Stark |
| B-Hord | KV1-KV2 | 5 | ** | Stark, Bearpaw |
| B-Hord | KV1-KV9 | 5 | RsaI | Stark |
| B-Hord | KV1-KV9 | 5 | ** | Bearpaw |
| D14 | L-R | 5 | HinfI, TaqI | Stark, Bearpaw |
| D14 | L-R | 5 | HhaI | Stark |
| Pst340 | L-R | 5 | HaeIII | Stark, Bearpaw |
| aHrth* | TB17-TB18 | 5 | HhaI, AluI, TaqI | Stark |
| Pst337* | L-R | 7 | HhaI, HaeIII HinfI, AluI TaqI, MspI | Stark |
| Pst319* | L-R | unmapped | TaqI, RsaI | Stark |

* Tested only with Stark
** Polymorphism due to differences is sizes of PCR amplified fragments.

TABLE 6. Chi-squared for aberrant ratios for markers in $F_2$ Stark/PI366444, $F_2$ Bearpaw/PI366444, and $F_2$ Bearpaw/PI366453.

| Marker | A1A1/A1A2/A2A2 Observed | Expected | X2 |
|---|---|---|---|
| $F_2$ Stark/PI366444 | | | |
| Hordein B | 14/21/5 | 10/20/10 | 4.15 |
| D14 | 14/18/7 | 9.75/19.5/9.75 | 2.74 |
| BCD175 | 11/14/13 | 9.5/19/9.5 | 2.84 |
| ABG8 | 14/19/9 | 10.5/21/10.5 | 1.57 |
| ABC358 | 11/16/11 | 9.5/19/9.5 | 0.95 |
| $F_2$ Bearpaw/PI366444 | | | |
| Hordein B | 12/15/9 | 9/18/9 | 1.50 |
| D14 | 12/17/6 | 8.75/17.5/8.75 | 2.09 |
| ABG8 | 15/13/7 | 8.75/17.5/8.75 | 5.97* |
| $F_2$ Bearpaw/PI366453 | | | |
| Hordein B | 7/16/12 | 8.75/17.5/8.75 | 1.69 |
| ABG8 | 15/11/8 | 8.5/17/8.5 | 7.12* |

* Significant at the 0.05 probability level.

TABLE 7. P-values, $r^2$ values and mode of action (A, additive; D, dominant; R, recessive) for markers that detect significant phenotypic variation in the $F_2$ Stark/PI366444, $F_2$ Bearpaw/PI366444, and $F_2$ Bearpaw/PI366453.

| Marker | Chlorosis | | | Rolling | | |
|---|---|---|---|---|---|---|
| | $r^2$ | P | Mode | $r^2$ | P | Mode |
| $F_2$ Stark/PI366444 | | | | | | |
| B-hordein | 0.38 | 0.0000 | A | 0.34 | 0.0002 | A |
| D14 | 0.49 | 0.0000 | D | 0.34 | 0.0001 | A |
| ABG8 | | | | 0.14 | 0.0177 | A |
| ABC358 | | | | 0.17 | 0.0360 | A |
| $F_2$ Bearpaw/PI366444 | | | | | | |
| B-hordein | 0.42 | 0.0001 | A | 0.40 | 0.0002 | A |
| D14 | 0.37 | 0.0004 | A | 0.45 | 0.0000 | A |
| $F_2$ Bearpaw/PI366453 | | | | | | |
| B-hordein | 0.29 | 0.0022 | A | 0.35 | 0.0003 | A |

# APPENDIX B

FIGURE 1. SDS-PAGE of the lines Bearpaw (lane 1), Stark (lane 2), PI366444 (lane 3) and PI366453 (lane 4). Resistant lines (lanes 3 and 4) had identical hordein patterns. Polymorphisms are observed among resistant and susceptible lines (lanes 1 and 2) for hordeins B, C and D.

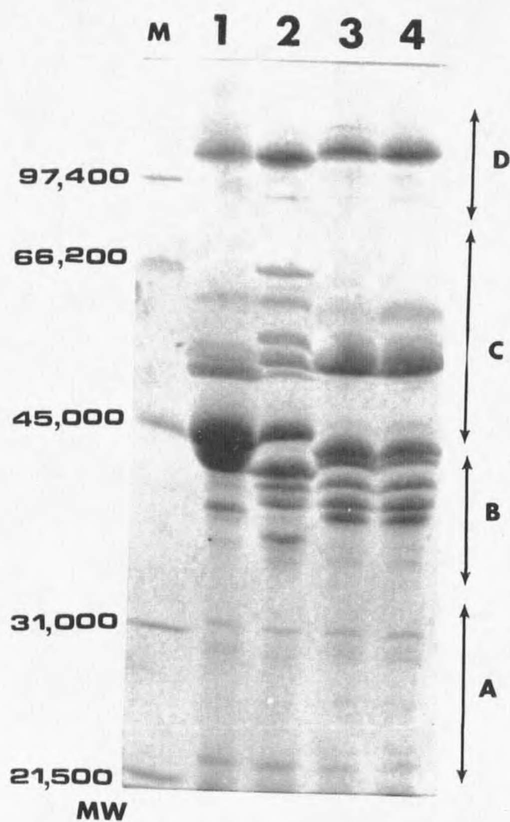
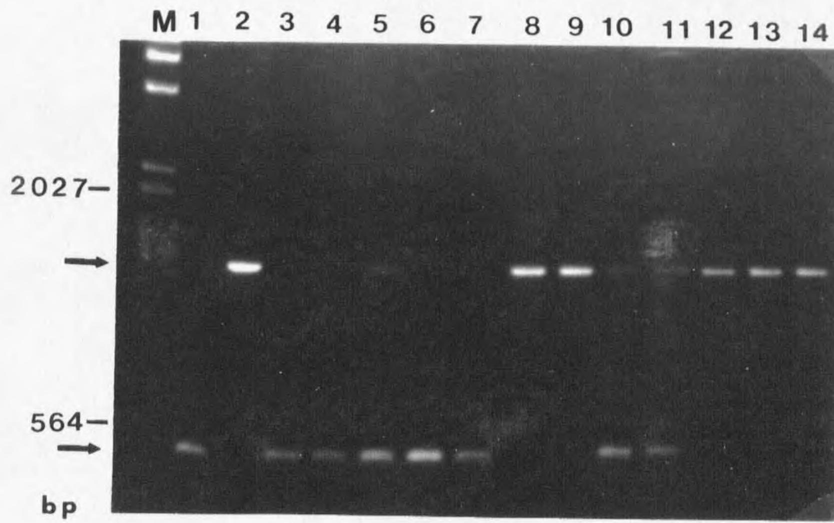


FIGURE 2. DNA fragments amplified with ABG8 L-R primers and electrophoresed in agarose gels of the lines PI366444 (lane 1), Stark (lane 2), and $F_2$ Stark/PI366444 (lanes 3-14).




M molecular weight markers
bp base pairs

FIGURE 3. Order of the markers BCD175, ABG358 and ABG8 and aproximate location in the map of chromosome 2 developed by NABGMP. Distances in cM between markers and % of variation attributable to each one from the QTL analysis. Rolling is indicated as a discontinuos line.

Chromosome 2

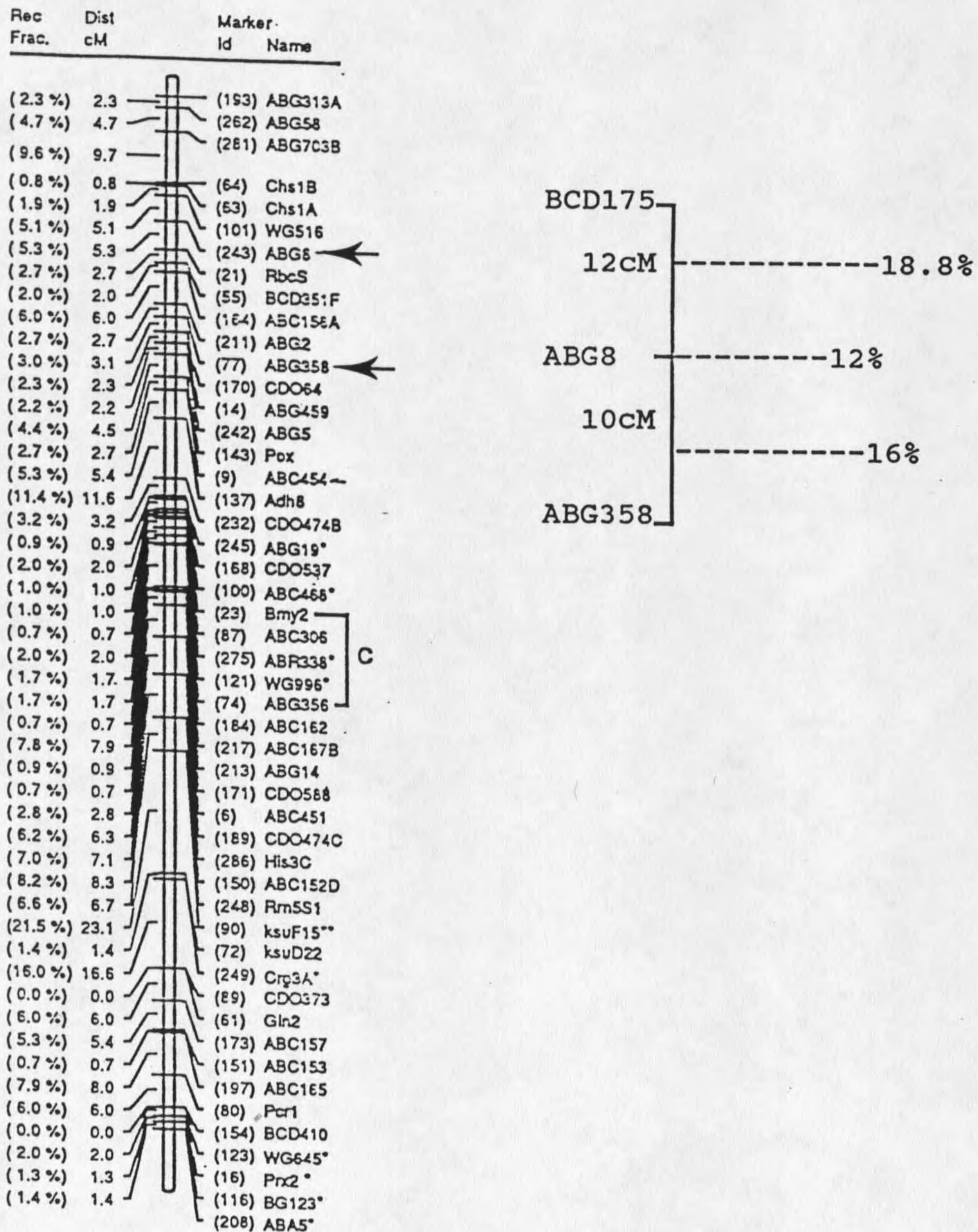
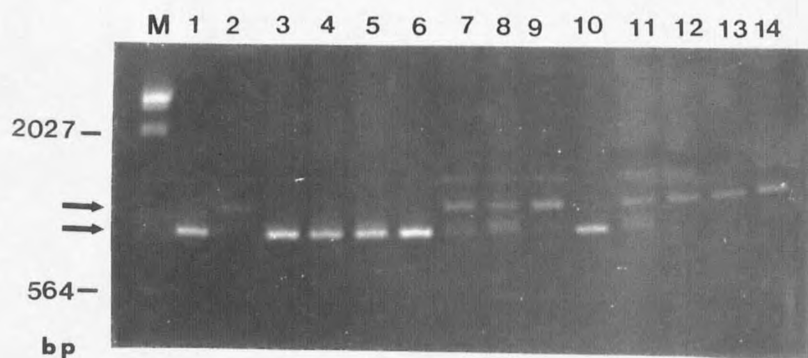
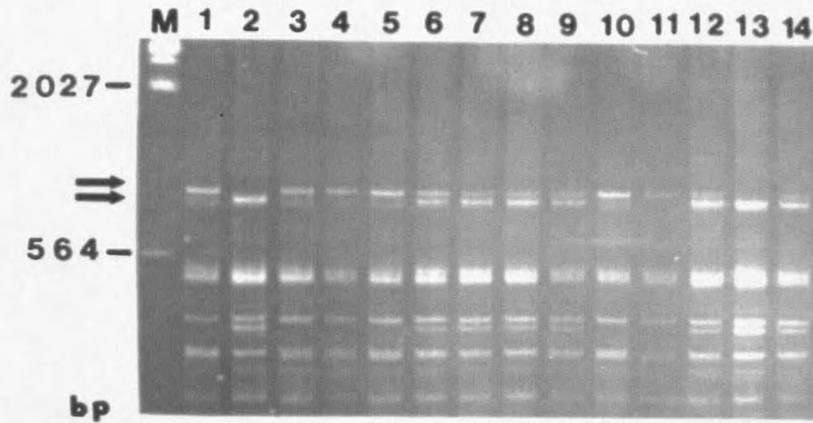


FIGURE 4. DNA fragments amplified with KV1-KV9 primers and electrophoresed in acrylamide gels of the lines PI366444 (lane 1), Bearpaw (lane 2), and $F_2$ Bearpaw/PI366444 (lanes 3-14).



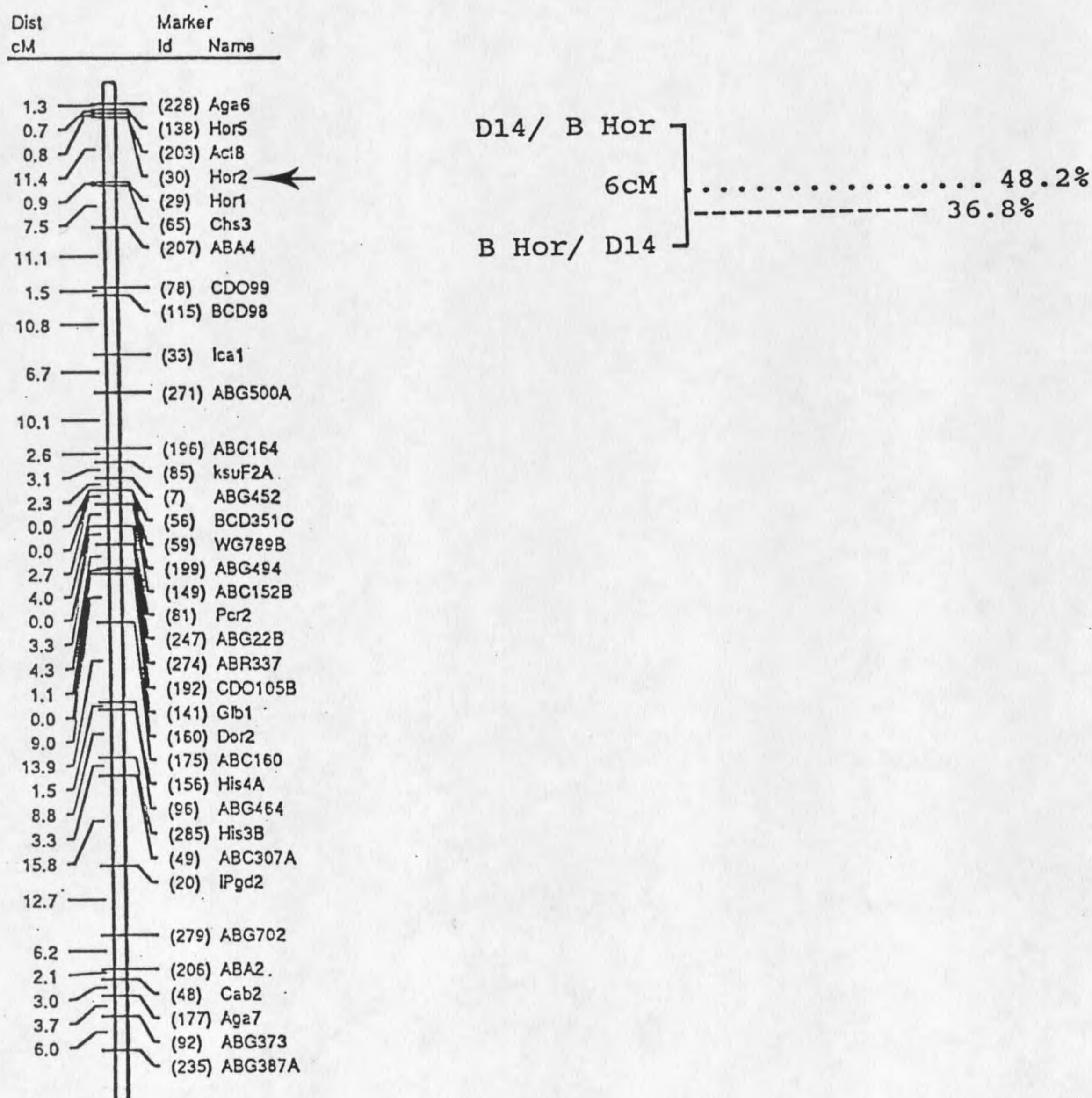

M molecular weight markers
bp base pairs

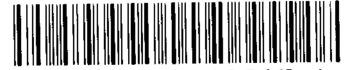
FIGURE 5. DNA fragments amplified with D14 L-R primers digested with the restriction enzyme <u>HinfI</u> and electrophoresed in acrylamide gels of the lines PI366444 (lane 1), Bearpaw (lane 2), and $F_2$ Bearpaw/PI366444 (lanes 3-14).




M molecular weight markers
bp base pair

FIGURE 6. Distance in cM between B hordein (B Hor) and D14 and location in the map of chromosome 2 developed by NABGMP. Percentage of variation attibutable to each marker was calculated in the QTL analysis. Chlorosis (...), rolling (---). Hor2=B Hor.

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