Agronomic and genetic characterization of winter wheat plant height isolines
by Stephen Gregory Allen

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Agronomy
Montana State University

Abstract:
The introduction of semidwarf wheat (Triticum aestivum L.) cultivars during the last 20 years has been credited with substantially increasing grain yields of both spring and winter wheats in many of the wheat-producing regions of the United States and throughout the world. At the present time, however, there are no adapted, semidwarf hard red winter wheat cultivars being grown in Montana, in spite of breeding efforts to incorporate shorter straw into new cultivars.

The primary source of semidwarfing genes is the Japanese cuItivar 'Norin 10' which carries two recessive semidwarfing genes, rht1 and rht2. The rht1 semidwarf has been reported to possess agronomic traits superior to the rht2 semidwarf; however, the two semidwarf genotypes cannot be distinguished from each other phenotypically. A biochemical technique, based on the reported gibberellic acid insensitivity of dwarf wheats, was investigated as a possible selection criterion for distinguishing between the two semidwarf genotypes. The results indicated that the semidwarf genotypes cannot be distinguished from each other on the basis of alpha-amylase activity in germinating seeds.

Other studies were initiated to ascertain which agronomic characteristics are responsible for the poor performance of semidwarf winter wheats in Montana. Selected agronomic traits were evaluated in 'Yogo' winter wheat isogenic plant height lines. Yogo is a hard red winter wheat adapted to Montana growing conditions. Emergence rate, total emergence, coleoptile length, and test weight were positively correlated with plant height. Crown depth was not associated with plant height. Grain yield was negatively correlated with plant height, and the tall isolines had the lowest grain yields at four Montana locations.

Test crosses initiated to determine the genotypes of the Yogo isolines, with respect to the rht1 and rht2 semidwarfing genes, were inconclusive due to the effects of minor genes for plant height and unexpected additive-like gene action of the rht1 and rht2 semidwarfing genes.

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

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ABSTRACT

The introduction of semidwarf wheat (*Triticum aestivum* L.) cultivars during the last 20 years has been credited with substantially increasing grain yields of both spring and winter wheats in many of the wheat-producing regions of the United States and throughout the world. At the present time, however, there are no adapted, semidwarf hard red winter wheat cultivars being grown in Montana, in spite of breeding efforts to incorporate shorter straw into new cultivars.

The primary source of semidwarfing genes is the Japanese cultivar 'Norin 10' which carries two recessive semidwarfing genes, rht1 and rht2. The rht1 semidwarf has been reported to possess agronomic traits superior to the rht2 semidwarf; however, the two semidwarf genotypes cannot be distinguished from each other phenotypically. A biochemical technique, based on the reported gibberellic acid insensitivity of dwarf wheats, was investigated as a possible selection criterion for distinguishing between the two semidwarf genotypes. The results indicated that the semidwarf genotypes cannot be distinguished from each other on the basis of alpha-amylase activity in germinating seeds.

Other studies were initiated to ascertain which agronomic characteristics are responsible for the poor performance of semidwarf winter wheats in Montana. Selected agronomic traits were evaluated in 'Yogo' winter wheat isogenic plant height lines. Yogo is a hard red winter wheat adapted to Montana growing conditions. Emergence rate, total emergence, coleoptile length, and test weight were positively correlated with plant height. Crown depth was not associated with plant height. Grain yield was negatively correlated with plant height, and the tall isolines had the lowest grain yields at four Montana locations.

Test crosses initiated to determine the genotypes of the Yogo isolines, with respect to the rht1 and rht2 semidwarfing genes, were inconclusive due to the effects of minor genes for plant height and unexpected additive-like gene action of the rht1 and rht2 semidwarfing genes.

INTRODUCTION

Many of the advancements in the breeding and production of wheat (*Triticum aestivum* L.) throughout the world in the last 25 years can be directly attributed to the utilization of semidwarfing genes. In areas with a high production potential semidwarf cultivars generally offer increased lodging and shattering resistance. The most successful source of semidwarfing genes has been the Japanese cultivar 'Norin 10', which carries two recessive semidwarfing genes, rht1 and rht2.

At the present time, however, there are no successful semidwarf hard red winter wheat cultivars being grown in the Northern Great Plains of the United States, in spite of breeding efforts to incorporate shorter straw into new cultivars.

To investigate the suitability of semidwarf winter wheats for Montana, a series of near isogenic lines for plant height in a 'Yogo' winter wheat background were studied. Several agronomic and genetic characteristics and their relationship with plant height were examined. The traits included plant height, emergence, coleoptile length, crown depth, yield, and test weight.

In addition, a new method for distinguishing between plant height genotypes, based on semidwarf gibberellic acid insensitivity, was investigated.

LITERATURE REVIEW

Short stature wheats have been observed by researchers for many years. Cutler (1919) reported the appearance of a dwarf wheat plant in a commercial field of 'Marquis' in 1914. Pao et al. (1944) investigated the inheritance of dwarfness in wheat during the 1940s.

It was not until the early 1950s that researchers began investigating the commercial potential of short stature wheats (Vogel et al., 1963, and Vogel et al., 1956). Derivatives of the Japanese dwarf cultivar Norin 10 were found to be particularly successful (Borlaug, 1968).

Semidwarf cultivars of both spring and winter wheat are presently being produced in many of the wheat-producing regions of the United States and throughout the world (Borlaug, 1968, and Porter et al., 1964). Borlaug (1968) stated that the Norin 10 semidwarfing genes are an important part of the success of the "green revolution" wheats of Mexico as well as Pakistan, India, Turkey, Afghanistan, and Tunisia.

The success of semidwarf wheats has been attributed to several factors. In areas of high rainfall, or irrigation, semidwarf cultivars generally have more resistance to lodging and shattering than normal tall cultivars (Vogel et al., 1956). Semidwarf cultivars are also reported to have a greater capacity for tillering and an increased number of grains per spike (Gale, 1978). Gale and Law (1976) proposed that the Norin 10 semidwarfing genes may have a favorable pleiotropic association with grain yield.

Genetics of Plant Height

Inheritance of Plant Height

Researchers began investigating the genetics involved in dwarfness in wheat in the 1940s. Pao (1944) studied F_2 and F_3 segregation ratios in a cross between a normal tall parent and a dwarf parent. He concluded that three genes were controlling plant height, and a homozygous recessive condition for all three genes was required for a dwarf plant to occur.

Based on the results of four crosses between semidwarf and tall genotypes, Allan et al. (1961) determined that one or two major semidwarfing genes, and several modifying factors in combination with the semidwarfing genes, produced a wide range of culm lengths.

Several years later, Allan and Vogel (1963) performed an F_2 monosomic analysis of culm length in crosses between the semidwarf 'Norin 10-Brevor 14' and the Chinese Spring series. The specific chromosomes which controlled semidwarfism in Norin 10-Brevor 14 could not be determined in this study. However, it was determined that at least 11 chromosomes appeared to influence culm length, and that the A and D genomes seemed to exert more influence on culm length than the B genome.

Further studies by Allan et al. (1968) into the inheritance of semidwarf culm length suggest that F_1 populations from crosses between semidwarf Norin 10-Brevor 14 and tall 'Brevor' expressed dominance or overdominance for tall culm length. F_2 culm lengths indicated that two recessive genes, rht1 and rht2, were responsible for control of the semidwarf character. However, the large number of minor factors affecting culm length made F_2 analysis of culm length difficult (Allen and Vogel, 1963, and Allan et al., 1968).

In similar studies involving Norin 10 based semidwarfs, Fick and Qualset (1973) also found dwarfness to be controlled by two recessive genes.

Gale, Law and Worland (1975) used the gibberellic acid insensitivity of Norin 10 type semidwarfs described by other researchers (Allan et al., 1959; Radley, 1970; and Gale and Marshall, 1973) to determine the chromosomal location of the semidwarfing gene rht2. A monosomic analysis for gibberellic acid insensitivity was completed for a cross between the semidwarf cultivar 'Maris Hobbit' and the Chinese Spring series. Results indicated that the rht2 semidwarfing gene was located on chromosome 4D.

Using a similar monosomic analysis for gibberellic acid insensitivity, it was soon found that the rht1 semidwarfing gene was located on chromosome 4A (Gale and Marshall, 1976).

It has been suggested that the rht1 and rht2 semidwarfing genes may be homeologous (Gale and Marshall, 1976, and McVittie et al., 1978).

Identification of Plant Height Genotypes

Plant height is associated with such agronomic characteristics as emergence (Allan et al., 1962, and Sunderman, 1964), coleoptile length (Allan et al., 1961; Allan et al., 1962; and Whan, 1976), crown depth (Allan and Pritchett, 1973), and yield (Gale, 1978; Gale and Law, 1976; and McNeal et al., 1972).

Furthermore, the Norin 10 based semidwarfing gene rht1 has been reported to be associated with superior agronomic characteristics compared to the rht2 semidwarfing gene (Allan, 1970, and Allan and Pritchett, 1975).

In an effort to better exploit the various plant height genotypes and their associated agronomic traits in breeding programs, researchers have developed several methods to identify the Norin 10 based plant height genotypes.

In most cases, the normal tall and two-gene dwarf genotypes can be distinguished from each other and from the two single-gene semidwarf genotypes phenotypically on the basis of plant height. The rht1 and rht2 semidwarf genotypes are phenotypically indistinguishable from one another, however.

With the identification of the two semidwarfing genes, rht1 and rht2 (Allan et al., 1968), it became possible to determine plant height genotypes on the basis of F_2 segregation for plant height using crosses onto tester lines of known plant height genotype. However, the numerous minor factors affecting plant height often made conclusive identification difficult (Allan and Vogel, 1963), especially when the crosses involved lines with divergent genetic backgrounds.

Allan and Vogel (1968) developed a technique for measuring the variance of the coleoptile lengths in F_2 populations from crosses onto tester lines. It was assumed that coleoptile length, which is highly correlated with culm length (Allan et al., 1961), was affected by a smaller number of genetic factors than culm length. In one study, only 40 of 71 F_2 lines could be identified using this technique (Allan, 1970). In a later test, however, the genotypes of all semidwarf lines tested were accurately predicted, and it was found that F_2 variances for coleoptile length were closely associated with F_2 variances for culm length (Allan and Pritchett, 1973b).

Gale and Gregory (1977) described another technique for determining plant height genotypes based on the gibberellic acid insensitivity of homozygous semidwarf genotypes.

F_2 seedlings from crosses of unknown semidwarf genotypes crossed onto known semidwarf genotypes were sprayed with GA_3 and 10-day plant heights were measured. Seedlings which were homozygous recessive for a semidwarfing gene showed no response to the GA_3 while heterozygous seedlings and homozygous dominant seedlings did show a growth response. F_2 segregation for growth response to GA_3 indicated a cross between parents carrying different semidwarfing genes.

O'Brien and Pugsley (1981) used semidwarf gibberellin insensitivity to select F_2 seedlings for F_3 yield response based on evidence of a positive pleiotropic relationship between yield and the Norin 10 semidwarfing genes (Gale and Law, 1976).

Gibberellic Acid Insensitivity and Alpha-amylase Synthesis

The discovery of the hormone gibberellic acid resulted from studies of the bakanae disease in rice (*Oriza sativa* L.) which caused tall, thin plants easily distinguished from normal rice plants (Varner and Tuan-Hua Ho, 1976).

In 1926, Kurosawa, as cited by Varner and Tuan-Hua Ho (1976), induced the bakanae disease in rice by treating plants with a culture medium of the fungus *Gibberella fujikuroi*. In 1938, Yabuta and Sumiki, also cited by Varner and Tuan-Hua Ho (1976), isolated biologically active compounds from *Gibberella fujikuroi* and named them gibberellins. Since then, over 45 different compounds with gibberellic acid-like activity have been reported (Varner and Tuan-Hua Ho, 1976).

Gibberellic acid affects the growth and development of many genera and species. Although Marth et al. (1956) found that the most obvious effect of gibberellic acid on

plants was on increasing stem elongation, differences in responses were noted between species.

Phinney (1956) observed that several genetically different dwarf maize (*Zea mays* L.) mutants could be induced to attain normal plant height after treatment with gibberellic acid. Brian and Hemming (1955) noted that dwarf cultivars of peas (*Pisum sativum* L.) and field beans (*Phaseolus vulgaris* L.) could all be induced to attain normal growth rates and plant height following applications of gibberellic acid.

Allan, Vogel and Craddock (1959) studied the effect of exogenously applied gibberellic acid on dwarf, semidwarf, and standard tall winter wheat cultivars. Little or no stem elongation was induced in the dwarf cultivar 'Tom Thumb' and three semidwarf cultivars, 'Seu Seun 27', 'Norin 10-Brevor 2238', and Norin 10-Brevor 14. Stem elongation was induced in two standard tall varieties, 'Burt' and 'Kharkof'.

The effects of gibberellic acid on the physiological processes occurring during germination of seeds have been studied extensively. Observations by Brian (1959) indicated that application of gibberellic acid to seeds of several species stimulates germination by breaking seed dormancy. Paleg (1960), using an amylase assay, found that production of reducing sugars from starch was significantly increased in barley (*Hordeum vulgare* L.) endosperm by exogenous application of gibberellic acid to de-embryonated barley seeds.

It is suggested that, in barley, gibberellic acids are synthesized in the embryonic axis of the seed (Radley, 1967, and Macleod and Palmer, 1967) and transported to the aleurone layer of the seed by way of the vascular system and the scutellum (Macleod and Palmer, 1966 and 1969). The gibberellic acids induce the de novo synthesis of alpha-amylase and other hydrolytic enzymes in the aleurone layer (Bennet and Chrispeels,

1972, and Jacobsen and Varner, 1967). The alpha-amylase enzyme is then secreted into the endosperm of the seed where it hydrolyzes the stored starch.

Chrispeels and Varner (1967a) and Jacobsen and Varner (1967) found that there is an 8- to 10-hour lag period between application of GA_3 and secretion of alpha-amylase into the incubation medium, indicating that gibberellic acids may be involved in a series of events leading up to alpha-amylase synthesis.

Varner (1964) found that alpha-amylase synthesis could be inhibited by application of protein synthesis inhibitors, which is consistent with the observation that alpha-amylase synthesis is de novo.

Chrispeels and Varner (1967b) observed that alpha-amylase synthesis could also be inhibited by inhibitors of RNA transcription such as actinomycin D and 6-methylpurine, which can be interpreted to indicate that alpha-amylase synthesis is dependent upon gibberellic acid-induced RNA synthesis.

Radley (1970) reported that seedling growth of Norin 10-based dwarf wheat cultivars was not stimulated by application of gibberellic acids, but seedling growth of tall cultivars was markedly stimulated by application of gibberellic acids. She also found that deembryonated grains of both the dwarf and the tall cultivars produced alpha-amylase in response to exogenous gibberellic acid. Germinating grains, seedlings, and developing stems of the dwarf cultivars were found to have significantly higher levels of endogenous gibberellic acid-like activity than the tall cultivars. Radley suggested that the dwarf cultivars have a metabolic block to the utilization of gibberellic acid in shoot growth, but not in the utilization of gibberellic acid in the synthesis of alpha-amylase.

Gale and Marshall (1973) confirmed Radley's results with Norin 10-based, dwarf wheat cultivars. They also compared the alpha-amylase response to applied gibberellic acid in two other genetically distinct dwarf wheats, 'Minister Dwarf' and Tom Thumb, which, like the Norin 10-based dwarf, were also found to be gibberellic acid insensitive for shoot growth. They found that only the Norin 10 dwarfs produced alpha-amylase in response to applied gibberellic acid, the other dwarfs were found to be gibberellic acid insensitive in their alpha-amylase response (Gale and Marshall, 1973, and 1975).

Many methods for measuring alpha-amylase activity may be found in the literature. The method used in this thesis is that described by Fox and Eslick (1980) as a rapid determination of cereal alpha-amylase using a blue-dyed amylose starch suspended in an agar medium.

Agronomic Characteristics

Emergence and Coleoptile Length

The establishment of a vigorous fall stand is a primary prerequisite for a successful winter wheat crop. In many dryland wheat growing areas, soil moisture may be the limiting factor in fall stand establishment of winter wheat (Helmerick and Pfiefer, 1954). Doneen and MacGillowray (1943) determined that seed germination and emergence were proportional to soil moisture at the time of planting.

Helmerick and Pfeifer (1954) found varietal differences in germination and emergence in winter wheat. The cultivar Yogo had significantly higher germination and emergence than 'Cheyenne' under limited moisture conditions, indicating genetic variability which could be used in selecting for drought tolerance.

Soon after the advent of semidwarf winter wheat cultivars in the Pacific Northwest, it became apparent that the short stature wheats had poorer fall emergence in the field than normal tall cultivars (Allan et al., 1961, and Burleigh et al., 1965). Two of the main factors involved in emergence of winter wheat are coleoptile length and rate of coleoptile growth (Allan et al., 1961). Allan et al. (1962) observed significant differences in the emergence rate index (ERI) for a group of standard height and semidwarf winter wheat selections. Their analysis indicated that associations between ERI and plant height, and ERI and coleoptile length were positively correlated.

In another comparison of semidwarf and standard height wheats, Whan (1976) found that at planting depths greater than the coleoptile length, emergence was significantly reduced. The semidwarf cultivars had shorter coleoptiles which adversely affected emergence at planting depths not detrimental to standard height cultivars.

The problem of poor emergence in dryland farming regions has caused many farmers to plant seed deeper so that it will reach moisture for germination. This practice has changed the problem from poor germination to poor emergence (Burleigh et al., 1965, and Sunderman, 1964), especially with the semidwarf wheat cultivars. Several researchers have studied the genetic relationship between plant height and coleoptile length in an effort to determine if satisfactory emergence, represented by a longer coleoptile, can be found in a semidwarf wheat.

Allan et al. (1961) stated that culm length in wheat is highly heritable and controlled by only a few genetic factors. The heritability of coleoptile length is much lower, indicating a more complex genetic control. These results suggest that selection for longer

coleoptiles within a culm length genotype may be possible, and that the association between culm length and coleoptile length may not be complete.

In a similar study, Sunderman (1964) observed that plant height and coleoptile length were, in general, positively correlated. Significant exceptions to this association were noted, however, confirming Allan's proposal that the association between plant height and coleoptile length may be broken (Allan et al., 1961).

Crown Depth

Researchers have studied the relationship of the crown node with factors such as disease, drought tolerance, and winterhardiness since the 1920s, often with conflicting results and observations.

Martin (1927) noted that crown tissue was the most hardy tissue in winter wheat, and that spring regrowth depended upon the winter survival of the crown tissue. Using laboratory studies, Pauli (1962) found that decreased survival was related to freezing temperatures and closely associated with decreased vascular connections between top and root tissue caused by death of the crown tissue in winter wheat. He also observed that percent survival was more closely associated with the condition of the crown tissue than either top or root tissue condition.

Marshall (1965) also found that crown tissue survival at freezing temperatures was most closely associated with winter survival of oats in the field. He developed a laboratory technique for winterhardiness using regrowth of frozen crown tissue which was positively correlated with field studies of winter survival.

Young and Feltner (1966) studied some biochemical and agronomic factors influencing winterhardiness of hardy and nonhardy cultivars of winter barley. They found that

later fall planting dates resulted in slower fall growth and enhanced winterhardiness. Planting date was also reported to influence the magnitude of the sugar components glucose, fructose and sucrose, as well as crown diameter and number of tillers. Winterhardy cultivars were found to have significantly higher fructose content in the crown tissue than nonhardy cultivars. It was proposed that winterhardy cultivars may have an enzyme or enzymes present for carbohydrate conversion that are not present in nonhardy cultivars.

The depth of the crown node may play an important role in winter survival. Taylor and McCall (1936) reported that crown depth was deeper for winterhardy than nonhardy cultivars of winter wheat in greenhouse studies. Webb and Stephens (1936) obtained similar results for winter wheat cultivars in field experiments. Dobrenz (1967) reported that winterhardy cultivars of barley appear to have deeper crowns than nonhardy cultivars.

Ashraf (1973) reported results which conflicted with the earlier findings of other researchers. Subcrown internode lengths were measured for six winter wheat cultivars which represented a range of winterhardiness. He found that subcrown internode length was positively correlated with spring field survival under Montana growing conditions. Significant differences for crown depth were also found between cultivars.

Gul and Allan (1978), using crown tissue regrowth as a criterion for winterhardiness, found that crown depth in winter wheat was expressed independently of crown tissue regrowth.

Several environmental and management factors appear to influence crown depth. Taylor and McCall (1936) observed that subcrown internode length was increased by deeper planting and higher temperatures. Webb and Stephens (1936) found the crown

depth in winter wheat was influenced by cultivar, temperature, and seeding depth. They also noted that winter wheats generally have a deeper crown than spring cultivars.

Ferguson and Boatwright (1968) reported that surface straw litter had a significant effect upon the location of crown node formation. As the straw rate increased, the crown node formed farther from the seed, in several cases forming above the soil surface. They also found that decreased light intensity and increased soil temperature caused the crown to form farther from the seed. They felt that surface straw may influence crown node location by changes in soil temperature and light intensity available to the emerging seedling.

Researchers have also studied some genetic aspects of crown depth in wheat in order to determine the heritability of crown depth for breeding purposes. Sallans (1961) concluded that crown depth is a heritable trait. He made crosses between parents with different crown depths. Randomly-selected lines from the cross were found to segregate for depth of crown, and selection of lines similar to each parent and intermediate to the parents was possible.

McKenzie (1971) reported similar results from four crosses of spring wheats. He concluded that crown depth is controlled by only one or two genes and a desirable crown depth could be transferred from one cultivar to another relatively easily.

Allan and Pritchett (1973) investigated the inheritance and association of subcrown internode length with coleoptile and culm length in a winter wheat cross. Results showed that subcrown internode length was closely correlated with coleoptile length and culm length. However, the genetic mechanisms governing crown depth, coleoptile length and culm length were somewhat divergent, implying that crown depth and coleoptile

length could be selected for within a given culm length genotype. However, unrestricted selection for short stature semidwarf genotypes may produce poor emerging or shallow crown types.

Grain Yield and Test Weight

The objective of all wheat breeding projects is increasing yield, whether it be by increasing disease and pest resistance, or by changing plant characteristics to suit changing management practices.

In the 1950s, researchers began to notice the potential value of semidwarf wheats for increasing grain yields. Vogel et al. (1956) found that semidwarf selections from the cross Norin 10 X Brevor outyielded Brevor and 'Elmar', two adapted cultivars in the Pacific Northwest. They also found that semidwarf cultivars could be sown earlier in the fall than tall cultivars with less lodging the following summer. This was viewed as a possible means to help prevent soil erosion during the fall.

McNeal et al. (1960) evaluated the yielding ability of semidwarf selections of spring wheat in Montana. The results showed that yield, as well as test weight, were similar for semidwarf and tall selections, indicating a potential usefulness for semidwarf spring wheat cultivars under Montana growing conditions.

Porter et al. (1964) studied the yield characteristics of semidwarf winter wheat selections under Texas growing conditions. Semidwarf selections were found to have higher yields than tall cultivars under irrigation and in dryland growing areas with a relatively high production potential. However, semidwarf yields were less than or equal to the tall cultivars in growing areas which received less than 20 inches of rainfall annually.

McNeal et al. (1972) compared the yield response of Norin 10-derived, two-gene dwarf, single-gene semidwarf, and standard height selections in a 'Centana' spring wheat background under a variety of yield levels in Montana. The two-gene dwarf selections produced the lowest grain yields at all yield levels. The single-gene semidwarf selections, however, showed the highest grain yields at all but the lowest yield level (679-1351 kg/ha), where tall Centana had the highest yield. The authors felt that plants below a certain critical plant height did not have enough foliage necessary to be an efficient grain producer, as evidenced by the low yields of the dwarf selections.

Gale (1978) evaluated some specific effects of the Norin 10 semidwarfing gene rht2 on yield. The most dramatic effect of the rht2 gene on yield was an increased number of grains per spike. A further yield promoting influence of rht2 was increased tiller number. Gale and Law (1976) proposed that the rht1 and rht2 semidwarfing genes may have an advantageous pleitropic association with yield.

The effect of the Norin 10 semidwarfing genes on test weight has been investigated by several researchers. McNeal et al. (1960) found no significant differences in test weights between standard tall and semidwarf selections of spring wheat originating from crosses of Norin 10 × Brevor, selection 14, with Centana and 'Thatcher'. Porter et al. (1964) also found no significant differences in test weight between short stature and tall wheat cultivars grown under Texas conditions.

Studies by McNeal et al. (1972) in which the two Norin 10 semidwarfing genes were backcrossed into a Centana spring wheat background indicated that test weight was positively associated with plant height. However, it was earlier suggested that high test

weights can be found in short statured wheats, as evidenced by commercial semidwarf varieties of spring wheat (McNeal et al., 1971).

Allan and Pritchett (1975) showed a positive association between plant height and test weight in near-isogenic plant height lines of Burt winter wheat. The Burt lines were selected only for plant height, however. This suggests that unrestricted selection for short stature wheats may reduce test weight.

MATERIALS AND METHODS

General

Two sets of near isogenic, Norin 10 derived plant height lines of winter wheat were used in the investigations reported in this thesis (Appendix Table 1).

The 20 near isogenic lines of hard red winter wheat used in Studies I and III were derived from the cross Norin 10/Brevor, selection 14//3* Yogo, selection 1231/3/3* Yogo (Yogo isolines). The last single plant selections were made in 1977, in the F_4 generation. The Yogo isolines used in the agronomic studies were in the F_6 generation in 1979 when these investigations were initiated. The Yogo isolines, of unknown plant height genotype, represent three phenotypic plant height classes, dwarf, semidwarf, and tall.

The second group of 20 near isogenic lines of hard white winter wheat, used in Studies I and II, were developed from the cross Burt/3/(Norin 10/Brevor, selection 14) //6* Burt (Burt isolines, Allan and Pritchett, 1975). The Burt isolines represent four plant height genotypes, dwarf (rht1rht1, rht2rht2), semidwarf (rht1rht1, Rht2Rht2; and Rht1-Rht1, rht2rht2), and tall (Rht1Rht1, Rht2Rht2).

The Burt isolines, of known plant height genotype, were used to study alpha-amylase activity in germinating seeds and as parents in test crosses to determine the genotypes of the 20 Yogo isolines with respect to the rht1 and rht2 semidwarfing genes.

Study I: Identification of Plant Height Genotypes

Four Burt isolines, one of each plant height genotype, were crossed with each of the 20 Yogo isolines, of unknown genotype, and the recurrent parent Yogo. The first

crosses were made on greenhouse grown plants in the spring of 1980. Poor seed set in the greenhouse dictated that some of the crosses be made on field grown material, near Bozeman, Montana, during the summer of 1980.

The literature gave no evidence of maternal effects upon plant height, so no effort was made to make reciprocal crosses.

Of the 84 crosses attempted, 81 were successful. F_1 seeds from the successful crosses were planted in the greenhouse during the fall of 1980. The F_1 seeds were planted in five randomized complete blocks, two seeds per cross in each block at a depth of approximately 4 cm. A greenhouse planting bench delineated a block, and the first two rows around the circumference of each bench were planted as a border.

F_1 seedlings were vernalized for approximately two months in greenhouse benches at temperatures ranging from 0 to 10 C. Sulfur pots were used intermittently to control mildew, and plants were sprayed with Malathion (active ingredient 0,0-dimethyl dithiophosphate of diethyl mercaptosuccinate), as needed, to control aphids.

F_1 plant heights were recorded to the nearest centimeter, excluding awns, and the heads of each plant were harvested during June 1981.

The difference in plant height between the two recurrent parents, Burt and Yogo, the influence of minor genes on plant height, and unexpected gene action of the rht1 and rht2 semidwarfing genes made a statistical analysis of F_1 plant height data impractical.

Study II: Relationship Between Plant Height and Alpha-amylase Activity During Seed Germination

Four Burt isolines from each of the four plant height genotypes, two-gene dwarf, two single-gene semidwarfs, and tall recurrent parent type, were used in this study.

The experiment was first performed in March 1981 and repeated in August 1981 to confirm the initial results. The techniques and materials used in both experiments were the same.

Three replications of 200 seeds of each of the 16 Burt isolines were placed on moistened blotters in covered plastic germination boxes. These were placed in a germinator at 15 C in a randomized complete block design, with an individual shelf in the germinator representing a block. Fifty seeds were removed from each germination box after periods of 24, 48, 72, and 96 hours. The seeds were oven dried for 24 hours at 65 C and ground into flour using a Cyclone Sample Mill produced by the UD Corporation.

Alpha-amylase activity was measured using the technique described by Fox and Eslick (1980). Flour samples of 0.25 g were mixed with 0.5 ml distilled water and placed in 5 mm diameter wells in an azure blue-dyed amylose starch agar. The agar medium contained a calcium acetate-sodium acetate buffer at pH 5.3. The agar plates were incubated at 65 C for four hours, and each agar plate represented a replication.

Alpha-amylase activity appeared as a clear ring around each flour sample. Ring diameters were measured to the nearest 0.25 mm and converted to areas minus the area of the agar well.

Four check samples of barley flour with known alpha-amylase activity were also placed in wells on each agar plate. A regression equation was formed relating the known

$\log_{10}$ of the dye release units of the barley checks to the areas of the activity on each plate. The regression equation was then used to estimate the $\log_{10}$ of the dye release units for the wheat samples. Results were converted to ng dye released per mg flour per minute.

Study III: Relationship Between Plant Height and Agronomic Characteristics

Plant Height

Mature plant height measurements of the 20 Yogo isolines plus the recurrent parent, Yogo, were recorded to the nearest centimeter at three Montana locations. Plant height data were recorded at the Bozeman and Hilltop Farm field yield trials (see Yield Materials and Methods) during the 1981 growing season. The third location was planted near Bozeman during the fall of 1979 in two row plots, each row 3 m long, with 30 cm between rows and a 60 cm spacing between plots. The plots were replicated four times in a randomized complete block design.

Plant heights were measured as the distance from the soil surface to the top of the spike, excluding awns, on the tallest plant in each row measured. Care was taken to make sure that the plant measured was representative of the rest of the row.

At the Bozeman 1981 and Hilltop Farm 1981 locations, plant height was measured in the middle, bordered rows of each plot in each of four replications. Plant height at the Bozeman 1980 location was measured in nonbordered rows of each plot in each of the four replications.

Emergence and Crown Depth

The 20 Yogo isolines and the recurrent parent Yogo were used in two separate field studies of emergence and crown depth. The first experiment was planted at Bozeman on 17 and 18 June 1980. Twenty-five seeds of each isoline were planted 7.6 cm apart in single row plots spaced 30 cm apart at a depth of 7.6 cm. Four replications were planted in a randomized complete block design.

All plots were irrigated on 18 June to insure adequate soil moisture for seed germination at the 7.6 cm planting depth. The average soil temperature during the first week after seeding was 19.3 C at a depth of 7.6 cm at 1:30 PM, MDT.

The second field experiment, planted at Bozeman on 24-26 September 1980, involved a planting of 50 seeds of each isoline using the same methodology as the previous experiment. Rains prior to planting provided adequate, uniform soil moisture at the 7.6 cm planting depth. The average soil temperature during the first week after seeding was 16.2 C at a soil depth of 7.6 cm at 1:30 PM, MDT.

Total emergence, emergence rate index, and depth to the crown node were measured in both experiments. Total emergence was calculated as the total number of seedlings emerged in a row 30 days after planting.

Emergence rate index was calculated by multiplying the number of seedlings emerged on the first, second, third, fourth, and fifth days after emergence had begun, by five, four, three, two, and one, respectively, and adding the products (Allan et al., 1965). This weighted index gave higher values to the rows which had the fastest emerging seedlings.

Crown depths were measured as the distance from the soil surface to the bottom of the crown node. Twenty-five seedlings from each row were extracted from the soil and measurements were made to the nearest millimeter approximately one month after planting. Crown depth analysis was done on the mean crown depth for each plot.

In experiment one, only 25 seeds were planted in each plot, compared to 50 seeds in each plot in experiment two. Therefore, the emergence rate index and total emergence data from experiment one were multiplied by two to put them on the same basis as experiment two.

Coleoptile Length

Coleoptile measurements were made on the 20 Yogo isolines plus the recurrent parent Yogo. Four Burt isolines, representing each of the four plant height genotypes, were used as checks. Plant height has previously been shown to be highly correlated with coleoptile length in the Burt isolines (Allan, 1970). Coleoptile measurements were made in a single growth chamber experiment.

Fifteen seeds of each isoline were row planted in a randomized complete block design, embryo down, at a depth of approximately 10 mm, in metal flats filled with vermiculite. Individual metal flats delineated each of three replications.

The flats were watered and placed in a darkened growth chamber at 18 C. Coleoptile measurements were made to the nearest millimeter 10 days after planting.

Analysis of coleoptile length used mean values for each isoline in each replication.

Yield and Test Weight

Yield trials for the 20 Yogo isolines, plus the recurrent parent Yogo, were planted in four locations during the fall of 1980. The four locations were Hilltop Farm, located 10 miles north of Three Forks, Montana, Bozeman, Moccasin, and Sidney, Montana. All locations were planted at a seeding rate of 0.67 quintals per hectare in a bordered, randomized complete block design with four replications. The seed planted at all four locations was treated with Vitavax (active ingredient carboxin) at the recommended rate prior to planting.

The Bozeman location was planted in four 3 m rows on 25 September 1980. The plots were sprayed with Bayleton (active ingredient triadimefon) during June 1981 to prevent stripe rust, *Puccinia striiformis* West. The two center rows were trimmed to 2.4 m and harvested with a mechanical row cutter on 13 August 1981, allowed to dry, and threshed on 27 August 1981.

Plots at Hilltop Farm were planted in three 3 m rows on 2 October 1980. The center row was trimmed to 2.4 m, harvested on 6 August 1981, and threshed on 18 August 1981.

The Moccasin plots were planted on 12 September 1980 in three 6 m rows. The center row of each plot was trimmed to 4.8 m and harvested on 21 August 1981.

Plots at Sidney were planted on 23 September 1980 in four 3 m rows. The two center rows were trimmed to 2.4 m and harvested with a plot combine.

Test weights of the yield rows were measured for each location. In the lower yield environments there was not enough grain to obtain test weight data for each plot in each

replication. Test weight results at all locations were, therefore, obtained by bulking the seed for each isoline over all replications.

Plant height measurements of the bordered yield rows were recorded at each of the four locations. Plant heights were measured as the distance from the soil surface to the top of the spike, excluding awns, on the tallest plant in each row. Care was taken to make sure that the plants measured were representative of the rest of the row.

Statistical Methods

An analysis of variance of alpha-amylase activity was calculated for all four germination periods in both experiments conducted in Study II, the relationship between plant height and alpha-amylase activity during seed germination. The mean square values of the random variables, blocks and isolines, as well as the mean square values for variation within each of the four genotypes, were tested with the error mean square. Variation between genotypes, the fixed variable, was tested using the within genotypes mean square even though variances within genotypes were not homogeneous. I believe this is a more conservative test than using the error mean square to test for variation between genotypes.

Mean alpha-amylase activity values of the four plant height genotypes, over both experiments and at each germination period, were compared using t-tests.

In Study III, the relationship between plant height and agronomic characteristics, the 21 Yogo isolines were separated into three plant height phenotypic groups based on the mean plant height values over three locations. Separation of plant height means was accomplished using the least significant difference method at the 0.10 probability level. Analysis of other agronomic traits for differences between and within phenotypes was

accomplished using the same grouping of the Yogo isolines into the three plant height phenotypic classes.

An analysis of variance was calculated for each location for each agronomic trait examined in Study III. For traits which were examined in more than one location (environment), a combined analysis of variance over locations was calculated to examine the interactions between environment and the other variables being analyzed.

In the location analyses of variance for each trait the mean square values of the random variables, blocks and isolines, were tested using the error mean square. Variation between phenotypes, the fixed variable, was tested using the within phenotypes mean square value. The within phenotypes mean square was used because it provided a more conservative test, although variances within phenotypes were not homogeneous for all of the agronomic traits examined.

In the combined analyses of variance, the mean square of each variable was tested using the mean square of the interaction between that variable and the environment, if the interaction was significant. If the interaction was not significant, the pooled error mean square was used to test the variable. The pooled error mean square was also used to test the interactions between all variables and the environment.

Comparison of means of each phenotype were accomplished with t-tests for each agronomic trait examined.

The relationships between plant height and emergence rate index, total emergence, coleoptile length, and crown depth were examined using a linear regression analysis. The plant height values used in these regressions were the means over three locations which

were used to separate the Yogo isolines into three plant height phenotypes. The values for the other agronomic traits were means over all locations in which they were studied.

The relationship between plant height and grain yield, and plant height and test weight were also examined using a linear regression. In this case, however, the plant height values used in the regression were from the same plots in which yield and test weight were measured. Consequently, regressions were calculated for plant height and yield, and plant height and test weight for each of the four locations in which the traits were examined.

RESULTS AND DISCUSSION

Study I: Identification of Plant Height Genotypes

One of the objectives of this study was to identify the genotypes, with regard to the Norin 10 semidwarfing genes, rht1 and rht2, of the 20 Yogo isolines. Information in the literature indicates that the semidwarfing genes are recessive (Allan et al., 1968), and the dominant condition results in tall, standard height plants. Consequently, it could be assumed that the heterozygous condition would result in tall plants.

Based on this assumption, it was believed that F_1 plant height data could be interpreted to identify the plant height genotypes of the 20 Yogo isolines.

The results of the F_1 plant height data (Table 1), however, indicate that the semidwarfing genes are probably not completely recessive. In the crosses involving MT 80271, for example, the F_1 plants were all expected to be of the tall phenotype since the MT 80271 parent (Table 2) is tall and assumed to be dominant for both semidwarfing genes.

The F_1 plants were expected to be heterozygous for both rht1 and rht2 in the cross with the dwarf CI 17317, heterozygous for rht2 in the cross with CI 17323, heterozygous for rht1 in the cross with CI 17329, and homozygous dominant for both genes in the cross with CI 17334. The plant heights for those crosses are 81.8, 105.5, 110.1, and 129.1 cm, respectively. If the semidwarfing genes were completely recessive, the F_1 plants would be expected to be about 129 cm, or near the height of the F_1 plants from the cross between the two dominant parents.

Another possible explanation of these results might be the influence of minor genes for plant height. This could occur if unintentional selection for minor genes occurred

Table 1. Mean F_1 plant heights from crosses of Yogo isoline and Burt isoline parents.

Yogo Isoline Parents	F_1 Plant Heights (cm)			
	Burt Isoline Parents			
	CI 17317 (rht1,rht2)	CI17323 (Rht1,rht2)	CI 17329 (rht1,Rht2)	CI 17334 (Rht1,Rht2)
MT 80244	49.0	77.5	71.8	58.2
MT 80245	50.0	72.2	77.5	99.3
MT 80249	54.9	68.0	71.7	100.8
MT 80250	48.9	72.0	76.5	102.9
MT 80252	48.9	64.6	58.4	86.0
MT 80253	56.0	82.3	83.6	109.9
MT 80256	88.5	90.1	103.1	112.9
MT 80257	64.5	99.2	114.1	119.2
MT 80258	65.2	89.9	89.4	107.5
MT 80259	61.1	130.6	96.2	122.9
MT 80260	100.9	98.4	133.3	114.9
MT 80262	102.4	110.4	100.4	120.7
MT 80264	100.4		139.2	141.7
MT 80266	62.6	90.8	99.0	127.9
MT 80267	74.4	118.2	116.5	133.8
MT 80268	104.9	118.9	127.4	131.6
MT 80269	71.4	107.6	110.4	120.8
MT 80270	100.5	118.3	116.6	
MT 80271	81.8	105.5	110.1	129.1
MT 80272		134.9	122.8	132.1
CI 8033	108.3	120.7	138.5	112.4

Table 2. Mean plant heights of Yogo isoline and Burt isoline parents*.

Parent	Mean Plant Height	Parent	Mean Plant Height
MT 80244	65.3	MT 80266	110.0
MT 80245	68.8	MT 80267	127.0
MT 80249	56.3	MT 80268	146.0
MT 80250	69.6	MT 80269	114.1
MT 80252	74.7	MT 80270	128.5
MT 80253	65.7	MT 80271	128.4
MT 80256	126.3	MT 80272	144.0
MT 80257	113.8	CI 8033	133.2
MT 80258	116.0		
MT 80259	107.0	CI 17317	34.5
MT 80260	139.4	CI 17323	81.3
MT 80262	140.2	CI 17329	84.1
MT 80264	130.3	CI 17334	105.4

*Grown in same environment as F_1 plants.

when the Burt isolines were selected for plant height. However, the difference in plant height in the crosses involving MT 80271, for example, are so great that this explanation seems unlikely.

Analysis of F_2 plant height data for segregation for plant height within crosses will be needed before the Yogo isolines can be classified as to plant height genotype.

Study II: Relationship Between Plant Height and Alpha-amylase Activity During Seed Germination

Alpha-amylase activity was measured in the Burt isolines, after seed germination periods of 24, 48, 72, and 96 hours, in two separate experiments. The purpose of these experiments was to examine the possibility of using alpha-amylase activity as a selection criterion for distinguishing between the two semidwarf genotypes as homozygous, homogenous lines.

The results of the two experiments showed similar trends in alpha-amylase activity for the 16 Burt isolines at each germination period.

Analysis of variance for alpha-amylase activity at the 24 hour germination period is given in Table 3. In both experiments there was significant variation for alpha-amylase activity between genotypes and within genotypes. The greatest variation occurred between genotypes and the least variation occurred with genotypes. Most of the variation within genotypes was found within the tall genotype.

Figure 1 shows the mean alpha-amylase activity of each genotype, averaged over both experiments, for the 24 hour seed germination period. In both experiments the tall

Table 3. Mean square values from analysis of variance for alpha-amylase activity in Burt isolines at 24 hours of germination.

Source	d. f.	M.S. Exp. One	M.S. Exp. Two
Between Blocks	2	504.40*	2,358.00**
Between Isolines	15	787.30**	1,597.00**
Between Genotypes	3	2,004.00**	3,875.00**
Within Genotypes	12	483.25**	1,027.75**
Dwarfs	3	69.42	19.00
rht2 S.D.	3	425.70*	665.40*
rht1 S.D.	3	85.53	509.70
Talls	3	1,351.80**	2,917.20**
Error	30	108.30	204.00

*,**Significant at .05 and .01 level, respectively.