LOCATION OF GENES CONDITIONING RESISTANCE OF BARLEY TO LEAF RUST (*PUCCINIA HORDEI* OTTH)

by

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CHAPTER ONE

INTRODUCTION

Barley leaf rust, incited by Puccinia hordei Otth is an important disease in most barley (Hordeum vulgare L.) producing regions of the world. Barley leaf rust was once quite prevalent in Virginia, where severe infections often occurred on 'Wong', a once widely grown cultivar. The inheritance of resistance to barley leaf rust has been subject to continued investigations at the Virginia Agricultural Experiment Station. Prior to 1958 no barley varieties recommended in Virginia had resistance to leaf rust, but today resistant cultivars provide a means to control leaf rust in this region.

Nine genes have been identified which condition resistance to specific races of P. hordei (45,58). These genes, designated Pa, Pa_2, Pa_3, etc., are assumed to operate on a gene-for-gene basis with virulence genes in P. hordei (47). Three have been assigned to chromosomes. The Pa gene in 'Speciale' and the Pa_7 gene in 'Cebada Capa' have, via primary trisomic analyses, been assigned to chromsomes 2 and 3, respectively (82,99). The gene Pa_4 (= Rph4) in the cultivar 'Gold' has been mapped on the short arm of chromosome 5 via linkage with the Ml_a (= Reglal) gene on chromosome 5 (36,56). Although the genes Pa and Pa_7 have been assigned to chromosomes 2 and 3, respectively, there have been as yet no reports linking these genes with any mapped genes. Several reports have dealt inconclusively with the location of the Pa_3 gene in the cultivar 'Estate' (61,82).

The genes Pa, Pa_3 and Pa_7 each condition seedling resistance to race UN4 of P. hordei, a physiologic race prevalent in Virginia. Since, to the author's knowledge, no prior reports indicated linkage of Pa, Pa_3 or Pa_7 to any mapped genes in the barley genome, these three genes were considered suitable for further investigation. The objectives of the present study were:

(a) to detect the linkage relationships of genes Pa and Pa_7 in Speciale and Cebada Capa, respectively, with recessive marker genes of chromsome 2 and 3; and

(b) to locate the Pa_3 gene in Estate by primary trisomic analysis.

Only viable recessive marker genes available in stocks susceptible as seedlings to race UN4 of P. hordei were used in linkage analyses. This enabled detection of linkages in coupling phases with the dominant resistance genes Pa and Pa_7. Primary trisomic analysis was employed to locate the Pa_3 gene because this technique can be more efficient than conventional linkage analyses for determining the chromosome on which a gene resides.

CHAPTER TWO

REVIEW OF THE LITERATURE

2.1 Barley Leaf Rust Studies

Barley leaf rust is found in most areas where barley is grown, but the causal fungus, P. hordei, is probably limited in its host range to cultivated barley and closely related wild grasses (10). The aecial stage occurs on a number of Ornithogalum spp. (42), but does not develop in abundance and appears unimportant to overwintering of the fungus in North America and temperate Europe (10,81). Lumbroso et al. (32) note, however, that in Mediterranean countries the alternate host is important in perpetuating the fungus and in originating new physiologic races. Puccinia hordei survives the cold months on winter barley through slowly sporulating uredial infections and as dormant uredo-mycelium (48,69,81). Parlevliet and Van Ommerman (48) note that in severe winters the dormant mycelium may bridge the winter, but often the size of the rust population in the early spring may be determined by the rate of winter leaf replacement in the host and winter sporulation rate of the pathogen.

Barley leaf rust is not a very destructive disease in North America, but has at times been severe in the eastern U.S. and now commonly occurs throughout the upper Mississippi Valley (22,54). When it develops early, the disease is particularly destructive in southern barley-growing regions of the United Kingdom where yield loss may be from 17 to 31% (25). Barley leaf rust is also of economic importance in New Zealand (2). Yield losses occur through a reduction of grain test

weight since the disease usually develops during the grain-filling period (83).

Physiologic specialization in P. hordei was first reported in the U.S. by Mains (33) who demonstrated the existence of two different races on several barley cultivars. D'Olivera (41) demonstrated the importance of the aecial stage in relation to physiologic specialization. He obtained six new physiologic races by selfing two known races on Ornithogalum umbellatum L. Physiologic specialization in this fungus had been reported in Australia, Canada, Germany, Argentina, and the United States (29). The physiologic races isolated in various parts of the world were not comparable since different sets of host cultivars were used to discriminate pathogen races in most cases. In order to consolidate the earlier work and to provide a unified basis for future studies on physiologic specialization in P. hordei., Levine and Cherewick (29) performed inoculation tests with all previously reported differential cultivars. These authors employed the infection type scoring system for this purpose in which types 0, 1, and 2 were indicative of host resistance while types 3 and 4 indicated susceptibility. This classification of infection type is essentially the same as that developed by Mains and Jackson (34). Levine and Cherewick found that 16 barley cultivars were essential to key out all reported races of barley leaf rust, but that only nine of these differentials were necessary for the identification of 52 races that had been consolidated from ingredient races. Half of these consolidated races were of North American origin, and of these, race 4 was found in the highest frequency, constituting nearly 30% of the isolates.

The consolidation of the known extent of physiologic specialization in P. hordei into 52 unified races was accomplished with very little knowledge about the inheritance of leaf rust resistance in barley. Waterhouse (102), in Australia, first reported resistance to barley leaf rust to be conditioned by a single dominant gene. Henderson (18) found that eight cultivars, including 'Weider' (C.I. 1021), 'Bolivia' (C.I. 1257), and 'Ricardo' (C.I. 6306), contained the same gene locus conditioning resistance, which he designated Pa (designated as such because P. hordei was at that time specified as Puccinia anomala). He found a different gene for leaf rust resistance in Estate (C.I. 3410) and designated it Pa_1. In her M.S. Thesis, Zloten (105) reported that 'Kwan' (C.I. 1016) and Ricardo (C.I. 6306) each have two genes for leaf rust resistance, one completely dominant and the other exhibiting incomplete dominance. The dominant genes in these two cultivars were independently inherited, whereas, the incompletely dominant gene was the same in both cultivars. Starling (76) found that seven cultivars, including 'Peruvian' (C.I. 2441) and Ricardo (C.I. 6306), carried a leaf rust resistance factor at a common locus and that Cebada Capa (C.I. 6193) had a single gene for resistance at a different locus.

Roane (54) noted that the cultivar Ricardo represented a connecting link among 14 cultivars from the three thesis studies of Henderson, Zloten, and Starling. These 14 cultivars were thereby demonstrated to contain the gene for leaf rust resistance at Henderson's Pa locus. In order to identify resistance genes at common loci, Roane crossed the nine North American differentials used by Levine and Cherewick (29) in diallel with each other and with 'Moore', a universally susceptible

cultivar. Three groups of cultivars having common loci for resistance to P. hordei, race 4, were recognized. The first group, consisting of Bolivia (C.I. 1247), 'Quinn' (C.I. 1024), and 'Reka 1' (C.I. 5051), had a common locus which Roane designated tentatively as the A locus. Both Quinn and Bolivia appeared to have second loci for resistance to race 4. Not knowing if these additional loci were identical, Roane designated the second locus in Quinn tentatively as B, and the second locus in Bolivia tentatively as X. The second group of differentials, including 'Oderbrucker' (C.I. 940), Speciale (C.I. 7536), and 'Sudan' (C.I. 6489), had a common locus for resistance to race 4 which was tentatively designated as the C locus. The locus for resistance to race 4 in common to the third group, 'Gold' (C.I. 1445) and 'Lechtaler' (C.I. 6488), was designated as the D locus. Roane (54) thus established that at least four and possibly five loci conditioning resistance to race 4 of P. hordei were present among the nine North American differential cultivars.

Roane and Starling (60) crossed the cultivars Weider and Estate, which contained resistance loci designated by Henderson as Pa and Pa_1, respectively, to differentials containing genes previously designated A, B, C, D and X. The gene in Weider was found to be identical or allelic to resistance conditioned at the A locus of Bolivia, Quinn, and Reka 1. The gene in Estate was not present in any of the other differentials. Roane and Starling observed that in Australia, Watson and Butler (103) had designated the resistance gene in Weider as Pa_2. This assignment was subsequent to the designation of this gene as Pa by Henderson (18), but Roane and Starling proposed that the Pa_2 designation for this locus

be retained since Henderson's work remained unpublished. A proposal was made to designate the C locus of Oderbrucker, Speciale, and Sudan as the Pa locus and the locus in Estate as Pa_3. The D locus of Gold and Lechtaler was designated as Pa_4 and the B locus in Quinn was designated as Pa_5. Until the X locus of Bolivia could be demonstrated as distinct, it was tentatively designated Pa_X. Roane and Starling (60) noted that the Pa_2 locus appeared to be widely distributed among barley cultivars and that an allelic series may occur at this locus. Pustular variations were observed among cultivars carrying resistance genes at a common locus. Whether such differences are due to the action of different alleles or to variations in the total genetic background in which the resistance genes are acting remains unknown.

Among the F_2 and F_3 progeny of crosses between Cebada Capa and differentials carrying genes Pa, Pa_2, Pa_3, Pa_4, Pa_5, and Pa_X, Roane and Starling (60) observed susceptible plants except from crosses involving Quinn, which contains both Pa_2 and Pa_5. Since Cebada Capa segregated in all other crosses they concluded that the resistance gene in Cebada Capa is at or very closely linked to the Pa_5 locus of Quinn. Crosses between Cebada Capa and Bolivia gave an acceptable fit to a three gene segregation ratio. From this, Roane and Starling concluded that the gene in Bolivia formerly designated as Pa_X could now be designated Pa_6.

Crosses between Cebada Capa and Estate, which carries the Pa_3 locus, produced a deficiency of susceptible progeny. Linkage was detected between the Cebada Capa gene and Pa_3 with a recombination value of $33.8 \pm 3.4\%$. In the various crosses involving Cebada Capa, Roane and Starling (60) noticed a characteristic trend toward a deficiency of

susceptible plants. Except in the cross with Estate, these deficiencies did not result in significant deviations from ratios expected for independent assortment. The authors (60) note that linkage between duplicate genes is difficult to estimate accurately and that the consistent deficiencies of susceptible plants may suggest that two closely linked genes condition leaf rust resistance in Cebada Capa.

By employing a different isolate of P. hordei than the race 4 culture used by Roane and Starling, Johnson (21) determined that the resistance gene in Cebada Capa was distinct from any of the known genes, Pa through Pa_6. Johnson designated the gene in Cebada Capa as Pa_y. It appeared to be the same gene also found in 'Forrajera Klein', 'LaEstanzuela', 'France 7', 'France 21', and 'H 2212'. Frecha (11) inoculated F_2 and F_3 progeny of crosses between Forrajera Klein and Quinn separately with races 8 and 30 of P. hordei. The Pa_2 gene in Quinn was known to condition resistance to race 8 and susceptibility to race 30, whereas both Pa_y of Forrajera Klein and Pa_5 of Quinn condition resistance to both races. Frecha determined that the Pa_y gene of Forrajera Klein is linked with an 8% recombination value to the Pa_5 gene of Quinn. This close linkage may explain why Roane and Starling (60) found no susceptible plants among 362 F_2 plants and 405 F_3 families from a cross between Cebada Capa and Quinn.

Johnson (21) found no evidence for linkage between the Pa_3 gene in Estate and the Pa_y gene. Independent assortment of these two genes was supported by Parlevliet (44) from crosses between Estate and four cultivars, including Cebada Capa, which were monogenic for the Pa_y gene as determined by crosses with a susceptible cultivar. Parlevliet (44)

designated the gene $\underline{Pa}_y$ in Cebada Capa as $\underline{Pa}_7$. Tan (82) also obtained independent assortment between $\underline{Pa}_3$ and $\underline{Pa}_y$ (now $\underline{Pa}_7$) from an array of crosses, similar to those made by Parlevliet. In addition, Tan found from primary trisomic analysis that $\underline{Pa}_3$ and $\underline{Pa}_7$ likely reside on different chromosomes. Parlevliet (44) reported a gene in the cultivar 'EP 75' which was apparently unlike genes $\underline{Pa}$, $\underline{Pa}_2$, $\underline{Pa}_4$, $\underline{Pa}_5$, and $\underline{Pa}_7$. He designated this gene tentatively as $\underline{Pa}_z$. Udeogalanya and Clifford (100) reported a previously undescribed gene in C.I. 1243 which they designated as $\underline{Pa}_9$. It is not known how this gene compares to the $\underline{Pa}_z$ gene reported in EP 75 by Parlevliet. Clifford (7) and Parlevliet (47) reported a resistance gene in 'Egypt 4' (C.I. 6481) which they designated $\underline{Pa}_8$, although most prevalent races of P. hordei are virulent on Egypt 4.

A total of at least nine gene loci conditioning resistance to P. hordei have been reported and only two of these, $\underline{Pa}_5$ and $\underline{Pa}_7$, have been shown to be linked, with a recombination values of approximately 8% (11, 82). Only three of the nine known genes conditioning resistance to P. hordei have been assigned to chromosomes. The $\underline{Pa}_4$ locus was assigned to chromosome 5 by virtue of 16% recombination with the Ml_a locus (36,38). The genes $\underline{Pa}$ and $\underline{Pa}_7$ in Speciale and Cebada Capa were found to be linked to chromosomes 2 and 3, respectively, by primary trisomic analyses (82, 99). Tan (82) confirmed by trisomic analysis the assignment of $\underline{Pa}_7$ to chromosome 3 and attempted to locate $\underline{Pa}_3$ of Estate by the same means. In crosses between Estate and plants trisomic for chromosomes 3 through 7, he found no excess of susceptible plants. From this he concluded

that the gene Pa_3 is not located on chromosomes 3 through 7, and suggested that it might be on chromosome 1 or 2.

The genes at the nine known loci in barley which condition resistance to P. hordei have been identified through studies involving a limited number of races of the pathogen. These genes condition the hypersensitive type of host response in seedling and adult plants and are thought to operate on a gene-for-gene basis with corresponding virulence genes in the pathogen (44,47). With the use of additional races of P. hordei, other resistance genes might be found in the host. In an examination of infection types produced on Speciale, Oderbrucker, and Sudan by each of 52 unified races, distinct differences among these three differentials have been observed in response to certain races (55). Thus, despite the fact that these three cultivars are known to contain a resistance gene at the Pa locus, they appear to have genetic differences in regard to reaction to various races of P. hordei. Roane and Starling (60) have noted the widespread distribution of genes at the Pa_2 locus among barley cultivars. In the absence of information on the inheritance of host resistance to each of the known races of P. hordei, it has not been established whether allelic series may be present at various loci in the host.

Roane and Starling (60) observed pustular variations between cultivars possessing a common locus for resistance to race 4, but in absence of clear-cut distinctions, such variation could be due to differences in genetic background as well as to presence of an allelic series. Resistance at the Pa_2 locus has been reported as incompletely dominant (18,69); however, Zloten (105) reported a gene at this locus as

being completely dominant. Genes at the Pa locus exhibit complete to partial dominance depending upon parents and environmental circumstances (55,57). D'Olivera (41) observed that temperature fluctuations influence the development of the mycelia of P. hordei. Levine and Cherewick (29) and Simkin and Wheeler (70) noted that P. hordei develops optimally on barley when temperature remains relatively constant at 20 C. Roane (54) recorded infection types produced by race 4 at an average of 14 days after inoculation in a greenhouse maintained at 20-21 C. In bright warm weather, rust reaction could be read in 13 days; but in cloudy weather pustule development was not optimal until 15-16 days after inoculation. Joshi (23) observed the influence of days after inoculation and temperature on infection produced by race 4. At 15-18 C, race 4 could be classified as race 11, 4, or 37 at 10, 12, or 17 days, respectively. At 29 C, race 4 was classified as race 14 when scored 12 days after infection.

Undeogalanya and Clifford (100) reported that race F of P. hordei develops more slowly at lower temperatures. Pustules appeared after 11 days under a 23/11 C day-night temperature regime, whereas, they developed within 9 days under a 25/20 C regime. They assessed a number of specific race-cultivar interactions according to response to temperature. The cultivar 'Midas' was susceptible, having infection type 4 (large pustule not surrounded by chlorosis or necrosis) at all temperatures to the three races used. A group of four cultivars, including Estate, was resistant at all temperatures. The resistant reaction in this group was characterized as slight chlorotic flecking with some scattered necrotic flecks not associated with any tissue collapse.

Another group of cultivars, including Cebada Capa, was found to be susceptible at 5 C and resistant at higher temperatures. In these cases susceptible reactions were of infection type 3, characterized by having some chlorosis surrounding the pustules. The resistant reactions produced at the higher temperatures were characterized as necrotic flecks associated with surrounding leaf tissue collapse. Surrounding the dead tissue were deep yellow patches, which were attributed to general stress resulting from the host-parasite interaction. Another cultivar, C.I. 1243, which carries the gene designated as Pa_9 by these authors (100), was susceptible at 25/20 C, but resistant at 23/11 C and 5 C. The susceptible reaction was again a type 3 infection characterized by some chlorosis surrounding medium sized pustules. Under the 23/11 C temperature regime, the reaction was intermediate with both chlorotic flecks and scattered type 3 pustules. At 5 C the rust reaction was characterized by chlorotic spots and scattered small type 2 pustules.

Variable expressivity is well known for the temperature sensitive gene Sr_6, which conditions resistance to stem rust (Puccinia graminis Pers. f. sp. tritici Eriks. and Henn.) in wheat (Triticum aestivum L. em. Thell.) at temperatures below 25 C (9). Loegering (31) notes that for wheat stem rust, full development of infection type 4 on 'Little Club' wheat requires 14 days at 27 C but at 16 C an additional 3 or 4 days are required for full expression of susceptibility. In 'Red Egyptian' wheat, which carries the Sr_6 gene, infection type 4 results in 14 days at 27 C, but at 16 C infection type remains 0; even after 20 days or more. Loegering (31) reported rust reactions in near isogenic lines for alleles Sr_6 and sr_6. Both races 19 and 139 of Puccinia

graminis tritici produced type 4 infections at both 27 C and 16 C on the sr_6 line. On the Sr_6 line, a type 4 infection was produced by race 139 at both temperatures, but race 19 produced a type 4 infection at 27 C and a type 0; infection at 16 C. The effect of temperature is apparently race specific in this case, but the development of the pustule depends upon interaction of both host and pathogen. The processes involved in this interaction which are so profoundly affected by temperature in the case of Sr_6 and race 19 are not known.

Roane (54) observed that the barley leaf rust differentials Reka and Sudan, when crossed with the susceptible cultivar Moore, produce F_2 progeny with infection type classes 1, 3, and 4. By grouping these progeny as non-4 versus type 4 reactions, monohybrid ratios were obtained, which were substantiated by F_3 data. He pointed out that previous studies had conformed to the system devised for identification of physiologic races in which infection types 0, 1, and 2 are classed as resistant reactions and infection types 3 and 4 are classed as susceptible. Departure from this system was based upon the behavior of cultivars such as Reka and Sudan which have monogenic resistance to race 4 of P. hordei, but still produce variable reactions among their progeny. Roane suggested that the infection type classification system employed to identify races of the pathogen may not be appropriate when studying inheritance in the host. Since temperature, daylength, and other environmental factors can influence the expression of infection type, the absence of major gene resistance in the host should result in type 4 infections, whereas, any other infection type may indicate the presence of a resistance gene. This system assumes some degree of

resistance is attributable to the host genotype when infection type is non-4, under conditions which uniformly produced type 4 infections in the susceptible check.

Infection type is a histological expression of host-parasite interaction. The seedling resistances to rust fungi governed by single genes have been commonly characterized by hypersensitive cell death, although histological studies have not always confirmed this (30,68). Genetic background can influence the histological expression of resistance conferred by known resistance genes in the wheat stem rust system (68). The resistance of Cebada Capa, which contains the $\underline{Pa}_7$ gene, is of the hypersensitive type, but, in addition, has some similarity to that of the slow rusting cultivar 'Vada' (8,43). Variation in the expression of hypersensitive resistance in different genotypes may be due to genetic background and/or to environmental differences. Parlevliet and Kuiper (46) concluded that, in addition to $\underline{Pa}_7$, Cebada Capa carries a number of genes governing the latent period, a component of partial resistance. In the wheat stem rust system, Skovmand et al. (73) reported that separate genetic systems were responsible for single gene resistance and slow rusting, although two major genes were found to be linked to genes which condition slow rusting.

Clifford and Roderick (8) noted that although genes which condition slow rusting or partial resistance have often been termed as minor genes, the nature of such genes may not be inherently different from the so-called major genes with large effects. They point out the possibility that slow rusting genes may, in fact, be the same as major genes, but when in combination with other genes produce a residual

additive effect. Roane and Starling (60) observed that the seedling progeny of crosses with Cebada Capa were characterized by a deficiency of susceptible plants which might suggest two closely linked genes. Parlevliet and Kuiper (46) point out that in crosses between Cebada Capa and completely susceptible cultivars, adult F_2 plants carrying the $\underline{Pa}_7$ gene range in infection type from 0 and 1 (like the resistant parent) up to 2^+ and 3^-. Since susceptible parents had short latent periods, the range of latent period found among F_2 and F_3 progeny was attributed to Cebada Capa which apparently contains a number of genes conditioning longer latent periods. The hypersensitive resistance conditioned by $\underline{Pa}_7$ tends in seedlings to obscure genes which condition an extended latent period. In the seedling stage, the $\underline{Pa}_7$ gene is expressed completely so that only small necrotic flecks result from infection. Genes which influence latent period do, however, influence adult plant infection type in the presence of $\underline{Pa}_7$. Parlevliet (43) noted that even in the absence of a major gene such as $\underline{Pa}_7$, an increase in latent period was associated with a lowered infection type from 4 to 3^-.

2.2 Linkage Studies

Since Robertson's (62) early linkage studies, inheritance and linkage relationships in barley have been studied extensively. Linkage studies have been summarized by Robertson and others (64,65,66,67). Smith (74) compiled information on approximately 175 simply inherited characters and Nilan (39) summarized an additional 200 genes. The known genes of barley were also summarized by Takahashi (77). Robertson et al. (64) established seven independent linkage groups in barley based

upon associations among known genes. Barley has seven chromosomes, but the cytological association of chromosomes with linkage groups required the use of translocations.

Ramage et al. (49) have summarized translocations in barley. Burnham developed a set of translocation tester stocks in which letters a, b, c, d, e, f, and g were arbitrarily assigned to designate each chromosome (6). The barley karyotype proposed by Tjio and Hagberg (84) numbered the chromosomes longest to shortest from 1 to 5 and designated the two satellited chromosomes as 6 and 7. This karyotype is used as standard, although Noda and Kasha (40) have proposed a minor revision concerning the relative lengths of chromosomes 1, 2, and 3. Burnham and others (5,14,26,49) established the relationship between the gene linkage groups and the chromosome's arbitrarily assigned letter designations via translocation testers. From this work, it became apparent that linkage groups III and VII were carried on a single chromosome, and also that one chromosome, designated g, had no corresponding linkage group. Ramage and Suneson (51) reported a gene associated with the g chromosome, thereby permitting a gene assignment to all seven chromosomes.

The development of self fertile primary trisomics in barley permitted positive association of the seven chromosomes of barley with the genetic linkage groups. The letter designation of the chromosomes was dropped in favor of arabic numbers, 1 through 7, based upon Tjio and Hagberg's (84) karyotype and upon cytogenetic studies of Burnham and Hagberg (5). The previously available trisomics in barley were developed in the cutlivar 'Mars'. These did not exhibit sufficient

fertility and germination to permit general usage in genetic analyses (90). At the Kihara Institute of Biological Research, Tsuchiya (85) developed a complete set of primary trisomics from the wild two-rowed Hordeum spontaneum. In contrast to the trisomics previously developed from translocation homozygotes, Tsuchiya's trisomics were obtained by selfing autotriploid plants. These trisomics were distinguishable in adult plants as seven distinct types which Tsuchiya (87) termed Bush, Slender, Pale, Robust, Pseudo-normal, Purple, and Semi-erect, corresponding to trisomics 1 through 7. Tsuchiya's trisomics had sufficient fertility to be utilized for genetic analysis.

It is well known that plants trisomic for a given chromosome will modify the expected genetic segregation ratios for genes residing on that chromosome (3,53,89). Burnham (4), Khush (27), and Hermsen (19) have discussed the types of genetic segregation that may be expected from trisomic plants of various allelic constitutions. Tsuchiya (86) employed the altered segregation in trisomics to determine the relationships between each of his trisomic types, 1 through 7, and the seven known linkage groups. His results confirmed translocation studies which had demonstrated that the seven known linkage groups were distributed over only six of the seven barley chromosomes. After the relationships between the linkage groups and seven trisomic types had been determined, Tsuchiya (88) cytologically identified the extra chromosome in each trisomic via crosses with Burnham's translocation stocks. At diakinesis, chromosome configurations were examined in trisomic F_1 plants produced by crossing trisomic plants with a series of reciprocal translocation homozygotes. A chain of five, plus five bivalents was

indicative that the extra chromosome was homologous to one of the translocated chromosomes. This work permitted use of the same numbering system for chromosomes as well as linkage groups.

In the trisomics of H. spontaneum, Tsuchiya (87) reports that upon selfing, seed set averages 80.2%, but trisomic types Bush and Slender (trisomic for chromosomes 1 and 2, respectively) have less than 70% self fertility. Pollen fertility is lowest for Pale (trisomic 3), being 72.3%. Germination percentages average 86.4% for seed produced on selfed trisomic plants and 85.6% from trisomic/disomic crosses. The extra chromosomes are not transmitted through the pollen of any of the seven trisomic types. Among progeny of selfed trisomic plants of H. spontaneum, 25.9% are found to be trisomic when averaged over all seven types. The egg transmission rate of the extra chromosome was 21.0% in selfed trisomic 3 plants. Among progeny of trisomic/disomic crosses, egg transmission of the extra chromosome ranged from 9.8% for chromosome 5 to 27.5% for chromosome 2, and averaged 22.7% for all seven types. Tsuchiya (87) noted that in barley, length of the extra chromosome and germination percentage did not seem correlated to rates of transmission; however, he found, as did Ramage (50), that seeds with the extra chromosome were often smaller. A frequent occurrence of plants with chromosome fragments, besides 2n = 14 or 15, was observed in the progeny of primary trisomics (87).

Tsuchiya (90) developed a second trisomic series in a two-rowed cultivated barley, 'Shin Ebisu No. 16' by selfing a triploid plant which had been derived by crossing an autotetraploid Shin Ebisu 16 with its diploid counterpart. Upon selfing trisomic plants, transmission rates

of the extra chromosomes through the female ranged from 12.5% for Bush (trisomic 1) to 36.7% in Robust (trisomic 4), and averaged 29.1%. Seed fertility was generally poorer in the selfed trisomics of Shin Ebisu 16 than was observed in the H. spontaneum trisomics. The range of seed set on selfed plants of trisomic 3 was 2-14%, and increased to 22-25% when self pollinations were performed by hand. The poor seed set is due to lower pollen fertility and poor anther dehiscence in the trisomic plants. Despite the poorer seed set of trisomics in Shin Ebisu 16, Tsuchiya (90) noted that the spring growth habit, nonbrittle rachis at maturity, and short post-harvest dormancy provide advantages over H. spontaneum trisomics.

Trisomic series have been developed in several other cultivated barleys (91). The trisomics of the two-rowed cultivar 'Betzes' were developed from apparent triploid plants selected on the basis of partial sterility from a commercial field of Betzes (101). The transmission and seed fertility aspects of trisomics in Betzes (C.I. 6398) are similar to those in Shin Ebisu 16 (95).

Singh and Tsuchiya (71) reported on the morphology, fertility, and transmission of seven monotelotrisomics in the genetic background of Shin Ebisu 16. Average transmission rate of the extra telocentric chromosome is higher than transmission rates of primary trisomics. Triplo 2S and Triplo 3S were very sensitive to cold temperatures which resulted in low seed set. Seed set on Triplo 3S ranges from 50 to 70.8%. Singh and Tsuchiya (72) reported that an improved Giemsa N-banding technique has permitted better identification of telocentric chromosomes. He noted that the previously designated telo 3S is now

telo 3L and that a newly obtained telocentric in chromosome 3 is designated 3S.

The use of trisomics in genetic studies has provided a powerful tool in the assignment of genes to chromosomes. Current linkage maps of barley include over 110 mapped genes (96). Gene location via trisomic analysis is more efficient than translocation analysis because if genes are linked distal to the translocation break point they can avoid detection (13). Primary trisomic analysis provides definitive evidence that a gene either is or is not located on a given chromosome, provided segregation ratios are measured in both disomic and trisomic progeny. The assignment of genes to linkage groups by conventional methods can be difficult because barley chromosomes have regions unmarked with known genes. In such cases, trisomic analysis has been effective in assigning unmapped genes to chromosomes (89). Conventional linkage analyses may also be complicated when different cultivars differ as to their content of duplications and inversions, but trisomic analysis is not affected by such differences.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Parent Stock Descriptions

The cultivars carrying the leaf rust resistance genes to be studied were provided by Dr. C. W. Roane, Department of Plant Pathology and Physiology, Virginia Tech, Blacksburg, VA. The stocks carrying genetic markers for chromosomes 2 and 3 were provided by Dr. T. Tsuchiya of the Barley Genetic Stocks Center, Colorado State University, Fort Collins, CO. Primary trisomic stocks were provided by Dr. Tsuchiya and by Dr. R. T. Ramage of the Plant Sciences Department, University of Arizona, Tucson, AR. These stocks are described in Sections 3.1.1, 3.1.2, 3.1.3, and 3.1.4.

3.1.1 Leaf Rust Resistant Cultivars

The cultivars Cebada Capa (C.I. 6193), Speciale (C.I. 7536), and Estate (C.I. 3410) were used as resistant parents. These are all six-rowed, awned, spring barleys which have been involved in genetic studies for reaction to various physiologic races of *Puccinia hordei* (2,36,34). The cultivar Moore (C.I. 7251) has been used as a susceptible check cultivar (54). Speciale, Estate, and Cebada Capa carry genes *Pa*, Pa_3, and Pa_7, respectively, each of which condition resistance to race UN4 of *Puccinia hordei*. The characteristic infection type conferred by each of these genes has been described by Roane (57). Since these genes are all dominant or partially dominant, the leaf rust resistant cultivars were used as pollen parents so as to verify crosses with recessive markers in the F_1.

3.1.2 Chromosome 2 Marker Stocks

The chromosome 2 markers used in this study are listed with chromosome arm designation and stock numbers as follows:

Gene designation	Description, location
(gs_5)	glossy sheath 5, chromosome 2L (BGS 0355, CSU T15-11)
(lr)	reduced laterals, chromosome 2L (BGS 0058, CSU B13-9)
(e)	wide outer glume, chromosome 2S (BGS 0057, CSU B3-5)

The glossy sheath 5 gene (gs_5) was reported by Rassmusson and Lambert (52) to be inherited as a monofactorial recessive, located on chromosome 2 with a 2.5 ± 0.79% recombination value with wide outer glume (e) and a 32.0 ± 2.55% recombination value with row number (v). Glossy sheath 5 originated as a radiation induced mutation in the cultivar 'Jotun' (37). The recessive phenotype is a bright glossy green color on the leaf sheaths, while the heads and stems display a normal waxy appearance.

The marker gene which conditions reduced lateral spikelet appendages (lr) was reported by Leonard (28) to be a mutant six-row type. The phenotype conferred by this gene is characteristic of 'Nudihaxtoni' (C.I. 2213), which is used as the seed stock for the lr marker. The lateral spikelets are fully fertile, but are awnless, and the central spikelets have long awns. This trait is inherited as a simple recessive, but F_1 heterozygotes are intermediate, with short awns

on the lemmas of the lateral spikelets and long awns on central spikelets. Leonard reported that classification of intermediate types among the F_2 segregates of crosses involving Nudihaxtoni was difficult and required the use of F_3 progeny tests.

There has been some confusion concerning the relationship of lr with v and lk (awnless). Robertson et al. (66,67) reported that all three of these genes constitute an allelic series at a single locus. They based this upon Leonard's crosses with Nudihaxtoni (28). Examination of Leonard's publication, as noted by Haus (16), reveals no mention of allelism of lr and v. Based upon reported recombination values between lr and other genes on chromosome 2, Haus (16) suggested that lr should be located about six to eight map units from the v locus, toward the end of the chromosome.

Recent linkage maps of chromosome 2 include all three genes (v, lk, and lr) at the same locus (96). Haus (16) cited crosses made by Glinyany (12) to substantiate the nonallelism between lr and v. Leonard (28) discussed Glinyany's crosses (12) between Nudihaxtoni and 'Trifurcatum', a hooded barley. According to Leonard, the F_2 progeny in Glinyany's (12) study exhibited a 1:2:1 ratio with respect to the lr phenotype and a 3:1 ratio of hooded plus intermediate, to awned plants. These data do not bear directly upon allelism between lr and v because the gene conferring the hooded phenotype is independent of both v and lr.

Genes v (six-row) and lk (awnless) appear either tightly linked or allelic. Robertson et al. (63) obtained no six-rowed awnless plants among 3,974 F_2 plants from crosses between 'Engleawnless' (C.I. 2504) a

two-rowed barley of the genotype (VVlklk) and six-rowed cultivars of the genotype (vvLkLk). They concluded that v must be tightly linked with lk. Wiebe (104) recovered no six-rowed awnless plants among 200,000 F_2 progeny of crosses between C.I. 3119 an awnless two-rowed barley, and C.I. 2330, a six-rowed fully awned line. He noted that it seems unusual for both row number and awning to be controlled by the Vv locus, and suggested the possible existance of a short inversion covering the Vv and awning loci.

The elongated or wide outer glume gene (e) is a monofactorial recessive (93). The phenotype conferred depends upon the genetic background in which it is located and has been the source of some confusion. Glumes are from 2.5 mm to 4.0 mm wide and glume awn length varies from zero (awnless) to long (92 mm). The seed stock for e is 'Triple Bearded Club Mariout' which has the long (92 mm) glume awns. Tsuchiya (92) suggested that the gene w, proposed by Hor (20) for wide glumes, is probably identical to the gene e. The gene proposed by Hor (20), however, was a dominant gene, W, influenced by a dominant inhibitor. Helgason (17) reported that the e gene for elongated glumes was epistatic to another locus (log) which influences glume length. Helgason (17) also reported a dominant inhibitor of e present in 'KB16, Ab. 1475'. Tsuchiya (92) grouped 22 cultivars into three groups based upon the width and length of glumes, glume awns, and lemma awns. None of the narrow glumed types has the extremely long glume awns; however, several wide glumed lines are awnless or have short glume awns. He noted the existence of at least two types of glume awn length, long (Log) and short (log) in E (narrow glumes) background. Although the

log and e loci control different traits, the Log log locus is apparently not expressed in ee genotypes.

The traditional designation, elongated glumes, for the wide glume gene e came into use apparently because long glume awns and wide glumes are absolutely correlated in crosses for which parent genotypes possess no inhibitors of the type suggested by Helgason's study (17). Tsuchiya and Singh (97) have demonstrated by telotrisomic analyses that e resides on the short arm of chromosome 2 and gs_5 resides on the long arm. These two genes were reported to be linked with a 2.5% recombination value (52); hence, they reside near the centromere.

3.1.3 Chromosome 3 Marker Stocks

The chromosome 3 markers used in this study are listed with chromosome arm designation and stock numbers as follows:

Gene designation	Description, location
(gs_2)	glossy sheath 2, chromsome 3L (BGS 0352, CSU T15-8)
(als)	absent lower laterals, chromosome 3L (BGS 0101, CSU T24-5)
(uz)	uzu or semi-brachytic, chromosome 3L (BGS 0102, CSU T30-32)
(yst_2)	yellow streak 2, chromosome 3S (BGS 0109, CSU T10-13)

The marker glossy sheath 2 (gs_2) is a monofactorial recessive which confers a bright glossy green color to the leaf sheaths, stems, and heads of the plant (37). The origin of glossy sheath 2 is a spontaneous mutation in several cultivars of which 'Atlas' (C.I. 4118) is used as the seed stock. Takahashi (78) reported a 37.7% recombination value

between gs_2 and the uz locus. He determined that the als gene resides between gs_2 and uz, with gs_2 more distal to the centromere. Through primary and telotrisomic analyses, the genes gs_2, als, and uz have been located on the long arm of chromosome 3 (96,98).

The absent lower laterals (als) marker was reported by Kasha and Walker (24) to be a monogenic recessive located on chromosome 3. The phenotype conferred by als is the absence of lateral spikelets on the base of the spike and the presence of only one or a few large coarse tillers. Kasha and Walker (24) reported these two diagnostic features are always associated as pleiotropic effects of a single gene or as effects of two tightly linked genes. They suggested that a small deletion may have occurred to produce this mutation since its origin was in cobalt^{60} irradiated 'Montcalm'.

The uzu or semi-brachytic gene (uz) is a monofactorial recessive, the phenotype of which is a pleiotropic dwarfing of all parts of the plant (94). The gene tends to shorten the length and broaden the width of the leaves, culms, and heads of the mutant. The coleoptiles of uzu plants are reported to show a prominent projection at the apex (94); however, this feature was not observed in the uzu mutants of the present study. This mutation occurs spontaneously in many Japanese cultivars. The cultivar 'Baitori 10' is used for seed stock.

The gene yellow streak 2 (yst_2) is a monofactorial recessive located on chromosome 3 (79). It occurred as a spontaneous mutation in the F_5 of the cross 'Kuromugi 148'/'Mensury C'. The mutant phenotype, which has many wide, white or yellow streaks on developing leaves, is expressed in response to a two-week period of low temperature (3 C)

immediately after sowing. Takahashi and Moriya (79) reported a 10% recombination value between $\underline{yst_2}$ and $\underline{uz}$. Through telotrisomic analyses, $\underline{yst_2}$ has been assigned to the short arm of chromosome 3 and $\underline{uz}$ to the long arm (96,98).

3.1.4 Primary Trisomic Stocks

The primary trisomic stocks used in this study are listed with their source and stock number as follows:

Triplo 1, Tsuchiya, Betzes Tri 1 '70

Triplo 2, Tsuchiya, Betzes Tri 2 '70

Triplo 3, Ramage, Betzes Tri 3 '80

Triplo 4, Tsuchiya, Betzes Tri 4 67-t-21-C

Triplo 5, Tsuchiya, Sin Ebisu 16 Tri 5 SE 71-10-14

Triplo 6, Tsuchiya, Betzes Tri 6 67-5-20-j

Triplo 7, Tsuchiya, Shin Ebisu 16 Tri 7, SE 71-15-18

The trisomics in Shin Ebisu 16 were isolated by Tsuchiya (90) from a selfed autotriploid plant and from reciprocal crosses between it and the normal diploid. The trisomics in the cultivar 'Betzes' (C.I. 6398) have been isolated from spontaneously occurring autotriploids (101). Both Shin Ebisu 16 and Betzes are two-rowed, spring barleys. The seven trisomic types each have distinguishing morphological features, especially conspicuous from tillering to heading (90). These phenotypes were helpful in confirming the identity of the trisomic parents used in the present study.

3.2 Procedures for Seedling Inoculations

Rust inoculations in this study were usually made on seedlings at the two leaf stage of growth using culture 57-19 of race UN4 of *Puccinia hordei*, which was provided by Dr. C. W. Roane of the Dept. of Plant Pathology and Physiology. Inoculations were made in the glasshouse by a dry spore method similar to that used by Roane (54). Urediospores mixed with talc were blown onto the plants in the late afternoon. The plants were then enclosed in a canvas tent with a mist machine timed to produce mist for 6 hours immediately following inoculation and for 1 hour at dawn. The plants were allowed to dry slowly within the closed tent until noon of the following day when the tent sides were lifted. So as to avoid exposing the plants to direct sunlight, the tent top was left in place until the next morning. Rust reaction data were taken from 13 - 16 days following inoculation, depending upon prevailing greenhouse temperatures. Bright, warm weather tended to hasten the development of the rust infections, whereas, cloudy weather tended to retard rust development. Greenhouse temperatures were maintained near 20 C, but exceeded 30 C at times during the April 1982 inoculation of trisomic-derived F_2 populations.

The method used to classify the seedling infection types was based upon descriptions provided by Levine and Cherewick (29) and was similar to the scale used by Parlevliet (44). The infection types are described as follows:

Infection type	Plant reaction
0	practically immune
0;	small dark flecks with little chlorosis or necrosis
0c	chlorotic flecks
0n	necrotic flecks
1	extremely resistant--minute uredia surrounded by mainly necrotic tissue
2	moderately resistant--small uredia surrounded by chlorotic tissue
3	moderately susceptible--medium size uredia surrounded by chlorotic rings
4	extremely susceptible--large size uredia with little or no chlorosis

The susceptible Moore was included as a check cultivar in all inoculations. The classification of resistant versus susceptible reactions was based upon the procedure used by Roane (54), who grouped infection types 0 - 3 into the resistant class and included only type 4 infections in the susceptible class under conditions which uniformly produce a type 4 reaction in the susceptible check. The present study involved crosses between resistant cultivars characterized by 0 - 0n infections and susceptible marker and trisomic stocks characterized by type 4 infections. Among the F_2 progeny of these crosses, segregation of infection type was primarily 0 - 0n types versus type 4 infections; thus, distinction between resistant and susceptible progeny was clear in most cases.

3.3 Crosses Between Marker Stocks and Leaf Rust Differentials

Both chromosome 2 and 3 marker stocks, the resistant parents Speciale and Cebada Capa, along with the susceptible Moore, were seeded into 15.2 cm clay pots in the glasshouse on 28 Nov. 1979. Potting soil was a mixture of topsoil, sand, and peat in 3:2:1 proportions by volume. Both a 14:14:14 balanced granular fertilizer and lime were mixed with the potting soil at approximately 5 and 10 kg per metric ton of soil mixture, respectively. Inoculations with culture 57-19, race UN4 of Puccinia hordei were made in January to determine the nature of the marker stocks' rust reactions. The marker stocks reported in this study all exhibited type 4 infections. Upon heading, the chromosome 2 marker stocks were used as seed parents in crosses with Speciale, and the chromosome 3 markers served as seed parents in crosses with Cebada Capa. The crossed seeds were harvested upon maturity.

During August 1980 the F_1 plants were started in 7.6 cm clay pots in the growth chamber at 10 hr, 16 C days and 10 C nights in order to provide an extended time period for tillering of the plants. Inoculation of the F_1 plants was accomplished in the growth chamber, after which the plants were transferred to the glasshouse in November and transplanted into 15.2 cm pots. Inoculations were made with a field collection of P. hordei when plants were at the jointing stage. Difficulties were encountered when attempts were made to increase race 4 inoculum during periods of warm weather which necessitated the use of a field collection of P. hordei for obtaining rust reactions in the F_1. The Cebada Capa crosses exhibited type 1 - 2 infections at 14 days, which developed into On type infections by the 18th day after

inoculation. The Speciale crosses exhibited type 4 infections. This suggests that inoculum collected in the field contained race 8 which has been prevalent in the Blacksburg field plots in recent years (59). The F_1 plants were harvested at maturity during March 1981.

After harvest the F_1 heads were dried at 36 C for two days, then threshed and transferred to a freezer for one week in an attempt to break seed dormancy. The F_2 populations were seeded into peat pots during April 1981 and transplanted into the field in May. The transplants were space-planted at a rate of six plants per 914 cm row, with rows spaced at 30 cm. The F_2 plants derived from a single selfed F_1 plant were designated as a single F_2 population. The F_2 populations were kept free of weeds by hoeing, and were provided moisture by sprinkle irrigation. The F_2 populations were observed for marker gene segregation, and individual plants expressing the mutant phenotype were tagged. Plants were harvested during mid-July when the majority were mature in each population. The remaining plants were harvested later. For each population, individual F_2 plants were pulled one at a time, and the heads put in a paper bag labelled with population and plant number. The harvested F_2 populations were dried and stored in these bags until the following winter.

During January, February, and March of 1982, a sample of about 20-30 F_3 progeny from each F_2 plant was planted in 7.6 cm pots for leaf rust inoculation. Each pot contained the progeny of a single F_2 plant. The segregation of individual F_3 families for reaction to race 4 of P. hordei enabled determination of F_2 genotypes with regard to the gene conditioning resistance. The remaining seed of each F_2 plant was then

threshed and put into coin envelopes for field planting. A preplant application of 14:14:14 balanced granular fertilizer at 336 kg/ha was made prior to disking the field. On 2 April 1982 the seed in each envelope was planted into a 914 cm row with a 30 cm spacing between rows. Granular Di-syston for aphid control was applied at 7.84 kg/ha after planting. In early May a mixture of liquid Di-syston and Brominal (for aphid and weed control) was applied at 7.84 and 1.68 kg active ingredient/ha, respectively.

Each row represented a single F_3 family. Marker gene segregation within a row permitted determination of F_2 genotypes for the gene in question. Sampling the F_3 progenies of F_2 plants, once for seedling rust reaction and once for marker gene segregation in adult plants, enabled the distinction of the nine genotypes expected in an F_2 population segregating jointly for two gene pairs. If the genes are linked, this procedure does not permit distinction between coupling and repulsion heterozygotes.

For crosses involving the seedling marker $\underline{yst_2}$, only one sampling of F_3 progenies was necessary to obtain the complete genotypic classification of F_2 plants. The F_3 progenies of these crosses were planted in 7.6 cm pots and transferred to the growth chamber where they were germinated at 2 C for two weeks. These populations were then returned to the glasshouse and inoculated with culture 57-19 of race 4. The segregation of the seedling marker and seedling rust reaction were then determined simultaneously. This procedure would permit, if F_3 families were sufficiently large, the complete genotypic classification of F_2 plants, including distinction between coupling and repulsion

heterozygotes in the presence of linkage. In addition to the F_3 progenies, several F_2 populations derived from $\underline{yst_2}$ crosses were grown and analyzed for the joint segregation of rust reaction and $\underline{yst_2}$.

For all crosses presented in Tables 1 through 21, the term population refers to the progeny of a single selfed F_1 plant. For the dihybrid segregation ratios presented in Tables 1 through 15, the letters "A" and "a" designate dominant and recessive alleles, respectively, of the marker gene locus under scrutiny, and the letters "B" and "b" designate dominant and recessive alleles, respectively, for the given locus conditioning reaction to race 4 of Puccinia hordei. In the following tables and text the resistant parents Cebada Capa, Speciale, and Estate are occasionally abbreviated CC, Sp, and Es, respectively.

3.4 Crosses Between Trisomic Stocks and Leaf Rust Resistant Cultivars

In January 1980, with the assistance of Mr. Allen Price, Research Supervisor, Agronomy Dept., a complete set (chromosomes 1 through 7) of trisomic parent plants was obtained from the stocks provided by Dr. Tsuchiya. Trisomic plants were identified by chromosome counts made from the tips of seminal roots taken from freshly germinated seed. To obtain the root tips, the seed was placed upon blotter paper in petri dishes and soaked with a thiram solution (to control fungi) for several minutes before draining. This was done in the evening and petri dishes were left on the counter at room temperature until the next afternoon, or until signs of germination were visible. At that time, the petri dishes were placed in the refrigerator at 4 C overnight and removed in the late afternoon of the following day. The seed at this point usually

appeared no different than when it was initially placed in refrigeration. Left on the counter at room temperature (20 C) overnight, the seed usually had sprouted seminal roots several cm in length by morning, at which time the root tips were collected. Two seminal roots were usually collected from each seed and placed with a label into vials containing a saturated solution of mono-bromonapthalene. The vials were then placed in the refrigerator for 4 hr in order to shorten the metaphase chromosomes. The root tips were then fixed in a solution of 3 ethanol:1 acetic acid and stored in the refrigerator until they were to be stained and examined. For Feulgen staining, the root tips were removed from the fixative and hydrolyzed in 1N HCl at 60 C for five to ten minutes. The root tips were then transferred to vials containing the leuco-basic fuchsin stain and stored in the refrigerator for microscopic examination.

As the root tips were collected, the seed was planted into peat pots and labelled with pot stakes. The seedlings were then transferred to the glasshouse until chromosome counts were completed. When trisomic plants were distinguished from diploids, they were transplanted into 15.2 cm clay pots. During the jointing stage, the trisomic plants were compared to normal diploid Betzes and Shin Ebisu 16 plants for the diagnostic traits described by Tsuchiya (90).

During April 1980 crosses were made with leaf rust resistant Estate, Speciale, and Cebada Capa using the trisomic plants as seed parents. The percent seed set on crossed heads was low. Viable trisomic F_1 plants were recovered only from crosses between Estate and Triplo 4, 5, and 6. During September 1980, chromosome counts were made

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to identify trisomic F_1 plants. These were planted in 7.6 cm pots and grown for 3-4 weeks at 16 C, 10 hr days in the growth chamber to promote tillering. The plants were then transplanted into 15.2 cm pots in the glasshouse.

During November 1980, another set of trisomic parents was obtained from Betzes stocks provided by Dr. Ramage. These newly obtained parents, along with the trisomic F_1's, were inoculated with P. hordei that had been collected in the field. At the time of inoculation the Betzes parent plants were in the tillering stage, whereas the F_1 plants were already in the jointing stage of growth. The Betzes parents exhibited type 4 pustules, but the trisomic F_1 plants had intermediate type 2 infections. The Betzes parents were checked for the trisomic phenotypes described by Tsuchiya (90), and crosses were made with Estate and Cebada Capa during January and February 1981. No crossed seed was obtained for trisomics 1, 2, and 7. Trisomic parents were again obtained in Betzes and were transplanted into the field in mid-April 1981, along with the leaf rust resistant cultivars. Granular Di-syston was applied to the soil between plants. Crosses were again attempted, but no viable trisomic F_1 seed was obtained from crosses between Estate and Triplo 1, 2, and 7.

In September 1981 the crossed seed obtained during the previous winter and spring were screened for trisomic F_1 plants. Trisomic F_1 plants and their diploid counterparts were transplanted into 15.2 cm pots during October 1981. Tsuchiya (90) has noted the poor self fertility of Triplo 3. Upon heading, the F_1 plants of Triplo 3 crosses were manually self pollinated and heads were bagged to prevent possible

outcrossing. These F_1 plants were harvested in February 1982. The F_2 seed derived from trisomic F_1 plants, grown in both the winters of 1980-81 and 1981-82, were planted into 7.6 cm pots in the glasshouse during late March 1982. F_2 populations derived from both trisomic F_1 plants and diploid checks, along with resistant and susceptible parents, were inoculated in the glasshouse with culture 57-19, race 4 of P. hordei on 6 April 1982. Leaf rust reactions were recorded during the interval 19 April through 22 April 1982.

Several trisomic derived F_2 populations were not included in the bulk F_2 inoculation of 6 April. It was desired to distinguish trisomic and diploid plants among the F_2 progenies of crosses involving Triplo 3 in order to detect segregation ratios separately among diploid and trisomic progenies. Chromosome counts were made for two F_2 populations of the cross Triplo 3/Cebada Capa and for six F_2 populations of the cross Triplo 3/Estate. These progenies were inoculated in the glasshouse together with parents and diploid checks on 1 June 1982. Immediately following inoculation, the seedlings were taken to a shaded spot at the Agronomy Farm Headquarters, where they remained until rust reaction data were collected on 15-16 June 1982.

3.5 Analyses

Progeny segregations in crosses involving marker stocks were analyzed by use of chi square goodness of fit tests in a manner described by Mather (35). The four-class data based upon F_2 phenotypes were analyzed using three orthogonal, single-degree-of-freedom comparisons. The three comparisons correspond to the three possible sources

of deviation which contribute to total deviation from the 9:3:3:1 dihybrid ratio. These three sources are: the two single factor segregations and their interaction or linkage. By use of single-degree-of-freedom comparisons the total chi square can be partitioned to remove deviations due to single factor segregations leaving the interaction portion as a sensitive test for linkage. The linkage portion of chi square is interpreted as follows: A high P value means there exists a high probability of having obtained a linkage chi square of the observed magnitude given that the genes are independent; whereas, a low P value for linkage means it is unlikely to have obtained a chi square of the observed magnitude given that the genes are independent. In the context used by Mather (35) P values lower than 0.05 for the linkage portion of chi square indicate a significant portion of the total chi square for goodness of fit to the 9:3:3:1 ratio is due to linkage between the two segregating genes. This interpretation of chi square analysis for linkage has been adopted in the present study. High P values in the linkage columns of tables 1 through 15 suggest independence, whereas, significantly low P values suggest the presence of linkage. Calculation of linkage intensity in the cross $\underline{gs}_5$/Speciale ($\underline{Pa}$) was by the method of maximum liklihood for F_2 and F_3 coupling data using the tables compiled by Allard (1).

Data derived from individual F_1 plants were analyzed as separate populations. Combined data are reported only when individual populations were homogeneous in segregation behavior as was determined by chi square tests for heterogeneity. In cases where single factor segregations departed significantly from expected ratios, heterogeneity tests

were based upon observed totals. A number of F_1 plants yielded too few F_2 progeny for valid use of chi square analyses. The P values reported in tests for which fewer than five observations were expected in a given class facilitate comparisons in these data, but cannot be accepted as being valid estimates for goodness of fit.

Trisomic analyses were based upon expectations for segregation of the simplex (Aaa) genotype since F_1 plants of the critical trisomic are of the constitution ($\underline{Pa}_7\ \underline{pa}_7\ \underline{pa}_7$) and ($\underline{Pa}_3\ \underline{pa}_3\ \underline{pa}_3$) for Cebada Capa, and Estate crosses, respectively. Segregation of the simplex causes departure from the expected 3:1 ratio due to an excess of susceptible plants. Detection of trisomic inheritance is most precisely accomplished by determining ratios separately for diploid and trisomic segregates. Crosses involving the critical trisomic should in the F_2 exhibit a much higher proportion of susceptibles among diploid plants than among trisomics. Diploid and trisomic plants were distinguished by chromosome counts in the F_2 of crosses involving Triplo 3.

For the cross Triplo 3/ Cebada Capa, the expected trisomic segregation ratios were calculated based upon random chromosome association and 15% transmission of the extra chromosome through the egg. This transmission rate was determined from the proportion of trisomic segregates found among the F_2 plants.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Linkage Analyses for Chromosome 3 Marker Stocks/Cebada Capa

Results from crosses between Cebada Capa and the four marker stocks for $\underline{gs_2}$, $\underline{als}$, $\underline{uz}$, and $\underline{yst_2}$ are presented in Tables 1 through 10. In all four crosses, F_2 genotypes have been determined by the classification of F_3 families for the combined segregation of the recessive marker and leaf rust reaction. The F_2 phenotypic classes in all tables have been extracted from F_3 family data, with the exception of Table 8, which presents the direct phenotypic classification of F_2 plants for populations involving the seedling marker $\underline{yst_2}$.

4.1.1 Glossy Sheath 2 ($\underline{gs_2}$)/Cebada Capa ($\underline{Pa_7}$)

In Table 1 are presented the joint segregation of $\underline{gs_2}$ and $\underline{Pa_7}$ from four selfed F_1 plants of the cross glossy sheath ($\underline{gs_2}$)/Cebada Capa ($\underline{Pa_7}$). All four populations exhibit good fit to the 3:1 ratios for each single gene pair, and have a good fit to the 9:3:3:1 ratio for independent assortment in the dihybrid. The interaction portion of the total chi square was small in each of the four populations, indicating there is no linkage between $\underline{gs_2}$ and $\underline{Pa_7}$.

In populations 1-1, 1-2, and 2-2 of Table 1, there are fewer than the required minimum of five observations expected in the double recessive class; therefore, the chi square probabilities reported for the total fit to a 9:3:3:1 ratio and those reported for the linkage portion in these three populations may not be accurate. The joint segregation of glossy sheath ($\underline{gs_2}$) and leaf rust resistance ($\underline{Pa_7}$) among the four

Table 1

F_2 phenotypes condensed from F_3 family data of the cross glossy sheath 2 (gs_2)/Cebada Capa (Pa_7)

Selfed F_1 plant	No. F_2 plants in phenotypic classes*				P from chi square for indicated ratio**			
	A-B-	A-bb	aaB-	aabb	9:3:3:1	3A-:1aa	3B-:1bb	Linkage†
1-1	35	11	6	3	.50-.30	.20-.10	.99-.98	.70-.50
1-2	17	4	8	4	.50-.30	.20-.10	.95-.90	.50-.30
1-5	37	11	17	5	.70-.50	.30-.20	.70-.50	.95-.90
2-2	7	2	5	1	.70-.50	.20-.10	.70-.50	.70-.50
Combined	96	28	36	13	.70-.50	.50-.30	.70-.50	.70-.50

*Phenotypes A-B-: normal, resistant
A-bb: normal, susceptible
aaB-: glossy sheath, resistant
aabb: glossy sheath, susceptible

**Less than five observations were expected in the double recessive class of 1-1, 1-2, and 2-2.

†P-values for the linkage portion of chi square indicate probabilities of the observed deviations from the dihybrid ratio if genes A and B are inherited independently.

populations is homogeneous and consistent with that observed in the combined data line of Table 1. The good fit of the combined data to 3:1 ratios for single gene segregations and to the 9:3:3:1 ratio for the joint segregation of two independent genes supports the evidence from the four individual populations that the genes $\underline{gs}_2$ and $\underline{Pa}_7$ are not linked.

The F_2 phenotypic classes were condensed from F_3 families sampled separately for segregation of leaf rust reaction and segregation of the glossy sheath trait. With the exception of population 1-5, it was difficult to distinguish in the field between F_3 families segregating for glossy sheath and those homozygous dominant for this trait. This was likely due to close plant spacing within family rows in combination with the early maturity of the glossy sheath ($\underline{gs}_2$)/Cebada Capa ($\underline{Pa}_7$) crosses. The failure to distinguish families segregating for $\underline{gs}_2$ from those homozygous dominant for this trait necessitated an analysis based soley upon F_2 phenotypes rather than genotypes. The one population (1-5) of the cross glossy sheath ($\underline{gs}_2$)/Cebada Capa ($\underline{Pa}_7$) for which all nine F_2 genotypic classes could be distinguished did exhibit a good fit to the expected 1:2:1 ratios for both single gene segregations, and to the 1:2:1:2:4:2:1:2:1 ratio expected for the joint segregation of two independent gene pairs.

4.1.2 Absent Lower Laterals ($\underline{als}$)/Cebada Capa ($\underline{Pa}_7$)

The genotypic classification of F_2 plants derived from two selfed F_1 plants of the cross absent lower laterals ($\underline{als}$)/Cebada Capa ($\underline{Pa}_7$) are presented in Table 2. Population 3-5 exhibited a poor fit to the

Table 2

F_2 classification via F_3 family data from the cross absent lower laterals (*als*)/Cebada Capa (*Pa*$_7$)

Selfed F_1 plant	Genotype of F_2 plants based upon F_3 family data*									P from chi square for indicated ratio**		
	AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb	1:2:1: 2:4:2: 1:2:1	1AA: 2Aa: 1aa	1BB: 2Bb: 1bb
3-2	5	5	4	6	5	9	1	3	1	.20-.10	.20-.10	.10-.05
3-5	13	24	4	10	24	10	9	5	3	<.01	<.01	.20-.10

*Genotypes AA = *Als Als* BB = *Pa*$_7$ *Pa*$_7$
Aa = *Als als* Bb = *Pa*$_7$ *pa*$_7$
aa = *als als* bb = *pa*$_7$ *pa*$_7$

**Less than five observations were expected in the double homozygote classes of 3-2.

expected 1:2:1 single factor segregation for the als gene. This was due to a large excess in the homozygous dominant class at the expense of both the heterozygous and homozygous recessive classes. Population 3-2 also had somewhat more homozygous dominants and fewer homozygous recessives for this trait than expected. In population 3-5, the large excess of homozygous dominant families may have resulted from misclassification of families segregating for the als gene. The phenotypic expression of als is somewhat variable in segregating materials and recognition of one or a few recessive (als als) plants within a family row may have been obscured by close plant spacing. The deficiency of recessive homozygotes for this trait is due primarily to partial sterility of the recessive phenotype in the F_2. The F_2 populations derived from the selfed F_1 plants of this cross revealed that these crosses segregated according to the expected 3 normal (Als-) to 1 recessive (als als). The seed produced on a number of the recessive (als als) plants was insufficient for adequate sampling in the F_3, which resulted in the deficiency of recessives (als als) as shown in Table 2.

The segregation of rust reaction in population 3-2 exhibited an excess of susceptible phenotypes, whereas, population 13-5 had a deficiency of susceptible plants. The genotypic array presented in Table 2 for population 3-2 reveals a trend toward an acceptable fit to the 1:2:1:2:4:2:1:2:1 ratio, although too few observations are available for proper use of chi square. In population 3-5 the observed genotypic array's poor fit to the 1:2:1:2:4:2:1:2:1 ratio is due to the poor single factor segregation in this cross.

The genotypic classifications presented in Table 2 for the cross absent lower laterals (*als*)/Cebada Capa (*Pa*$_7$) have been condensed into phenotypic classifications for the chi square analyses presented in Table 3. The poor fit to expected single factor segregations in both populations contribute to the rather low Pvalues (.10-.05) for fit to the 9:3:3:1 dihybrid segregation ratio. The higher P values for the linkage portion of chi square indicate that the low P values for fit to the 9:3:3:1 ratio trace primarily to the poor fit of single gene segregations to the expected 3:1 ratios. The low P value for 3:1 segregation of the *als* gene presented in the combined data line of Table 3 reflects the deficiency of the (*als als*) recessive phenotype in both data sets. The P value for the expected 3:1 segregation of rust reaction is higher in the combined data line of Table 3 than that for the two individual populations. This reflects some compensation by population 3-2 for the deficiency of susceptibles in 3-5. The two populations are homogeneous in their overall segregation behavior which does not suggest that the genes *als* and *Pa*$_7$ are linked. The high P value in the independence column of the combined data set is consistent with the data from the individual data sets, suggesting that *als* and *Pa*$_7$ are inherited independently.

4.1.3 Uzu or Semi-brachytic (*uz*)/Cebada Capa (*Pa*$_7$)

The genotypic classifications of F_2 plants based upon F_3 family data are presented in Table 4 for the cross uzu or semi-brachytic (*uz*)/ Cebada Capa (*Pa*$_7$). Poor fits to the 1:2:1:2:4:2:1:2:1 ratio are primarily due to a deficiency of uzu type plants. The deficiency is

Table 3

F_2 phenotypes condensed from F_3 family data of the cross absent lower laterals (*als*)/Cebada Capa (*Pa*$_7$)

Selfed F_1 plant	No. F_2 plants in phenotypic classes*				P from chi square for indicated ratio**			
	A-B-	A-bb	aaB-	aabb	9:3:3:1	3A-:1aa	3B-:1bb	Linkage
3-2	21	13	4	1	.10-.05	.10-.05	.20-.10	.30-.20
3-5	71	14	14	3	.10-.05	.10-.05	.10-.05	.70-.50
Combined	92	27	18	4	.10-.05	<.01	.50-.30	.90-.80

*Phenotypes A-B-: normal, resistant
A-bb: normal, susceptible
aaB-: absent lower laterals, resistant
aabb: absent lower laterals, susceptible

**Less than five observations were expected in the double recessive class of 3-2.

Table 4

F_2 classification via F_3 family data from the cross uzu or semi-brachytic (uz)/Cebada Capa (Pa_7)

Selfed F_1 plant	Genotype of F_2 plants based upon F_3 family data*									P from chi square for indicated ratio**		
	AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb	1:2:1: 2:4:2: 1:2:1	1AA: 2Aa: 1aa	1BB: 2Bb: 1bb
4-2	17	18	10	14	41	16	9	9	10	.10-.05	.20-.10	.80-.70
4-4	3	8	5	8	24	10	2	0	3	.10-.05	<.01	.70-.50

*Genotypes AA = Uz Uz BB = Pa_7 Pa_7
Aa = Uz uz Bb = Pa_7 pa_7
aa = uz uz bb = pa_7 pa_7

**Less than five observations were expected in the double homozygote classes of 4-4.

particularly extreme for population 4-4 and is reflected by the low P value for the single factor segregation of the uzu locus (Table 4). Rust reaction fits the expected segregation ratio of 1:2:1 well for both populations of this cross (Table 4). Although progeny number is lower than that required for satisfactory use of chi square in population 4-4, the low P value observed for fit to the 9 class joint segregation ratio is due primarily to the deficiency of uzu plants. The deficiency of uzu plants in population 4-2, however, cannot entirely account for the poor fit to the 1:2:1:2:4:2:1:2:1 ratio expected for two independently inherited loci.

In Table 5, the F_2 phenotypes condensed from the F_3 family data are presented for both populations of the cross uzu(uz)/Cebada Capa (Pa_7). A moderate P value for fit to the expected 9:3:3:1 dihybrid ratio is reported for population 4-2. The segregation pattern is not homogeneous for both populations. The P values reported for population 4-4 indicate that the poor fit to the 9:3:3:1 ratio is accounted for by the poor fit to a 3:1 caused by a deficiency of uzu plants. In population 4-2 the segregation of rust reaction conforms exactly to the expected 3:1 ratio (Table 5). In this population (4-2), deviation from the expected 9:3:3:1 dihybrid ratio is accounted for entirely by chi square values from the single factor segregation of uzu, and from the interaction or linkage portion of the total chi square. On the basis of the phenotypic frequencies in population 4-2 alone, a loose degree of linkage might be postulated between genes uz and Pa_7.

In Table 4, the distribution of F_2 genotypes presented for population 4-2 suggests a possible trend toward coupling phase linkage

Table 5

F_2 phenotypes condensed from F_3 family data of the cross uzu or semi-brachytic (uz)/Cebada Capa (Pa_7)

Selfed F_1 plant	No. F_2 plants in phenotypic classes*				P from chi square for indicated ratio**			
	A-B-	A-bb	aaB-	aabb	9:3:3:1	3A-:1aa	3B-:1bb	Linkage
4-2	90	26	18	10	.30-.20	.20-.10	exact fit	.20-.10
4-4	43	15	2	3	.05-.02	<.01	.70-.60	.50-.30

*Phenotypes A-B-: normal, resistant
A-bb: normal, susceptible
aaB-: uzu, resistant
aabb: uzu, susceptible

**Less than five observations were expected in the double recessive class of 4-4.

in that the parental-homozygote and double-heterozygote classes contain greater numbers than expected based upon independent assortment. The recombinant homozygotes are, however, present in greater frequency than would be expected if the genes were linked. Although the parental phenotypic classes presented for population 4-2 in Table 5 contain somewhat greater numbers of plants than expected based upon independent assortment, the P value for linkage is nonsignificant. No trend toward linkage is discernable in the data of population 4-4 when organized as a genotypic array in Table 4, nor when condensed into the phenotypic classes presented in Table 5. In light of the inconsistencies regarding the segregation behavior observed in populations 4-2 and 4-4 and the limited number of observations presented for the cross uzu (uz)/Cebada Capa (Pa_7), inferences regarding linkage of genes uz and Pa_7 cannot be made with confidence.

4.1.4 Yellow Streak 2 (yst_2)/Cebada Capa (Pa_7)

The genotypic classification of F_2 plants based upon F_3 family data for two populations (13-1 and 13-4) of the cross yellow streak (yst_2)/Cebada Capa (Pa_7) are presented in Table 6. The same two populations are presented in Table 7 as phenotypic classes condensed from F_3 family data. The low P values reported in Tables 6 and 7 for the single factor segregation ratios are due to a deficiency of yellow streaked plants in both populations and a deficiency of susceptible plants in 13-1. Both single factor segregations in population 13-1 also exhibited a greater number of heterozygotes than expected for a 1:2:1 segregation. This resulted in considerably more double heterozygotes than expected for the

Table 6

F_2 classification via F_3 family data from the cross yellow streak 2 ($\underline{yst_2}$)/Cebada Capa ($\underline{Pa_7}$)

Selfed F_1 plant	Genotype of F_2 plants based upon F_3 family data*									P from chi square for indicated ratio		
	AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb	1:2:1: 2:4:2: 1:2:1	1AA: 2Aa: 1aa	1BB: 2Bb: 1bb
13-1	9	15	7	15	46	9	5	10	2	.05-.02	.05-.02	.05-.02
13-4	6	23	6	17	26	14	3	5	3	.05-.02	<.01	.70-.50

*Genotypes AA = $\underline{Yst_2}$ $\underline{Yst_2}$ BB = $\underline{Pa_7}$ $\underline{Pa_7}$
Aa = $\underline{Yst_2}$ $\underline{yst_2}$ Bb = $\underline{Pa_7}$ $\underline{pa_7}$
aa = $\underline{yst_2}$ $\underline{yst_2}$ bb = $\underline{pa_7}$ $\underline{pa_7}$

Table 7

F_2 phenotypes condensed from F_3 family data of the cross yellow streak 2 ($\underline{yst_2}$)/Cebada Capa ($\underline{Pa_7}$)

Selfed F_1 plant	No. F_2 plants in phenotypic classes*				P from chi square for indicated ratio			
	A-B-	A-bb	aaB-	aabb	9:3:3:1	3A-:1aa	3B-:1bb	Linkage
13-1	85	16	17	2	<.01	.05-.02	.02-.01	.95-.90
13-4	72	20	8	3	<.01	<.01	.70-.50	.70-.50
Combined	157	36	25	5	<.01	<.01	.05-.02	.70-.50

*Phenotypes A-B-: normal, resistant
A-bb: normal, susceptible
aaB-: yellow streaked, resistant
aabb: yellow streaked, susceptible

segregation ratio (1:2:1:2:4:2:1:2:1) in Table 6. The failure of the F_2 phenotypes to conform to the 9:3:3:1 ratio is explained by the poor fit to the expected 3:1 ratio for single factor segregations (Table 7). The P values for linkage reported in Table 7 are high for both populations; thus, no trends toward linkage between $\underline{yst}_2$ and $\underline{Pa}_7$ are suggested by these data.

In Table 8 are presented F_2 phenotypes read directly from F_2 seedlings for 6 populations of the cross yellow streak ($\underline{yst}_2$)/Cebada Capa ($\underline{Pa}_7$). Five of these populations give an acceptable fit to the 9:3:3:1 ratio expected for the segregation of two independent genes. The poor fit to the 9:3:3:1 ratio in population 14-3 is caused primarily by a deficiency of yellow streaked plants, which is evident from the low P value for the segregation of this single factor (Table 8). All six populations exhibit acceptable fits to 3:1 segregation for rust reaction. The P values for linkage are nonsignificant for all populations, with somewhat low values in populations 14-1 and 14-4. The combined data from all six populations summarized in Table 8 exhibit a barely acceptable fit to the 9:3:3:1 ratio. The poor fit is caused primarily by the linkage portion of the total chi square value. These F_2 data taken together suggest a trend toward loose coupling phase linkage between $\underline{yst}_2$ and $\underline{Pa}_7$ which was not evident in the data reported in Tables 6 and 7.

In order to bring more evidence to bear on the nature of the joint segregation of $\underline{yst}_2$ with $\underline{Pa}_7$, F_3 seedlings in those families originating from double heterozygote F_2 plants were analyzed. These results are presented in Table 9. In families from population 13-1, these data do

Table 8

F_2 phenotypic classification of the cross yellow streak 2 (yst_2)/Cebada Capa (Pa_7)

Selfed F_1 plant	F_2 seedling phenotype*				P from chi square for indicated ratio			
	A-B-	A-bb	aaB-	aabb	9:3:3:1	3A-:1aa	3B-:1bb	Linkage
13-1	81	32	32	11	.80-.70	.40-.30	.40-.30	.80-.70
14-1	161	39	53	22	.20-.10	.50-.30	.30-.20	.10-.05
14-2	65	23	21	8	.99-.98	.98-.95	.80-.70	.90-.80
14-3	335	92	71	26	<.01	<.01	.20-.10	.30-.20
14-4	77	26	21	14	.30-.20	.95-.90	.30-.20	.10-.05
Combined	719	212	198	81	.10-.05	.20-.10	.70-.50	.05-.02

*Phenotypes A-B-: normal, resistant
A-bb: normal, susceptible
aaB-: yellow streaked, resistant
aabb: yellow streaked, susceptible

Table 9

Segregation of normal vs. yellow streak and resistant vs. susceptible in F_3 families segregating for both character pairs from the cross yellow streak 2 (*yst*$_2$)/Cebada Capa (*Pa*$_7$)

Selfed F_1 plant	F_3 seedling phenotype*				P from chi square for indicated ratio			
	A-B-	A-bb	aaB-	aabb	9:3:3:1	3A-:1aa	3B-:1bb	Linkage
13-1	742	214	237	92	.20-.10	.70-.50	.50-.30	.05-.02
13-4	513	157	198	48	.10-.05	.20-.10	.10-.05	.20-.10
Combined	1255	371	435	140	.20-.10	.30-.20	.10-.05	.50-.30

*Phenotypes A-B-: normal, resistant
A-bb: normal, susceptible
aaB-: yellow streaked, resistant
aabb: yellow streaked, susceptible

exhibit a trend toward coupling phase linkage as is reflected by the low (.05-.02) P value for linkage. The trend in population 13-4 appears, however, to be in an opposite direction from that in 13-1. This is evident by the larger P values for linkage in the combined data line of Table 9. If linkage is actually involved in the joint segregation of $\underline{yst}_2$ and $\underline{Pa}_7$, any linkage trends could be masked in the data sets of Table 9 since both parental and recombinant type heterozygotes serve as the source of segregating F_3 families.

In Table 10 an attempt has been made to distinguish the segregation of repulsion from coupling heterozygotes based upon inspection of the segregation patterns of F_3 families. The distinctions between coupling and repulsion segregation patterns were based upon only 30 - 40 seedlings per family and cannot be considered entirely accurate. Of the families in population 13-1, segregating for both $\underline{yst}_2$ and $\underline{Pa}_7$, 36 appeared to be of coupling origin and six appeared to be derived from repulsion heterozygotes. In population 13-4, 18 F_3 families were postulated to be of coupling origin and eight families of repulsion origin. The postulated coupling and repulsion segregations for both populations 13-1 and 13-4 deviated significantly from the 9:3:3:1 ratio (Table 10). Single factor segregation for rust reaction deviated significantly from the expected 3:1 ratio in the repulsion families of 13-1 and in the coupling families of 13-4. In both cases this was due to a deficiency of susceptible plants. Chi square values for linkage are highly significant, except for the coupling families of population 13-4, for which there was an extreme deficiency of susceptible plants. The highly

Table 10

Segregation of yellow streak and rust reaction in F_3 families with postulated origins from both coupling and repulsion heterozygotes of the cross yellow streak 2 (*yst*$_2$)/Cebada Capa (*Pa*$_7$)

F_1 plant	Segregation pattern/(no. of F_3 families)	F_3 seedling phenotype*				P from chi square for indicated ratio			
		A-B-	A-bb	aaB-	aabb	9:3:3:1	3A-:1aa	3B-:1bb	Linkage
13-1	coupling/(36)	652	186	190	89	.02-.01	>.98	.80-.70	<.01
	repulsion/(6)	90	28	47	3	<.01	.20-.10	.05	<.01
13-4	coupling/(18)	367	86	132	37	.02-.01	.30-.20	<.01	.70-.50
	repulsion/(8)	146	71	66	11	<.01	.70-.50	.30-.20	<.01

*Phenotype A-B- = normal, resistant
A-bb = normal, susceptible
aaB- = yellow streak, resistant
aabb = yellow streak, susceptible

significant chi square values for independence support the hypothesis that $\underline{yst_2}$ and $\underline{Pa_7}$ are linked in coupling phase.

Recombination percentages estimated by the product method from the coupling data of Table 10 are 43% in population 13-1 and 48% in population 13-4. The repulsion families provide lower recombination values, 29% in population 13-1 and 36% in 13-4. The discrepancy between the recombination values is very likely due to misclassification of coupling versus repulsion segregation within F_3 families. The estimate of recombination between $\underline{yst_2}$ and $\underline{Pa_7}$ from the combined F_2 data of Table 8 is approximately 46%. The genotypic and phenotypic classification of the F_2 (Tables 6 and 7) do not suggest genes $\underline{yst_2}$ and $\underline{Pa_7}$ are linked, but the populations presented in Tables 6 and 7 are small and exhibit poor single gene segregations. The total available data on the cross yellow streak 2 ($\underline{yst_2}$)/Cebada Capa ($\underline{Pa_7}$) are not consistent enough to permit a reliable estimate of the recombination value between $\underline{yst_2}$ and $\underline{Pa_7}$, yet the data do suggest that $\underline{yst_2}$ and $\underline{Pa_7}$ are loosely linked in the coupling phase.

4.2 Linkage Analyses for Chromosome 2 Marker Stocks/Speciale

The results from crosses between Speciale and the three marker stocks for $\underline{lr}$, $\underline{gs_5}$, and $\underline{e}$ are presented in Tables 11 through 15. The F_2 phenotypic classes in all tables have been extracted from F_3 family data.

4.2.1 Reduced Lateral Spikelet Appendage on the Lemma ($\underline{lr}$)/Speciale ($\underline{Pa}$)

The phenotypic classification of F_2 plants condensed from F_3 family data is presented for three populations of the cross reduced

laterals (lr)/Speciale (Pa) in Table 11. Population 21-2 exhibits a poor fit to the 9:3:3:1 ratio which is accounted for by the linkage portion of chi square, indicating a trend toward linkage in this population. Populations 21-5 and 21-6 exhibit good fits to the 9:3:3:1 ratio for joint segregation. All three populations exhibit acceptable single factor segregations. The combined data of Table 11 exhibit good fit to both single gene segregations and the joint segregation expected for two independently inherited genes. The small population sizes in this cross render the chi square values valid for only the combined data line of Table 11, which suggests no evidence of linkage between the genes lr and Pa.

In Table 12 are presented F_2 genotypic arrays based upon F_3 family data for the three populations and combined data of the cross reduced laterals (lr)/Speciale (Pa). The poor fit in population 21-2 to the 9:3:3:1 joint segregation ratio of Table 11 can be traced primarily to deviations in two genotypic classes in Table 12. There is an excess in the double recessive class and deficiency in the single heterozygote class (Lr lr pa pa). In other respects, examination of this genotypic array suggests no evidence of a trend toward coupling linkage. In the combined data of Table 12, nonparental homozygotes are present in higher frequency than expected for independent assortment; yet these genotypes are expected to be less frequent if two genes are linked in coupling. The data for this cross, reduced laterals (lr)/Speciale (Pa), suggest that genes lr and pa are independently inherited, although small population sizes render these results tentative.

Table 11

F_2 phenotypes condensed from F_3 family data of the cross reduced laterals (lr)/Speciale (Pa)

Selfed F_1 plant	No. F_2 plants in phenotypic classes*				P from chi square for indicated ratio**			
	A-B-	A-bb	aaB-	aabb	9:3:3:1	3A-:1aa	3B-:1bb	Linkage
21-2	39	10	7	9	.05-.02	.95-.90	.50-.30	<.01
21-5	40	17	13	4	.90-.80	.70-.50	.70-.50	.70-.50
21-6	16	4	8	3	.70-.50	.20-.10	.80-.70	.70-.50
21-5, 21-6 Combined	56	21	21	7	.95-.90	.70-.50	.70-.50	.90-.80

*Phenotypes A-B-: normal, resistant
A-bb: normal, susceptible
aaB-: reduced lateral awns, resistant
aabb: reduced lateral awns, susceptible

**Less than five observations were expected in the double recessive class of the three populations 21-2, 21-5, and 21-6.

Table 12

F_2 classification via F_3 family data from the cross reduced laterals (lr)/Speciale (Pa)

Selfed F_1 plant	Genotype of F_2 plants based upon F_3 family data*									P from chi square for indicated ratio**		
	AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb	1:2:1: 2:4:2: 1:2:1	1AA: 2Aa: 1aa	1BB: 2Bb: 1bb
21-2	3	6	5	8	17	3	2	5	9	.10-.05	.95-.90	.80-.70
21-5	1	6	7	9	23	9	5	7	4	.70-.50	.50-.30	.70-.50
21-6	3	4	1	2	7	2	5	3	3	.50-.30	.30-.20	.70-.50
Combined	7	16	13	19	47	14	12	15	16	.30-.20	.80-.70	.90-.80

*Genotypes AA = $\frac{Lr}{Lr}\ \frac{Lr}{Lr}$ BB = $\frac{Pa}{Pa}\ \frac{Pa}{Pa}$
Aa = $\frac{Lr}{Lr}\ \frac{lr}{lr}$ Bb = $\frac{Pa}{Pa}\ \frac{pa}{pa}$
aa = $\frac{lr}{lr}\ \frac{lr}{lr}$ bb = $\frac{pa}{pa}\ \frac{pa}{pa}$

**Less than five observations were expected in the double homozygote classes of the three populations 21-2, 21-5, and 21-6.

4.2.2 Glossy Sheath 5 (*gs*$_5$)/Speciale (*Pa*)

The genotypic classification of F_2 plants based upon F_3 data for two populations of the cross glossy sheath 5 (*gs*$_5$)/Speciale (*Pa*) are presented in Table 13. Both populations 18-1 and 18-8 exhibit an acceptable fit to the 9 class genotypic array based upon independent assortment, but the P value is rather low for population 18-8. Both populations exhibit acceptable fits to the expected single gene segregation ratios, although the rather low P value for population 18-1 reflects a deficiency of susceptible plants. The combined data exhibit a poor fit to the 1:2:1:2:4:2:1:2:1 genotypic ratio expected for the independent assortment of two gene pairs. Both single factor segregations in the combined data show acceptable fits to the expected 1:2:1 ratios. Examination of genotypic frequencies in Table 13 suggests a trend in both 18-1, 18-8, and in the combined data toward coupling linkage between genes *gs*$_5$ and *Pa*. With the exception of the double dominant homozygote of population 18-8 all parental classes occur with greater frequency than expected for independent assortment. One reason there is no excess of double dominant homozygotes in population 18-8 is that in both populations there occurred for the *gs*$_5$ locus an excess of heterozygotes at the expense of the dominant homozygote. This accounts for the excess of double heterozygotes above that expected for coupling linkage between two genes. In both populations nonparental homozygotes are present in lower frequency than expected for independent assortment.

The F_3 family data from this cross, glossy sheath 5 (*gs*$_5$)/Speciale (*Pa*), are condensed into F_2 phenotypes in Table 14. This enables the

Table 13

F_2 classification via F_3 family data from the cross glossy sheath 5 (gs_5)/Speciale (Pa)

Selfed F_1 plant	Genotype of F_2 plants based upon F_3 family data*									P from chi square for indicated ratio		
	AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb	1:2:1: 2:4:2: 1:2:1	1AA: 2Aa: 1aa	1BB: 2Bb: 1bb
18-1	7	8	3	12	27	4	4	9	6	.50-.30	.80-.70	.10-.05
18-8	11	26	14	36	65	22	9	27	24	.10-.05	.70-.50	.95-.90
Combined	18	34	17	48	92	26	13	36	30	.05-.02	.50-.30	.80-.70

*Genotypes AA = Gs_5 Gs_5 BB = Pa Pa
Aa = Gs_5 gs_5 Bb = Pa pa
aa = gs_5 gs_5 bb = pa pa

Table 14

F_2 phenotypes condensed from F_3 family data of the cross glossy sheath 5 (gs_5)/Speciale ($\underline{Pa}$)

Selfed F_1 plant	No. F_2 plants in phenotypic classes*				P from chi square for indicated ratio			
	A-B-	A-bb	aaB-	aabb	9:3:3:1	3A-:1aa	3B-:1bb	Linkage
18-1	54	7	13	6	.10-.05	.80-.70	.10-.05	.10-.05
18-8	148	41	43	25	.10-.05	.70-.50	.90-.80	.02-.01
Combined	202	48	56	31	.02-.01	.80-.70	.70-.50	<.01

*Phenotypes A-B-: normal, resistant
A-bb: normal, susceptible
aaB-: glossy sheath, resistant
aabb: glossy sheath, susceptible

inclusion of several F_2 plants that were not completely classified via F_3 families. This also permits a simple partitioning of the total chi square for deviation from independent segregation into a portion due to single gene segregations and a portion due to interaction or linkage. When the data are grouped in this way (Table 14) both populations exhibit marginal fit to the 9:3:3:1 ratio. In population 18-1 this is due to both a deficiency of susceptible plants and to the linkage portion of the total chi square. In population 18-8, the poor fit to the 9:3:3:1 independent segregation is due primarily to the linkage portion of chi square. The combined data in Table 14 exhibit good fit to expected single gene segregations, but do not fit the 9:3:3:1 ratio because of the highly significant chi square value for linkage, which suggests the presence of linkage.

The data for the joint segregation of $\underline{gs}_5$ and $\underline{Pa}$ are homogeneous for populations 18-1 and 18-8 of the cross glossy sheath 5 ($\underline{gs}_5$)/ Speciale ($\underline{Pa}$), and strongly suggest that these genes are linked in coupling. The maximum liklihood estimate of the recombination value between genes $\underline{gs}_5$ and $\underline{Pa}$ is 41.0 ± 2.2% when the data from both Tables 13 and 14 are employed for analysis.

4.2.3 Wide Glume ($\underline{e}$)/Speciale ($\underline{Pa}$)

Genotypic classification of F_2 plants based upon F_3 data is presented in Table 15 for three families of the cross wide glume ($\underline{e}$)/ Speciale ($\underline{Pa}$). All three families appear to conform to the 1:2:1:2:4:2:1:2:1 segregation expected for independent assortment, although too few observations are available in families 17-2 and 17-5

Table 15

F_2 classification via F_3 family data from the cross wide glume (e)/Speciale (Pa)

Selfed F_1 plant	Genotype of F_2 plants based upon F_3 family data*									P from chi square for indicated ratio**		
	AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb	1:2:1: 2:4:2: 1:2:1	1AA: 2Aa: 1aa	1BB: 2Bb: 1bb
17-2	6	2	1	7	9	8	4	8	3	.50-.30	.50-.30	.30-.20
17-5	1	7	1	1	8	5	0	8	2	.30-.20	.70-.50	.05-.02
17-7	5	12	11	15	34	16	7	12	7	.90-.80	.70-.50	.50-.30
17-2, 17-7 Combined	11	14	12	22	43	24	11	20	10	.95-.90	.70-.50	.50-.30

*Genotypes AA = EE BB = Pa Pa
Aa = Ee Bb = Pa pa
aa = ee bb = pa pa

**Less than five observations were expected in the double homozygote classes of both 17-2 and 17-5.

for an accurate test of goodness of fit to this ratio. Population 17-5 exhibits a poor fit to the expected 1:2:1 segregation for rust reaction because the Pa allele is nearly exclusively present in the heterozygous condition. The single gene segregations fit the expected ratios in all other cases. Over the three populations combined, the data fit the 9 class ratio for independent segregation of two gene pairs quite well. Population 17-5, however, has been omitted from the combined data line in Table 15 since the near absence of homozygous resistant genotypes resulted in an aberrant segregation for rust reaction in this population. No linkage trends are present in this data and the good fit to the 1:2:1:2:4:2:1:2:1 for all cases suggests that the genes e and Pa are independently inherited.

When linkage relationships reported among genes lr, gs_5, and e are taken into account, a discrepancy becomes apparent in the results obtained in the present study for crosses involving these genes. The results reported in Tables 11 through 16 suggest that the gene Pa of Speciale is independent of both lr and e, but linked in loose coupling to gs_5 by a 41.0 ± 2.2% recombination value. Rasmusson and Lambert (54) obtained a 2.5 ± 0.79% recombination between genes e and gs_5. Tsushiya and Singh (97) have presented evidence from telotrisomic analyses which suggests that e and gs_5 are on opposite sides of the centromere of chromosome 2. In recent linkage maps the genes e and gs_5 are placed close to each other on opposite sides of the centromere (96). By virtue of the close linkage between e and gs_5, and the 41.0 ± 2.2% recombination between gs_5 and Pa observed in the present study, one might expect

to observe a loose linkage between genes e and Pa. In the present study, however, e and Pa appear to be independently inherited.

The gene lr has been mapped at or near the v (6-rowed) locus (96). Various reports have linked the v locus with e by recombination values between 24 and 34% (24,80). The gs_5 locus resides between e and lr. Linkage between gs_5 and Pa would accordingly be expected to imply the existence of linkage between Pa and either e, lr, or both. No clear evidence of linkage between Pa and, either e, or lr was obtained in the present study; however, population 21-2 of the cross lr/Sp did exhibit a trend towards linkage between lr and Pa (Table 11). The limited population sizes in these crosses, particularly in lr/Sp, may have precluded accurate detection of loose linkages with the Pa gene. According to Hanson (15), the minimum family size required to detect linkage so that the probability of concluding independence is 0.05, if the recombination value is in fact 35%, is 225 individuals. A population size of 540 is required to detect a 40% recombination value with similar reliability. If the true recombination value is 45%, a population size of more than 2000 is required for accurate linkage detection.

It should also be emphasized that the map positions of the chromosome gene markers used in this study are only tentative. In most instances the recombination values between the various markers such as lr, gs_5, and e have been derived from two-point linkage data. Very few results have been reported in barley for linkage data derived from three point test crosses. In this light one might expect inconsistencies between different reports. Precise mapping of barley chromosomes will

be accomplished only when multiple marker stocks are available for all 14 chromosome arms.

The population sizes employed in the present study were too small to reliably clarify the linkage relationships between genes Pa and lr, gs_5, and e. The data presented in Tables 13 and 14 do, however, suggest that Pa is loosely linked with the gs_5 gene of chromosome 2L. Crosses between Speciale, Nudihaxtoni, and a susceptible 2-rowed cultivar such as Betzes could in the future clarify relationships between the Pa gene and the v and lr loci.

4.3 Trisomic Analyses for Genes Pa_3 in Estate and Pa_7 of Cebada Capa

F_2 populations derived from trisomic F_1 plants were obtained for crosses Triplo 3/Cebada Capa and for Estate crosses with Triplo 3, 4, 5, and 6. The Triplo 3/Cebada Capa cross was made principally as an experimental control to confirm reports of others (82,99) concerning the location of the Pa_7 gene of Cebada Capa. Crosses with Estate were made for the purpose of locating the gene Pa_3. Trisomic analyses for Cebada Capa crosses are presented in Tables 16 and 17, and the data from trisomic crosses with Estate are presented in Tables 18 through 21.

4.3.1 Triplo 3/Cebada Capa (Pa_7)

In Table 16 are presented the F_2 segregations for resistant versus susceptible rust reactions among progeny of the cross Triplo 3/Cebada Capa (Pa_7). In crosses with Cebada Capa the resistant and susceptible phenotypes were easily distinguished since infection types were either On-1, or 4. Rust reactions are presented for total F_2 populations and

Table 16

F_2 segregation of rust reaction in the cross Triplo 3/Cebada Capa (*Pa*$_7$)

Selfed F_1 plant	Total F_2 segregates			2n segregates			2n + 1 segregates		
	Res	Sus	P*	Res	Sus	P*	Res	Sus	P**
Trisomic crosses									
T 3-1/CC	47	23	.20-.10	34	21	.05-.02	12	2	— — —
T 3-2/CC	33	22	.02-.01	29	21	<.01	4	1	— — —
Combined	80	45	<.01	63	42	<.01	16	3	— — —
Control crosses									
D 3-1/CC	34	11	.95-.90						
D 3-2/CC	74	18	.30-.20						
Combined	108	29	.30-.20						

*P-values are based on chi square for a 3:1 ratio.

**Less than the minimum required number of observations for chi square among the 2n + 1 progeny.

for the diploid and trisomic segregates separately in Table 16. Both populations (T3-1/CC and T3-2/CC) exhibit poor fit to a 3:1 ratio among their 2n segregates. Among the total F_2 population, T3-2/CC exhibits poor fit to the 3:1 ratio and T3-1/CC shows an acceptable fit to the 3:1. The small number of observations for trisomic (2n + 1) plants prevents use of chi square, but the observed ratios of resistant to susceptible trisomic plants are greater than 3:1 in both T3-1/CC and T3-2/CC, and in the combined data. Control crosses represent F_2 populations from diploid F_1 plants which originated from the same crossed spike that served as the source of the trisomic F_1's. For both controls in Table 16, rust reaction conformed to the 3:1 ratio expected for the diploid segregation of a single gene.

The data of Table 16 clearly confirms that the gene $\underline{Pa}_7$ segregates with an excess of susceptible plants in F_2 populations originating from trisomic F_1 plants having an additional chromosome 3. The frequency of susceptible plants is highest among the diploid (2n) plants and lowest among the trisomic (2n + 1) plants of Table 16. This is strongly indicative of trisomic inheritance and implies that the $\underline{Pa}_7$ gene resides on chromosome 3. Further evidence that the excess of susceptible plants in the Triplo 3/Cebada Capa cross is due to trisomic inheritance is presented in Table 17. Goodness of fit of the observed segregations to that expected for trisomic inheritance based upon random chromosome association is good for both the diploid and total F_2 groups. In unpublished data, Starling (75) observed a highly significant excess of susceptible plants in each of four F_2 populations of the cross Triplo

Table 17

Goodness of fit to a trisomic segregation of rust reaction among the F_2 progeny of the cross Triplo 3/Cebada Capa (*Pa*$_7$)

Selfed F_1 plant	Total F_2 segregates				2n segregates			
	Res	Sus	Ratio tested*	P	Res	Sus	Ratio tested*	P
T 3-1/C.C.	47	23	1.43:1	.20-.10	34	21	1.25:1	.50-.30
T 3-2/C.C.	33	22	1.43:1	.90-.70	29	21	1.25:1	.90-.70
Combined	80	45		.30-.20	63	42		.50-.30

*The ratio tested is based upon random chromosome association and 15% transmission of the extra chromosome through female gametes.

3/Cebada Capa. The data reported here are also consistent with that of others (82,99) which indicate that $\underline{Pa_7}$ is located on chromosome 3.

The relationship of $\underline{Pa_7}$ with other loci known to reside on chromosome 3 is not known, although data from this study (Tables 8, 9 and 10) suggest that $\underline{Pa_7}$ may be loosely linked to the gene $\underline{yst_2}$. Takahashi and Moriya (79) reported a 10% recombination between $\underline{yst_2}$ and $\underline{uz}$ which reside on opposite sides of the centromere. By Giemsa N-banding combined with telotrisomic analysis, the gene $\underline{yst_2}$ has been assigned to the short arm of chromosome 3 and the gene $\underline{uz}$ to the long arm of this chromosome (72). The data presented in Table 5 of this study for the joint segregation of $\underline{uz}$ and $\underline{Pa_7}$ are not consistent in exhibiting a trend toward linkage between these genes. If the results reported in Table 5 had clearly indicated independence between $\underline{uz}$ and $\underline{Pa_7}$, inference would suggest the short arm of chromosome 3 as the likely location of $\underline{Pa_7}$. As yet, there is no conclusive evidence to bear on the chromosome arm location of $\underline{Pa_7}$.

4.3.2 Triplo 3/Estate ($\underline{Pa_3}$)

In Table 18 are reported the observed numbers of resistant and susceptible plants in F_2 populations derived from trisomic F_1's and diploid controls of the cross Triplo 3/Estate ($\underline{Pa_3}$). Among the six test populations, T3-1/ES through T3-7/ES, the results are heterogeneous, primarily due to the extreme observed deficiency of susceptible plants in population T3-7/ES. In contrast, an excess of susceptible plants was observed in population T3-1/ES. Among the four remaining test populations the observed segregations are consistent with the expected

Table 18

F_2 segregation for rust reaction in the cross Triplo 3/Estate (Pa_3)

Selfed F_1 plant	Total F_2 segregates			2n segregates			2n + 1 Segregates		
	Res	Sus	P*	Res	Sus	P*	Res	Sus	P**
Trisomic crosses									
T 3-1/Es	21	13	.10-.05	18	13	.05-.02	3	0	— — —
T 3-2/Es	50	23	.20-.10	30	13	.50-.30	5	4	— — —
T 3-3/Es	33	12	.80-.70	30	8	.70-.50	3	4	— — —
T 3-5/Es	70	16	.20-.10	54	10	.10-.05	18	5	.90-.70
T 3-6/Es	49	12	.50-.30	38	11	.70-.50	11	1	— — —
T 3-7/Es	68	6	<.01	48	5	<.01	19	1	.05-.02
Combined							59	15	.50-.30
Control crosses									
D 3-1/Es	169	108	<.01						
D 3-2/Es	115	61	<.01						
D 3-3/Es	17	12	.05-.02						
D 3A-1/Es	43	24	.05-.02						
Combined	344	205	<.01						

*P-values are based on chi square for a 3:1 ratio.

**Four crosses have less than the minimum required number of observations among 2n + 1 progeny.

3 resistant:1 susceptible monogenic ratio. Differences between diploid (2n) and trisomic (2n + 1) groups are not apparent with regard to the segregation of rust reaction, although in most cases too few trisomic plants were available for chi square analysis. The control crosses, all derived from diploid F_1 plants of the cross Triplo 3/Estate, consistently exhibit an excess of susceptible plants for reasons unknown.

The susceptible phenotype was classified as such based upon the exclusive presence of type 4 pustules on the first and second seedling leaves. Included in the resistant classes were infection types 0, 0c-n, 1 and 2. This range of infection types within the resistant class was unexpected. The Estate parent, included as a check in these inoculations, exhibited the 0-0c infection type; whereas, the parent Betzes and the susceptible Moore exhibited type 4 infections. Moore, however, exhibited a range of pustule sizes with large pustules being less frequent than medium to small pustules. The observed range of infection types was not expected in these progenies (Table 18) since the gene $\underline{Pa_3}$ in Estate has been reported to confer the seedling infection type 0-0c, with complete dominance (83).

The test populations presented in Table 18 do not exhibit any trend toward trisomic interitance of the $\underline{Pa_3}$ gene. If the $\underline{Pa_3}$ gene were located on chromosome 3, an excess of susceptible plants should be particularly evident in the diploid segregates. This is not the case, since four of the six populations exhibit a ratio greater than 3 resistant:1 susceptible among segregates. Among total F_2 segregates, those populations derived from trisomic F_1's exhibit closer adherence to a 3:1 ratio than do the controls, which all exhibit an excess of

susceptibles. Resistance is not apparently inherited through the trisomic chromosome in these populations; therefore, $\underline{Pa_3}$ is not likely located on chromosome 3.

The unexplained excess of susceptible plants in the control crosses (Table 18) may have arisen from misclassification of segregates. Since the Betzes parent uniformly exhibited type 4 pustules, only type 4 infections were classed as susceptible. There was, however, more pustular variation than expected among the resistant progenies. Large temperature fluctuations, with day-time temperatures sometimes exceeding 30 C, occurred in the glasshouse during the 14 day period following inoculation of these materials. High temperatures are known in some instances to produce altered infection types in both wheat stem rust (31) and barley leaf rust (23,100). The resistance conferred by Estate, however, has been demonstrated to remain stable over temperature regimes from 5-25 C (100). No results have been published to my knowledge concerning the behavior of Estate derived progenies at temperatures exceeding 30 C, although the Estate parent included in this test did exhibit an immune rust reaction.

4.3.3 Triplo 4/Estate ($\underline{Pa_3}$)

F_2 segregations for rust reaction among test populations and controls for the cross Triplo 4/Estate are presented in Table 19. Significant deviations from a 3:1 ratio occur in both trisomic test population T4-5/ES and in the control population D4-3/ES. In both populations the deviation is due to an excess of susceptible plants. Except for a deficiency of susceptible plants in test population T4-6/ES, there is an

Table 19

F_2 segregation of rust reaction in the cross Triplo 4/Estate (*Pa*$_3$)

Selfed F_1 plant	Total F_2 segregates		P from chi square for a 3:1 ratio
	Res	Sus	
Trisomic crosses			
T 4-3/Es	103	48	.10-.05
T 4-4/Es	92	38	.50-.30
T 4-5/Es	97	49	.02-.01
T 4-6/Es	154	37	.20-.10
T 4-9/Es	64	25	.70-.50
Control crosses			
D 4-2/Es	212	82	.30-.20
D 4-3/Es	130	60	.05-.01
D 4-4/Es	166	69	.20-.10
D 4-10/Es	67	28	.50-.30
Combined	575	239	<.01

overall trend in both trisomic testers and control crosses toward an excess of susceptibles. Pustule size was quite variable among susceptible plants which made classification difficult. Occasional progeny plants were encountered which had very small pustules not associated with any surrounding chlorosis. These were classed as resistant since the susceptible parent exhibited larger pustules. Although the high glasshouse temperatures were not favorable for proper disease development, four of the five trisomic test populations presented in Table 19 exhibit acceptable fit to the 3:1 ratio. This indicates that the $\underline{Pa}_3$ locus is not likely on chromosome 4.

4.3.4 Triplo 5/Estate ($\underline{Pa}_3$)

In Table 20 are presented the segregations for rust reactions among progenies derived from trisomic F_1's and diploid controls for the cross Triplo 5/Estate. An excess of susceptibles is apparent in both trisomic test populations and diploid control populations of the cross Triplo 5/Estate. Four of the five test populations do, however, exhibit acceptable fits to a 3:1 ratio, which suggests that the $\underline{Pa}_3$ locus does not reside on chromosome 5.

4.3.5 Triplo 6/Estate ($\underline{Pa}_3$)

In Table 21 are presented the segregations for rust reaction in test popoulations derived from trisomic F_1 plants and control populations of the cross Triplo 6/Estate. Here, as with the Estate crosses involving stocks trisomic for chromosomes 4 and 5, both test and control populations exhibit an excess of susceptible plants. In this case (Table 21), both diploid control populations D6-15/ES and D6-16/ES have

Table 20

F_2 segregation of rust reaction in the cross Triplo 5/Estate (Pa_3)

Selfed F_1 plant	Total F_2 segregation		
	Res	Sus	P*
Trisomic crosses			
T 5-25/Es	33	10	.90-.70
T 5-42/Es	86	33	.70-.30
T 5-46/Es	111	48	.20-.10
T 5-50/Es	61	20	.95-.90
T 5-49/Es	102	72	<.01
Control crosses			
D 5-4/Es	136	56	.20-.10
D 5-5/Es	65	23	.90-.70
D 5-45/Es	199	88	.05-.02
D 5-47/Es	78	45	<.01
D 5-52/Es	108	55	<.01
Combined	586	267	<.01

*P-values are based on chi square for a 3:1 ratio.

Table 21

F_2 segregation of rust reaction in the cross Triplo 6/Estate (*Pa*$_3$)

Selfed F_1 plant	Total F_2 segregates		
	Res	Sus	P*
Trisomic crosses			
T 6-2/Es	42	27	<.01
T 6-3/Es	125	42	.98-.95
T 6-4/Es	37	15	.70-.50
T 6-21/Es	40	30	<.01
Control crosses			
D 6-15/Es	81	55	<.01
D 6-16/Es	139	65	<.05
Combined	220	120	<.01

*P-values are based on chi square for a 3:1 ratio.

excess susceptibles, while two of the test populations have excess susceptibles and two, T6-3/ES and T6-4/ES, exhibit good fit to a 3:1 ratio. Since two test populations do exhibit good fit to a 3:1 ratio, these data indicate that the Pa_3 locus does not likely reside on chromosome 6.

4.4 Discussion of Trisomic Analyses for Location of Genes Conditioning Resistance to P. hordei

The data presented in Tables 18 through 21 reflect the poor test conditions under which these rust segregations were observed, by virtue of an excess of susceptible plants in one to several diploid control populations of each cross. The susceptible trisomic parent plants were of the Betzes cultivar, except those of Triplo 5 which were Shin Ebisu 16 stocks. Since the individual crosses were made in three attempts over a two-year period, several seed lots of the Estate parent were utilized. Except for the crosses involving Triplo 3 for which chromosome counts were performed on each F_2 seedling, the F_2 segregations reported in these tables were obtained in the glasshouse 13 - 16 days subsequent to a single inoculation with culture 57.19, race 4 of Puccinia hordei on 6 April 1982. A common feature of all segregations observed in this test was the tendency toward an excess of susceptible progenies.

A high degree of outcrossing among the F_1 plants might explain the excess of susceptible F_2 segregates. The F_1 plants were grown in the glasshouse in different years, fall and winter of 1980-81 and 1981-82. To attribute the observed excess of susceptible segregates to

outcrossing would mean that considerable outcrossing had to occur in the glasshouse during both years. There were no visible signs of this, such as opened glumes, in any of the F_1 plants for Triplo 4, 5, and 6 crosses with Estate. A number of tillers on F_1 plants derived from crosses with Triplo 3 did, however, exhibit a high degree of sterility. Triplo 3 is known to exhibit some sterility in crosses (90,95).

In both years F_1 plants from trisomic crosses were grown on the glasshouse bench, and were located closer to other resistant F_1 plants than to susceptible parents. There is no basis to assume that outcrossing would have led to higher than normal proportions of susceptible segregates. A more likely explanation of these data is misclassification of rust reaction due to poor expression of infection type when glasshouse temperatures exceeded 30 C. Under these conditions the susceptible check cultivar Moore generally exhibited type 4 infections, but with varied pustule sizes.

Two diploid control populations, D3-1/ES and D3-2/ES, derived from crosses between Triplo 3 and Estate, presented in Table 18, were included in the glasshouse inoculation on 6 April 1982. All other populations presented in Table 18 were included with the Cebada Capa derived populations (Table 16) in the 1 June 1981 inoculation for which rust development took place outdoors in a shaded spot at the Agronomy Farm. The control populations D3-1/ES and D3-2/ES (Table 18) exhibit a more extreme excess of susceptible segregates than do controls D3-3/ES and D3A-1/ES for which rust development occurred outdoors. This gives some credence to the suggestion that high glasshouse temperatures may have contributed to the excess of susceptible segregates observed in most of

the populations included in the April inoculation. High temperatures apparently interfered with normal plant-parasite interaction to the extent that rust reaction was misclassified for many plants.

Infection type is conditioned not only by host and parasite genotypes, but also by the environmental conditions which prevail during the disease incubation period. Joshi (23) reported that Speciale, when incubated with race 4 of P. hordei at 12-18 C, exhibits type 0-1 infections, but type 3 infections result when incubation temperatures are above 30 C. Whether high temperatures alter infection type by affecting host cell properties or fungal properties, or both, remains unknown.

Despite the apparent excess of susceptibles among diploid check populations, acceptable fits to the 3:1 ratio were obtained in trisomic test populations for crosses between Estate and Triplo 3, 4, 5, and 6. This indicates that the Pa_3 locus does not likely reside on chromosomes 3, 4, 5, or 6. This agrees with data reported by Tan (82) who, by using the same inoculum culture 57-19, found no excess of susceptible segregates in crosses between Estate and Triplo 3 through 7. In unpublished data, Starling (75) observed no significant departures from a 3:1 ratio in crosses between Estate and Triplo 2 through 6. In conjunction with the data obtained in the present study and that reported by Tan (82), it appears that Pa_3 is not located on chromosomes 2 through 7 and should, therefore, reside on chromosome 1. Data from the cross Triplo 1/Estate are not available to substantiate this.

The evidence available to date suggests that the Pa_7 gene in Cebada Capa resides on chromosome 3. As yet no marker genes have shown close linkage with Pa_7, although in the present study a possible loose

association was reported between *Pa*$_7$ and *yst*$_2$, a chromosome 3S marker. Tuleen and McDaniel (99) and Starling in unpublished data (75) found evidence through trisomic analyses suggesting that the *Pa* gene in Speciale is located on chromosome 2. In the present study a recombination value of 41.0 ± 2.2% was reported between *Pa* and *gs*$_5$, a chromosome 2L marker. Available evidence suggests that the *Pa*$_3$ gene in Estate may possibly be located on chromosome 1, although critical data from neither trisomic analyses nor linkage studies are available to substantiate this.

The *Pa*$_4$ gene in the cultivar Gold is known to reside on chromosome 4 and is the only gene conditioning resistance to *P. hordei* which has been mapped (36). The gene *Pa*$_5$ has been linked to *Pa*$_7$ by 8% recombination and therefore must reside on chromosome 3 (11,82). None of the genes *Pa*$_2$, *Pa*$_6$, *Pa*$_8$, or *Pa*$_9$ have been associated with their respective chromosomes. The unpublished data of Starling (75) for crosses between Reka 1 containing *Pa*$_2$, and Triplo 2, 3, 4, 6, and 7 exhibit no excess of susceptible segregates. This suggests the possible location of *Pa*$_2$ on either chromosome 1 or 5. The available data suggest that additional information concerning the location of genes conditioning resistance to *P. hordei* might be garnered through attempts to link *Pa*$_3$ with chromosome 1, *Pa*$_2$ with chromosome 1 or 5, and through crossing Speciale and Cebada Capa with additional marker stocks for chromosomes 2 and 3.

CHAPTER FIVE

SUMMARY AND CONCLUSION

Nine genes are known which condition resistance to Puccinia hordei. Only one of these, Pa_4, has been mapped. Genes Pa and Pa_7 have been shown to reside on chromosomes 2 and 3, respectively, by primary trisomic analyses. Although once reported to reside on chromosome 3, the Pa_3 gene has never been successfully located.

The objectives of the present study were: (a) to detect linkage relationships of the Pa gene of Speciale and the Pa_7 gene of Cebada Capa with recessive marker genes on chromosomes 2 and 3, respectively; and (b) to locate the Pa_3 gene in Estate by primary trisomic analysis.

Three stocks carrying marker genes for chromosome 2 were used as seed parents in crosses with Speciale. Both genes e (wide outer glume) and lr (reduced lateral spikelet appendages) appeared to be inherited independently of the Pa gene in Speciale. A recombination value of 41.0 ± 2.2% was observed between gs_5 (glossy sheath 5) and Pa. This result was difficult to interpret because previous work has indicated that gs_5 resides between the e and lr loci (96). The small population sizes obtained for crosses involving chromosome 2 marker genes, particularly for lr, resulted in little clarification of the relationship between Pa and the genes e, gs_5, and lr. There does, however, appear to be a loose association between Pa and the gs_5 gene which has been mapped near the centromere on the long arm of chromosome 2.

Four chromosome 3 gene stocks were used as seed parents in crosses with Cebada Capa. No evidence of linkage was found between Pa_7 and either gs_2 (glossy sheath 2) or als (absent lower laterals), although

population sizes were small in these crosses. The two populations reported for the crosses involving uz were inconsistent in their segregation behavior, making inference regarding linkage between uz and Pa_7 difficult. The larger of the two populations (4-2) did exhibit a slight tendency toward coupling phase linkage between uz and Pa_7. Crosses involving the seedling marker yst_2 (yellow streak 2) and Cebada Capa produced varied results, but segregation ratios obtained from F_2 seedlings and segregating F_3 families suggested the existence of a loose association between genes yst_2 and Pa_7. The gene yst_2, reported to reside on the short arm of chromosome 3 (96), has been linked to the uz locus by a 10% recombination value (79). Data of the present study suggest that Pa_7 may be linked to these genes. Since no trends toward linkage were observed between Pa_7 and either gs_2 or als (both located on the long arm of chromosome 3), crosses to additional markers on the short arm of chromosome 3 may help clarify the map position of the Pa_7 gene. Additional crosses between stocks carrying uz and Pa_7 would also be helpful.

The map positions of chromsome marker genes are tentative, since most recombination values have been reported from two point linkage data. Accurate determination of gene order requires three point test crosses. Until multiple marker stocks are available for test crosses involving each chromosome arm, the map positions of genes residing on the same chromosome arm cannot be considered final.

Data for trisomic analyses were obtained for chromosomes 3, 4, 5, and 6. No data were obtained for trisomic analyses involving chromosomes 1, 2, and 7 because of difficulties encountered in obtaining

viable F_1 seed. Results from crosses between Triplo 3 and Cebada Capa confirmed the location of $\underline{Pa}_7$ on chromosome 3. The highly significant excess of susceptible plants among diploid segregates and deficiency of susceptible plants among trisomic segregates of the cross Triplo 3/ Cebada Capa was indicative of the trisomic inheritance of $\underline{Pa}_7$ through chromosome 3.

In crosses between Triplo 3 and Estate no evidence of trisomic inheritance of resistance was observed. Five out of six test populations of the cross Triplo 3/Estate exhibited either no departure from a 3:1 ratio or had a deficiency of susceptible plants. One population (T3-1/ES) exhibited an excess of susceptible diploid segregates, but population size was small (31 plants). This suggests that the $\underline{Pa}_3$ gene in Estate is not on chromosome 3.

There was an overall trend in both the test and control populations of crosses between Estate and Triplo 4 through 6 toward an excess of susceptible segregates. During the two-week incubation period following inoculation temperatures at times exceeded 30 C. These conditions apparently interfered with normal plant-parasite interaction to the extent that rust reaction was frequently misclassified. Despite the apparent excess of susceptible segregates, particularly among diploid controls, acceptable fits to the 3:1 ratio were obtained in a number of trisomic test populations for crosses between Estate and Triplo 3, 4, 5, and 6.

This result is in agreement with findings of others (70,82). Accrued data lend evidence that the $\underline{Pa}_3$ gene in Estate is not on chromosomes 2 through 7. This would suggest $\underline{Pa}_3$ resides on chromosome 1, but

no data are available for crosses between Estate and Triplo 1 to substantiate this.

In conclusion, a 41.0 ± 2.2% recombination value was found between *Pa* in Speciale and gs_5, a chromosome 2L marker located near the centromere. A loose association was observed between Pa_7 in Cebada Capa and yst_2, a chromosome 3S marker. The assignment of Pa_7 to chromosome 3 was confirmed by primary trisomic analysis. By the same means Pa_3 in Estate was shown to be independent of chromosomes 3 through 7. Suggestions were made for additional work that may clarify the location of genes conditioning resistance to *P. hordei*.

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LOCATION OF GENES CONDITIONING RESISTANCE OF BARLEY TO LEAF RUST (*PUCCINIA HORDEI* OTTH)

by

JAMES RUSSELL LARKINS

(ABSTRACT)

Conventional linkage and primary trisomic analyses were undertaken to locate genes conditioning resistance of barley (*Hordeum vulgare* L.) to *Puccinia hordei* Otth. The linkage relationships of resistance genes *Pa* in Speciale (C.I. 7536) and Pa_7 in Cebada Capa (C.I. 6193) with genes known to reside on chromosomes 2 and 3 were studied in crosses involving Speciale and three chromosome 2 markers (*e*, gs_5, and *lr*), and in crosses involving Cebada Capa and four chromosome 3 markers (gs_2, *uz*, *als*, and yst_2). The location of the resistance gene Pa_3 in Estate was studied by primary trisomic analysis.

Inheritance of leaf rust resistance was determined by seedling reaction to culture 57-19, race UN4 of *Puccinia hordei*. The inheritance and linkage relationships of marker genes and rust resistance were analyzed from F_2 and F_3 coupling data. A recombination value of 41.0 ± 2.2% was found between *Pa* and gs_5, a marker for chromosome 2L. No evidence was obtained linking *Pa* with genes *e* or *lr*, but population sizes were too small to detect reliably loose linkages in these populations. A loose association was observed between Pa_7 and yst_2, a marker for chromosome 3S; however, the data were not sufficiently homogeneous to permit estimation of a recombination value. The data from crosses

involving $\underline{Pa_7}$ and $\underline{uz}$ were not consistent but one population exhibited a trend which suggested these genes might be linked. No evidence of linkage was observed in crosses between $\underline{Pa_7}$ and the genes $\underline{gs_2}$ and $\underline{als}$, although population sizes were limited.

The assignment of $\underline{Pa_7}$ to chromosome 3 was confirmed by primary trisomic analyses. By the same method, the $\underline{Pa_3}$ gene in Estate was shown to be independent of chromosome 3. High temperatures during leaf rust inoculation and incubation periods resulted in poor expression of infection type in crosses involving Estate and Triplo 4, 5, and 6. Despite this the data from these crosses indicated that $\underline{Pa_3}$ is not likely inherited through chromsomes 4 through 6. Accrued data suggest that $\underline{Pa_3}$ may be located on chromosome 1.