PRE-HARVEST SPROUTING RESISTANCE IN WHITE WHEATS: TESTING METHODOLOGY AND INHERITANCE

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BY

DEBBIE L. JONES

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

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I would like to dedicate this thesis to my mom. I miss you.

Abstract

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Pre-harvest sprouting resistance has been investigated for many years and is still a problem to this day. This is due in part to the difficulty in testing for pre-harvest sprouting resistance and also the lack of agreement on the inheritance of sprouting resistance. The objectives of this study were to develop a testing methodology for sprouting resistance and to follow the inheritance of sprouting resistance in white-kernelled wheat genotypes

Twelve wheat genotypes representing a range of sprouting resistance were used in determining a testing methodology to distinguish resistant from susceptible genotypes. Three maturation temperatures (19/15°C, 24/15°C, 31/19°C) were used in conjunction with four germination testing temperatures (15°C, 20°C, 25°C and 30°C). Optimum testing conditions were a combination of an intermediate maturation temperature of 24/15°C and a germination temperature of 20°C.

In order to follow the inheritance a field study was conducted on populations produced from two white sprouting resistant parents, RL4555 and Bihar 124 crossed to two white sprouting susceptible parents, Hy611 and 87W164. It was found that there are two major genes in both RL4555 and Bihar 124. A third gene which is variable in expression is also found in both resistant parents.

Doubled haploid populations produced from RL4555/3/Alpha*2// Hy612*4 / Biggar BSR, SC8019R1/3/Alpha*2//Hy612*4/Biggar BSR and SC8021V2/3/Alpha*2 //Hy612*4

/Biggar BSR were analysed to determine the number of genes conferring sprouting resistance in each of the resistant parents. RL4555 displayed the same inheritance as was found in the genetic analysis of the field material in that there are two or three genes conveying sprouting resistance. Both SC8019R1 and SC8021V2 may possess two or three genes for dormancy.

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1.0 Introduction

Pre-harvest sprouting has been defined as the germination of the grain while it is still in the ear as it encounters weather conditions which are unfavourable, usually rain and high relative humidity (Mares, 1984). Wheat, barley, rye, oats, triticale as well as rice and maize can suffer from pre-harvest sprouting. The producer suffers as losses arise from a reduction in yield, lower test weights and down-grading based on visible sprouting. The processor suffers as end use quality is lowered. The damage which arises from pre-harvest sprouting resistance arises mainly from the fact that the levels of germinative enzymes rise, most notably alpha-amylase which leads to reduced end use quality. Although the producer is penalized on the percentage of sprouted grain in the sample the processor has to deal with the level of starch degradation. However, there is some ambiguity as to whether the number of sprouted grains correlates (Bhatt et al., 1976) or does not correlate (Moss et al., 1972) with the degree of starch degradation.

Of the mechanisms conferring pre-harvest sprouting resistance, dormancy is the most important (Mares and Ellison, 1989). Dormancy is defined as the inability of threshed grain to germinate under favourable conditions (Derera, 1982). There has long been known an association between grain coat colour and pre-harvest sprouting resistance. The red-kernelled genotypes are much more resistant to pre-harvest sprouting than the white-kernelled genotypes (McCaig and DePauw, 1992). However, white-kernelled wheats are desired by many countries, especially the middle East, Near-East and Africa. White-kernelled wheats are often favoured because of higher flour extraction rates, more valuable bran due to less pigmentation and greater aesthetic appeal of whole-wheat products

(DePauw and McCaig, 1988). The attempt to generate white-kernelled wheat genotypes with an acceptable level of dormancy has been a problem to breeders because of the association with the genes for red-seed coat colour and dormancy. However, other mechanisms not associated with the colour genes have been found such as the sensitivity of the caryopsis to gibberellins, the level of endogenous gibberellins, the sensitivity of the starch to hydrolytic enzymes (Derera, 1982 and DePauw and McCaig, 1983a) as well as new sources of resistance in white-kernelled genotypes (DePauw et al., 1989; Derera et al., 1977).

Pre-harvest sprouting resistance is one of the characters of interest to breeders which is strongly influenced by environmental factors which leads to difficulty in screening genotypes. The maturation temperature can act to reduce the level of dormancy at high temperatures and increase the level of dormancy at low temperatures (Belderok, 1968). It is important to plant breeders in screening for pre-harvest sprouting resistance to have a test which is repeatable, quick, relatively easy and will clearly distinguish resistant and susceptible lines. A second confounding problem is that the temperature used to preform many of the tests will also affect the expression of the level of resistance (Hutchinson et al., 1948).

The first objective of this study is to devise a method which will clearly differentiate between pre-harvest sprouting resistant and susceptible varieties. This method is to take into account the affect of maturation and germination temperatures.

The second objective is to follow the inheritance of sprouting resistance in white-kernelled wheat genotypes and determine the number of genes involved in the resistance.

In addition to a field study, a number of doubled haploids were also produced to conduct a genetic study on the inheritance of sprouting resistance.

2.0 Review of Literature

2.1 Effect of Seed Coat Colour on Pre-harvest Sprouting Resistance

Several authors have shown that there is an association with the genes for seed coat colour and dormancy (Gfeller and Svejda, 1960; Freed et al., 1976; Dyck et al., 1986). When hybrids are produced from a red dormant parent and a white non-dormant parent the white progeny are less dormant than the red progeny. There appears to be a tight linkage or pleiotropic gene action between kernel colour and tolerance to pre-harvest sprouting which has been described in hexaploid wheat and durum (Freed et al., 1976; Soper et al., 1989).

Seed coat colour is controlled by three genes located on homeologous group three chromosomes (Metzger and Silbaugh, 1970). Red seed coat colour is dominant to white. Freed et al. (1976) believes that the different colour genes contribute differently to the level of dormancy as they found that cultivars with three genes were more dormant than cultivars containing two genes while cultivars with different combinations of two genes for seed coat colour had different levels of dormancy. It is thought by some that the seed coat colour genes are additive as a darker pigment is seen in a cultivar with three genes than a cultivar with one or two genes (Freed et al., 1976). DePauw and McCaig (1988) also found that when using

sodium hydroxide to determine kernel colour the intensity of the reaction is related to the number of genes for kernel colour. However, in a study by Flintham (1989), no correlation was found between the number of genes for red seed coat colour and sprouting which still leaves some question as to the additivity of the colour genes.

2.2 Genetic Control of Pre-harvest Sprouting Resistance

There may be other genes or modifying genes associated with the genes for red seed coat colour which may affect dormancy. RL4137 which has a strong dormancy has three genes for red seed colour (Baker, 1981) while Neepawa which also has three genes for red seed coat colour only has an intermediate dormancy (Noll et al., 1982). The sprouting tolerance of RL4137 may be controlled with another mechanism in addition to the one associated with seed coat colour (DePauw and McCaig, 1983b). The sprouting resistance mechanism which is independent of the seed coat colour mechanism found in RL4137 may be controlled by more than one gene as a high degree of sprouting resistance was found in a population generated from a white-kernelled genotype with poor sprouting resistance backcrossed twice to RL4137 (DePauw and McCaig, 1986). Reitan (1980) has also shown by use of an 8 x 8 diallele cross that different varieties with the same seed colour genotype had differing levels of dormancy which demonstrates that another mechanism exists which influences dormancy other than the one associated with seed coat colour.

Attempts have been made to incorporate dormancy into white-kernelled cultivars. McCaig and DePauw (1992) tested a number of white and red-seeded genotypes and found

that there are some white-seeded genotypes that have considerable tolerance to sprouting. In attempting to recombine the long dormancy of RL4137 into a white-seeded genotype, DePauw and McCaig (1983b) were able to attain a higher level of dormancy than the white parent (7722, a cross of NB320/NB402), but they were not able to attain the dormancy associated with RL4137.

Paterson and Sorrells (1990) in their attempt to determine the inheritance of grain dormancy in white-kernelled wheats suggest that dormancy is largely dominant. In a study by Mares and Ellison (1989), dormancy in white-grained wheats was found to be recessive although dormancy in red-grained wheats appeared to be partially dominant. McEwan (1976) compared red-grained and white-grained cultivars for a number of parameters and found the white group to have less dormancy, higher tendency for sprout damage, lower capacity to maintain test weight, more visible sprout damage to grain and lower seed viability under sprouting conditions. Bhatt et al. (1983) studied the inheritance of dormancy in three white-kernelled wheat crosses for the F_1, F_2, BC_1 and BC_2 generations and found segregation ratios which fit the model for two recessive genes in two of the three crosses examined. In the BC_2 generation for the third cross the segregation ratio did not fit the expected 3:1 which may be due to other modifying genes.

In crosses with a red sprouting tolerant line crossed to a susceptible amber line, Sharma et al. (1994) determined that pre-harvest sprouting tolerance was controlled by one dominant gene. They also demonstrate that through the attainment of amber-grained progeny which are pre-harvest sprouting tolerant that sprouting tolerance is not a pleiotropic effect, but rather it is closely linked to the red-kernelled trait although no estimate of linkage

is given.

2.3 Influence of Maturation Temperature

The development of dormancy is altered by a variety of environmental conditions, the most important being the maturation temperature. Many researchers have shown that a relationship exists between the temperature encountered after fertilization and the development of dormancy (Reddy et al., 1985; Plett and Larter, 1986). Takahashi (1980) performed a series of experiments in which the maturation temperature was altered at various periods after fertilization. It was found that if the seed was allowed to mature at a warmer temperature applied for a period of ten days beginning at 15 days post-fertilization the dormancy would be greatly reduced. While a warm maturation temperature will reduce the level of dormancy, a cooler maturation temperature will aid to increase the expression of dormancy. Reddy et al. (1985) demonstrated that cultivars that were matured at 15°C had a much lower germination percentage than cultivars matured at 26°C. Reddy et al. (1984) were also able to demonstrate that alpha-amylase synthesis is also influenced by maturation temperature. The time and duration of the temperature during the period of maturation is still somewhat vague. Strand (1989) found that 10-40 days prior to harvest was the time in which the temperature has the greatest effect on seed dormancy. Contrary to what is commonly believed, Lallukka (1976) concluded that the temperature encountered prior to ripening has little effect on sprouting.

2.4 Influence of Germination Temperature

In order to determine the level of sprouting tolerance in a genotype the germination temperature that is used will have a dramatic effect on the amount of germination. Hagemann and Ciha (1987) found that the percent germination encountered when kernels were germinated at 15°C was higher than the percent gemination of seeds germinated at 30°C. In general, when lower germination temperatures are used there is often a lower expression of dormancy while conversely if warmer germination temperatures are used there is a greater expression of dormancy. This phenomenon has been reported by many researchers (Reddy et al., 1985; Belderok, 1968) and on a variety of cereals; oats, wheat and barley (Strand, 1989)

By altering the germination temperature dormancy can be broken. When imbibed grains are exposed to low temperatures for a period of time (temperature and duration are both genotype dependant) and then placed at warmer germination temperatures of approximately 25°C, dormancy is broken (Black et al., 1986). This "chilling" effect occurs mainly when grain moisture contents are above 25% and also occurs more readily if the grains are dried and then reimbibed (Black et al, 1986). Mares (1984) has demonstrated that this chilling temperature can be as much as 15°C which implies that there could be a natural chilling condition in the field which may lead to the breakage of dormancy especially if this condition occurs after the grains have already reached a low moisture content and then are wetted again. The speed at which maximum germination is attained can be altered by temperature as Mares (1984) demonstrates that after-ripened grain which is germinated at a range of temperatures will all reach 100% germination with the only difference being the

length of the lag phase.

2.5 Factors Affecting Pre-harvest Sprouting

2.5.1 Spike Characteristics

There are many spike characteristics which may have an effect on pre-harvest sprouting. King and Richards (1984) in their study of the effect of awns on germination and ear water uptake confirmed other studies (Pool and Patterson, 1958b; King and Chadim, 1983) that awned ears do indeed take up more water than awnless ears. The presence of awns accounts for faster wetting and increased in-ear sprouting. In using isogenic lines for the awned/awnless character, King and Richards (1984) found that when the awns were removed from the awned lines there was no difference in water uptake between the awned and de-awned lines. This implies that the awns themselves do not act to increase water uptake, rather it may be the structure of the bracts (lemma, palea and glumes) associated with the awned characteristic which is the important factor.

Waxiness of the glumes may also have some affect on the uptake of water and thus sprouting. Pool and Patterson (1958b) determined that waxy glumes will slow the rate of water uptake although waxy glumed genotypes tend to dry more slowly than the non-waxy genotypes. This conflicts with the results of King and Richards (1984) who found that ear uptake of water is not affected by surface glaucousness. However, King and Licis (1989) found that ear water uptake was greater for a waxy line than a nonwaxy line when grown under cool conditions.

Shape of the head as well as the nodding angle have been implicated in pre-harvest

sprouting. Heads which are club shaped greatly affect the uptake of water (King and Richards, 1984). Heads which droop down from the horizontal position appear to shed rain and absorb less water than those heads which are vertical (King and Licis, 1989). In barley, if the ear nodding angles are greater than 120° from vertical then there is a decrease in water absorption as compared to vertical ears. However, in wheat the ear nodding angles do not exceed 90° (King and Licis, 1989).

There appear to be inhibitors found in the bracts of some genotypes which aid in retarding germination. Upadhyay et al. (1988) found that when the bracts from a genotype were milled and placed in the germination dish with the grain from that genotype there was a decrease in the speed of germination. Similar results were found by Derera et al. (1977) as milled bracts when added to seed in a germination test would induce highly significant differences compared to germination tests without the addition of milled bracts. Derera and Bhatt (1980) have shown it is relatively easy to transfer the germination inhibitor that is found in the bracts into a genotype that does not posses the bract inhibitor.

2.5.2 Dormancy

Dormancy is the most important factor when considering pre-harvest sprouting resistance. In the study by DePauw and McCaig (1991) 60-80% of the variation for sprouting tolerance could be attributed to dormancy. Belderok (1968) divides dormancy into three types which are seed coat imposed, embryo and secondary dormancy.

2.5.2.1 Seed Coat Imposed Dormancy

Seed coat imposed dormancy occurs when no germination or a slow germination results, provided the seed coat layers are intact (Belderok, 1968). The assumption of the mechanism of seed coat dormancy is that the seed coat provides inadequate oxygen permeability as removal of the pericarp and testa which in turn acts to facilitate oxygen transport breaks dormancy. Other ways to facilitate oxygen transport include damaging the seed coat mechanically or adding hydrogen peroxide to the water in the germination test. Wellington (1956) suggests that the seed coat may be a physical constraint. In a comparison of non-dormant white and dormant red grained cultivars, it was found that the dormant red cultivar could not continue embryo expansion after 24 hours of water uptake.

2.5.2.2 Embryo Dormancy

If no germination occurs even if the seed coat layers are damaged or removed then embryo dormancy is present. This type of dormancy is thought to be controlled by germination inhibitors in the embryo. The response of the embryos to germination inhibitors is important in aiding against pre-harvest sprouting resistance. Abscisic acid acts to inhibit germination in mature grains. In the developing kernel abscisic acid is found in the pericarp at very high levels. Abscisic acid may be transported to the embryo once the kernel takes up a significant amount of water (Derera, 1982). Upadhyay et al. (1988) found that abscisic acid acted to decrease the germination promptness index for all the genotypes they were studying and also acted to decrease the germination percentage in all but two of the genotypes. Abscisic acid when applied exogenously will act to prevent embryonic

germination as well as blocks the expression of germinative enzymes (Walker-Simmons, 1987). Water stress acts to increase the level of abscisic acid in wheat (Goldbach and Goldbach, 1977). Walker-Simmons (1987) has demonstrated that abscisic acid levels are higher in less germinable cultivars of wheat.

2.5.2.3 Secondary Dormancy

Quite often during the storage of grain when it is undergoing after-ripening to remove dormancy there is a shift to a dormant state. The conditions conducive to this are a high seed moisture content as well as low temperatures in the field (Strand, 1989), high relative humidities in the field (Belderok and Habekotté, 1980) and high germination temperature (George, 1967 and Buraas and Skinnes, 1985). In order for secondary dormancy to be induced there is no need for a drying period before rehydration (Skinnes and Sorrells, 1990). This type of dormancy can be induced during periods other than storage of harvested material as Belderok and Habekotté (1980) assert that the minimum temperatures encountered and a high relative humidity act to induce a secondary dormancy in the field. George (1967) found that when harvest-ripe samples of grain were tested at a temperature of 30°C there was a dramatic decrease in germination which he called high-temperature dormancy. Skinnes and Sorrels (1989) suggest that secondary dormancy may arise when an increase in moisture content of the grain alters the permeability of the grain coat to oxygen. The reduction of oxygen may retard some biochemical events which would otherwise lead to germination of the kernels.

2.5.3 Movement of Water into Spikes

The movement of water into the spike has an important effect on pre-harvest sprouting. Spike water uptake is the first stage in pre-harvest sprouting. As previously mentioned there are characteristics of the spikes which may inhibit the uptake of water. In a study by King and Richards (1984) spike water uptake was significantly different between genotypes when measured after 24 hours. Mares (1983) also found differences in grain water uptake as well as the time taken for moisture content to reach a threshold level for germination.

2.5.4 Movement of Water into Grains

The second event leading to pre-harvest sprouting is the uptake of water into the kernel. Skinnes and Sorrells (1990) noted that there were significant interactions between seed moisture content and genotype. At high seed moisture the germination was lower compared to the germination percentage at low moisture content. Differences in grain water uptake for isolated grain for the first two hours of imbibition have been reported by King and Richards (1984). This also correlates with germination. This difference is thought to be related to grain hardness (Butcher and Stevnert, 1973) although using isogenic lines King and Richards (1984) found that there was no influence of grain water uptake related to grain hardness. The wetting drying cycles in grain are able to significantly affect the sprouting. This may be due to retention of water in the grain, not a sufficient amount for sprouting, but enough to reduce the requirement of future wetting cycles, or the wetting drying cycles may increase the rate of imbibition of later wetting cycles (Bauer and Black, 1983).

2.5.5 Compounds Affecting Dormancy

2.5.5.1 Alpha-amylase

Alpha-amylase is one of many hydrolytic enzymes that are found in grains. There are high levels of alpha-amylase in the developing kernel, but as ripening occurs the alpha-amylase level decreases to very low levels. Alpha-amylase is found primarily in the pericarp of the developing kernel (Kruger, 1972a) and can be induced when kernels take up sufficient moisture. Besides the pericarp, alpha-amylase has also been found in the aleurone layer, scutellum and the embryo (Mares, 1986). Much of the sprouting alpha-amylase in embryonic and aleuronic. Errors can arise when testing for alpha-amylase if there is a presence of green kernels in which the alpha-amylase level has not decreased. This would affect the test for alpha-amylase activity in a negative manner (Kruger, 1972b).

2.5.5.2 Gibberellic Acid

Gibberellic acid acts to induce the synthesis of hydrolytic enzymes, most notably alpha-amylase. This synthesis can occur in the aleurone layer when gibberellic acid is applied exogenously to the kernel. Gibberellic acid has been shown to have an association with the level of pre-harvest sprouting. Stoy and Sundin (1976) found that sprout susceptible genotypes had a high level of sprouting as well as a high gibberellic acid response. If the grain water content is elevated then there can be an increase in gibberellic acid and alpha-amylase production (Stoy and Sundin, 1976).

There are a number of Rht alleles which aid in conferring gibberellic acid insensitivity, these being, Rht1, Rht2, Rht3, Rht10 and Rht15 (Derera, 1982). Gale and

Marshall (1975) found that kernels from varieties containing the Rht3 (dwarfing) gene retained a low alpha-amylase production as well as being insensitive to exogenous gibberellins. The Rht genes are associated with gibberellin insensitive genes (Gai). The Rht3 gene is closely linked or pleiotropic to the Gai3 gene although this mechanism may not be useful as the plants produced are too short for practical purposes (Derera, 1982). The gene controlling gibberellin insensitivity (Gai3) operates in the aleurone of the grain. In the endosperm, which is triploid, there appears to be a dosage effect as the more alleles that are present, the greater the response (Gale and Marshall, 1975).

2.5.6 Maturity

Maturity is important when considering dormancy in cereals. The time to maturity is genotype dependent although temperature will effect the speed of attainment. Gordon et al. (1977) states that harvest ripe grain is below approximately 20% moisture, is fully mature, has a low alpha-amylase activity and will readily germinate in the absence of dormancy. In a comparison of grain which is considered harvest ripe at 17.5 % moisture and 20 % moisture there is a difference in germination potential with a reduced germination potential at the higher moisture content (Gordon et al., 1977). In studies of the development of dormancy in wheat Buraas and Skinnes (1985) found that at early harvest stages there was a higher level of dormancy compared to later harvest stages.

2.6 Methods of Testing for Pre-harvest Sprouting Resistance

2.6.1 Germination Tests

In order to visually assess the level of pre-harvest sprouting resistance in a variety one of two methods can be utilized. These two methods involves germination tests of threshed grain or intact spike testing.

2.6.1.1 Threshed Grain

Germination tests on threshed grain can be considered a test of dormancy. It is preferred that the threshing of the grain should be done by hand as Pool and Patterson (1958a) have shown that mechanical threshing damages the grain. The microfissures that are caused by mechanical damage can lead to increased rate of water uptake and lead to erroneous germination data. Once the grain is rubbed out of the heads many methods of performing the germination test can be used. Germination tests on sand (Hagemann and Ciha, 1984) and blotter paper in petri dishes (Mares, 1984) are very common. Germination can be reported as percent germination or by a dormancy index.

2.6.1.2 Intact Spikes

Intact spikes can be given a artificial rain treatment in a rain simulator and kept at a high relative humidity. The sprouting chamber which has been simulated by many is that based on the model by McMaster and Derera (1976). The heads are kept upright and misted from above for a designated period of time while being maintained at a high relative

humidity.

The advantage of using intact heads is that it more closely simulates natural conditions and takes into affect the head characteristics which may affect pre-harvest sprouting resistance. There are also many disadvantages to using intact spikes. These include requiring large amounts of space, occasional rewetting of the spikes, because of seed position in the spikes not all the seeds receive the same treatment, in general it is more difficult and time consuming.

Intact heads have also been germinated on moist sand, covered with cloth and were wetted as required in order to determine the length of dormancy (Harrington, 1949). Another method of germinating intact spikes is by rolling the spikes in paper towels and then keep the paper towels moist until the completion of the germination test (Hagemann and Ciha, 1984). The means by which germination is presented from germination of intact heads can take the form of sprouting scores or percentages of germinated kernels after the heads have been dried and threshed or the number of spikes with visible sprouts (DePauw and McCaig, 1991). They also found significant correlations between the percentage of germinated kernels and the number of spikes with visible sprouts.

2.6.2 Measures of Alpha-Amylase

There are a number of methods which can be used in order to determine the levels of alpha-amylase in a sample of grain with the following being a list of the most utilized.

Method	Reference
a.) Briggs' Assay	Briggs, 1961
b.) Fluorometric method	Marchylo and Kruger, 1978
c.) Gel-Diffusion method	Hejgaard and Gibbons, 1979
d.) Hagberg Falling number	AACC 1976
e.) Nephelometric method	Campbell, 1980
f.) Colorimetric method	Mathewson and Pomeranz, 1977
g.) Kinetic microplate assay	Kruger and Hatcher, 1992

2.6.3 Comparison of Methods

The advantages of using enzymatic tests are that they are often faster to perform and relate to the level of sprouting. Alpha-amylase and sprouting are positively correlated and the starch quality may be lowered before actual visual sprouting. McCrate et al. (1981) found a high correlation between the colorimetric method and a modified falling number method (smaller sample size and less water) and states that either would be useful to test for pre-harvest sprouting damage. However, the advantage of using the colorimetric method is that it is faster, requires a smaller sample size, is less affected by other grain traits such as protein, amylose and fibre content and measures a wider range of alpha-amylase activity. Falling number is more correctly a measure of endosperm degradation rather than of alpha-amylase activity (Gordon et al., 1977).

In a comparison of evaluation methods for pre-harvest sprouting resistance, which included germination tests of both intact and threshed heads, and measures of alpha-amylase, Hagemann and Ciha (1984) only found two methods which were considered to be useful. The criteria for their test were that it had to show significant differences among cultivars and environments at a given germination temperature and it must have a low coefficient of

variability. These two methods were a germination test using intact heads rolled in paper towels and another using threshed seeds in petri dishes. They also showed that the germination index used was significantly correlated to germination percentage. Henry et al. (1989) describes the method used by Barnes and Blakeney as not simple, but able to assess a wide range of alpha-amylase values very accurately.

3.0 Materials and Methods

3.1 Genetic Study

Four white wheat genotypes (*T. aestivum*) of which two were considered sprouting susceptible (87W164 and Hy611) and the other two being sprouting resistant (RL4555 and Bihar 124) were used as parents. Each of the resistant genotypes was crossed to the susceptible genotypes along with the production of the reciprocal crosses. These crosses were advanced to an F_7 generation through a modified single seed descent procedure (Figure 3.1.1) . The number of F_2 derived lines are listed in Table 3.1.1. Two selections of each line were taken in the F_3 generation to increase the number of lines studied. Two heads were taken from each of the F_6 rows and used to sow the early and late F_7 plots in 1993.

Figure 3.1.1 Modified single seed descent procedure with generation designation referring to the plant.

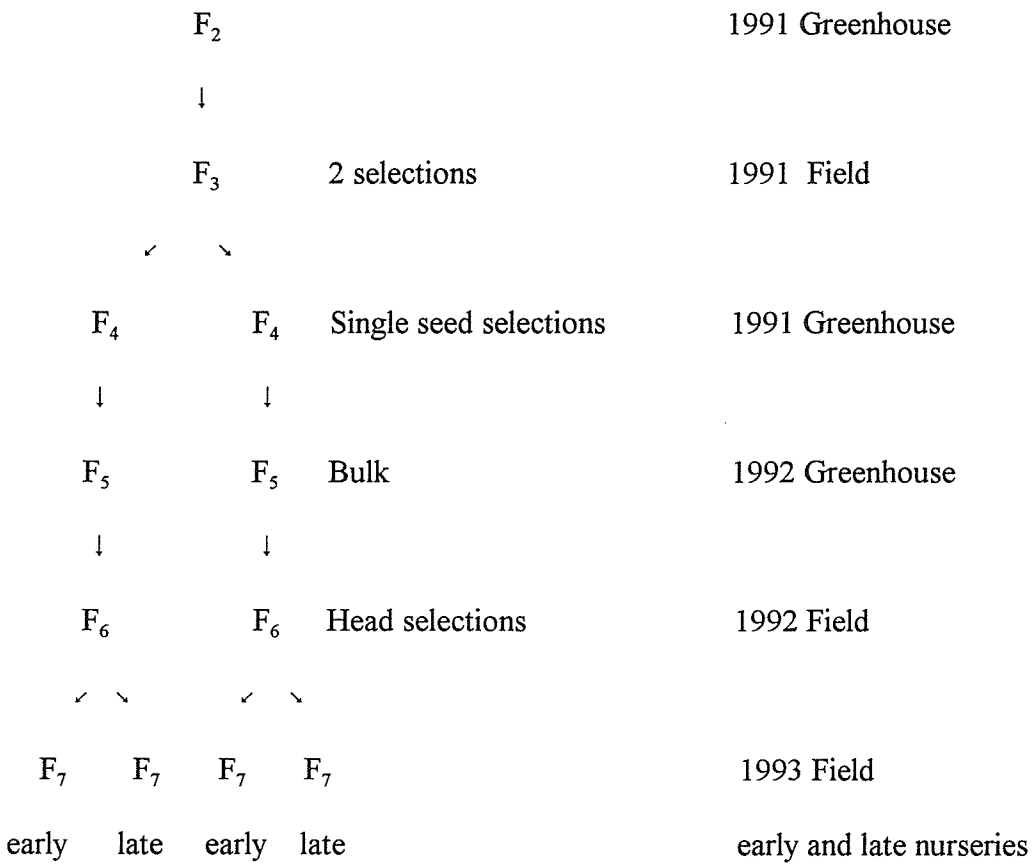

Table 3.1.1 The number of F_2 derived lines for each of the crosses used for the genetic study.

Cross	Number of F_2 derived lines	Total number of lines taken from F_3
RL4555/87W164	85	170
87W164/RL4555	87	174
Bihar 124/87W164	59	118
87W164/Bihar 124	56	112
RL4555/Hy611	84	168
Hy611/RL4555	86	172
Bihar 124/Hy611	53	106
Hy611/Bihar 124	56	112

3.1.1 1992 Field Season

The F_6 generation was planted as F_5 derived head rows on May 20, 1992 at the Agriculture Canada research station at Glenlea, Manitoba. Seeding was done using a Wintersteiger Seedmatic with a row spacing of 23 cm and row length of 1 m. The parents of the crosses were also planted repeatedly along with other selected sprouting resistant genotypes (Columbus, SC8019R1 and SC8021V2) to be used as checks.

Leaf and stem rust were highly prevalent and in order to deter some of the rust damage a fungicide, Tilt (CIBA-Geigy) was applied at a rate of 1.5 mL/L four times during the growing season. In order to prevent the occurrence of aphid transmissible diseases a pesticide, Cygon-2E (ICI Chipman, 1.5 mL/L) was applied twice during the growing season. Two heads were selected from each row in order to provide F_6 derived F_7 seed for the 1993 field season.

In order to provide seed for germination testing six heads with approximately 15 cm of stem from each row were cut by hand. These heads were considered ready to be harvested when there was no chlorophyll remaining on the glumes or on the peduncle. After harvest the heads were placed in a lab at room temperature with a fan for air circulation for a period of seven days. Drying down to less than 13% moisture content was important to ensure long term cold storage viability. After the drying period the heads were placed in a -15° C freezer. Threshing was done using a 20 cm length of bicycle tubing in which the six heads were placed and the seed was rubbed out. This hand threshing was done in order to prevent damage to the seeds.

3.1.2 1993 Field Season

Seed produced from the 1992 field season was used for planting in 1993. Each of two heads harvested in 1992 were threshed separately by hand. The F_6 derived F_7 seed from one head was used to plant the early sprouting nursery on May 13, 1993. Seed from the second head was used to plant the late sprouting nursery approximately two weeks later on May 26, 1993. Tilt (CIBA-Geigy) was applied five times during the growing season in attempt to control various diseases. There was not much control of the prevalent diseases, fusarium head blight *(Fusarium graminarim)* and leaf and glume blotch (*Septoria tritici and Septoria nodorum*) with this particular fungicide. Ten heads from each row were cut by hand. These heads were handled in the same manner as the heads from the 1992 field season.

3.1.3 Germination Tests

Germination tests were carried out in 9 cm glass petri plates with two sheets of Whatman #3, 9 cm qualitative filter paper with the addition of 8 ml of water to each plate. Twenty-five kernels were placed crease side down and were allowed to germinate in the dark. The plates were placed in a growth cabinet at a constant temperature with the temperature being measured at the level of the plates and was accurate to +/- 1.0°C. Only 25 kernels were tested in the 1992 study. In 1993, the germination tests were duplicated for a total of 50 seeds tested. A kernel was considered sprouted when the pericarp was clearly ruptured and there was evidence of protrusion of rootlets. The number of sprouted kernels was recorded on a daily basis for a maximum of eight days. The 1992 material was tested

at 21.0°C and the 1993 early and late material was tested at 22.5°C.

The classification of dormant and non-dormant lines of the field material from 1992 as well as the early and later seeded lines from 1993 for each of the reciprocal crosses is presented in Table 4.8.6. It is always difficult to determine cut off points for germination tests to enable one to separate a dormant from a non-dormant line. In this study, classification was performed by first taking all lines which have a germination percentage that is less than or equal to the mean of the dormant parent (Table 4.2.1). Secondly, to get the upper cut off point among the lines studied, 80% of the area under the distribution curve of the dormant parent was selected. This proportion falls between one and two standard deviations of the mean. For example, the mean ± 1 standard deviation includes 68% of the population, ± 1.96 standard deviations includes 95%. For the desired cut off, 80% of the population is equivalent to 1.28 standard deviations. Since there were a number of plots of the dormant parent, it was possible to calculate the standard deviation for it and thus to calculate a cut off point among the progeny. The proportion of the population that is considered dormant include all lines with a germination percentage less than or equal to the mean of the dormant parent + 1.28 standard deviations (Table 4.2.5).

The cut off point which included 80% of the area under the frequency distribution curve of the dormant parent was chosen to potentially avoid choosing the heterozygotes or lines with fewer dormancy genes than the dormant parent. For example if three genes are conferring dormancy then it is not known what the germination percentages would be if only two of the three dormancy genes are present. Presumably it would be significantly lower than the dormant parent, but it is not known for certain how much lower it actually is.

3.2 Growth Cabinet Sprouting Study

Twelve wheat (*T. aestivum*) genotypes representing a wide range of sprouting resistance and both red and white kernel colour entries were included in this study (Table 3.2.1). Three seeds of each entry were planted in 5" plastic pots containing 200 ml of soil and 300 ml of soilless mix (Metro Mix). Upon reaching the two leaf stage the weakest of the seedlings was removed, leaving two plants per pot. Thirteen pots of each of the twelve genotypes were placed in a completely randomized design in a controlled environment chamber. At approximately the three leaf stage the plants were fertilized with 26-13-0. Afterwards the plants were fertilized with a solution of 20-20-20 (Plant Prod 400g/23L) on a weekly basis until the onset of flowering. In order to control powdery mildew an application of Milgo was given in the form of a soil drench at approximately the four leaf stage. If powdery mildew developed later in the plants life cycle a foliar application of Milgo was applied as necessary. To control aphids, Cygon was applied as required. All plants were grown under the same conditions which consisted of a 16 hour photoperiod and a dark/light temperature cycle of 17°C/11°C (light/dark) until two weeks post anthesis of the earliest genotype. At this point the maturation temperature was altered. Three maturation temperatures were utilized. The first was considered cool at 19°C/15°C (light/dark), the intermediate condition was 24°C/15°C while the warm condition was 31°C/19°C. These growth cabinet temperatures were measured at the height of the heads in the cabinet with a maximum/minimum thermometer.

At the beginning of head collection the plants were grouped together by genotype in order to facilitate harvesting. Heads were collected when there were no traces of chlorophyll on the glumes or peduncle. This harvesting was done approximately every two days until all heads were taken. Heads from all plants of a genotype on each day of harvest were bulked together then divided into two lots. The first lot was placed in a dryer for two days and depending on the time of year this was done the dryer would range from approximately 22°C in winter to 30°C in summer . The second lot was placed in a lab at ambient temperature with a fan for air circulation for a period of seven days. After the drying regimes were complete the heads were placed in a -15°C freezer until testing. All the heads were hand threshed in the same manner as that for the genetic study.

Table 3.2.1 Pedigrees and sprouting tolerance designations for the wheat genotypes used in the growth cabinet sprouting study.

Genotype	Colour	Sprouting Tolerance	Pedigree
RL4137	red	highly tolerant	Frontana/3/McMurachy/Exchange/2/ 2*Regent/Canus/4/Thatcher*6/Kenya Farmer
RL4555	white	tolerant	Kenya Farmer*2/Kenya 321.BT.1.B.1
Bihar 124	white	intermediate	Unknown
SC8019R1	white	tolerant	Kenya 321/Takahe
SC8021V2	white	tolerant	Kenya 321/Peck
Katepwa	red	intermediate	Neepawa*6/RL2938/3/Neepawa*6// C18154/2*Frocor
Roblin	red	susceptible	(Manitou/Tobari 66, BW15)/2/(BW38, CT615/Neepawa)/4/(CT615/Neepawa, BW40)/3/(RL4353, CT934/ Neepawa /2/Era/Park)
Hy612*4/ Biggar BSR	white	susceptible	NB406//Era*4/CT932/3/ Glenlea*6/CT932//Vireo*4/4/Biggar BSR
Hy611	white	susceptible	NB406//Era*4/CT932/3/ Glenlea*6/CT932//Vireo
87W164	white	susceptible	NB406//Era*4/CT932/3/Glenlea*6/ CT932//Vireo
Fielder	white	susceptible	Yaktana54a*4/2/Norin 10 / Brevor/3/2*Yaqui 50/4/Norin 10/ Brevor/2/Baart/Onas
Hy386	white	susceptible	hard selection from Genesis = Hy320/NB402

3.3 Doubled Haploid Sprouting Study

F_1 plants of Hy612*4/Biggar BSR crossed with three sprouting resistant parents (Table 3.3.1) were grown in 5" plastic pots containing 200 ml of soil and 300 ml of soilless mix (Metro Mix). The spikes were emasculated when fully out of the boot and pollinated the next day with maize pollen. 2,4-D was applied at emasculation, pollination and the following day. Gibberellic Acid (GA3) was applied on each of the next two days followed by an application of 2,4-D on the next day. Ten days after pollination both 2,4-D and GA3 were applied. At 18 days following pollination the embryos were excised and placed on an agar medium fortified with Gamborg's minerals and sucrose. (Aung and Howes, unpublished). Once the embryos grew into plantlets approximately three cm tall they were transplanted into 5" plastic pots. Upon reaching the four tiller stage the haploid plants were immersed in colchicine in order to double their chromosome complement. Seed produced off the doubled haploids were used to plant this study. Plastic pots, 6" in diameter containing 300 ml of soil and 400 ml of soilless mix were used. Two seeds per pot were planted with two pots representing each doubled haploid line. Population sizes for each of the crosses are given in Table 3.3.1. Fertilizer, fungicide and pesticides were applied in the same manner as that for the sprouting study. The plants were initially grown at a light / dark temperature of 15°C /11°C with a 16 hour light period. At two weeks post-anthesis the pots were divided into two groups with one group matured at a temperature of 19°C/17°C while the other group matured at 25°C/17°C. Heads were collected on a per pot basis in a manner similar to the sprouting study. Twenty-five seeds from each doubled haploid line were placed crease side down with 6 ml of water in a 9 cm petri dish containing a double layer of

filter paper. The germination tests were carried out in a controlled environment cabinet in the dark at 15 °C and 20 °C.

Table 3.3.1 Double haploid populations utilized in the genetic study.

Cross	Number of doubled haploids
SC8019R1/3/Alpha*2//Hy612*4/Biggar BSR	10
SC8021V2/3/Alpha*2//Hy612*4/Biggar BSR	33
RL4555/3/Alpha*2//Hy612*4/Biggar BSR	21

4.0 Results and Discussion

4.1 Growth Cabinet Sprouting Study

As the selection of lines with adequate pre-harvest sprouting resistance is difficult, it is important to develop a test which is reproducible at specific maturation and germination temperatures. When grain is ripened under relatively warm conditions there is a dramatic decrease in dormancy. Conversely when grain is ripened under cooler conditions there appears to be a greater degree of dormancy. This study includes three genotypes which have a red seed coat, RL4137 which is highly sprouting tolerant, Katepwa which has an intermediate resistance and Roblin which is susceptible. The sprouting tolerant white seeded genotypes, RL4555, SC8019R1, SC8021V2 and Bihar 124 do not have the same level of resistance of the red resistant genotypes. The remaining genotypes, Hy612*4/Biggar BSR, Hy611, 87W164, Fielder and Hy386 are susceptible white seeded genotypes. Figures 4.1.1,4.1.2, 4.1.3 and 4.1.4 represent the same twelve genotypes whose seed has been tested at 15°C, 20°C, 25°C and 30°C after having been matured under three sets of conditions. The maturation conditions include a cool set of conditions of 19°C light/ 15°C dark, a medium set of conditions of 24°C light/15°C dark and warm conditions of 31°C light/19°C dark. In Figure 4.1.1 each genotype can be seen to exhibit the same general trend. As the temperature increases at which the genotypes are matured there is an increase in the germination percentage, thus a decrease in the appearance of dormancy. RL4137, RL4555, Bihar 124, SC8019R1 and SC8021V2 all have low germination percentages when matured under a cool set of conditions when tested at 15°C.

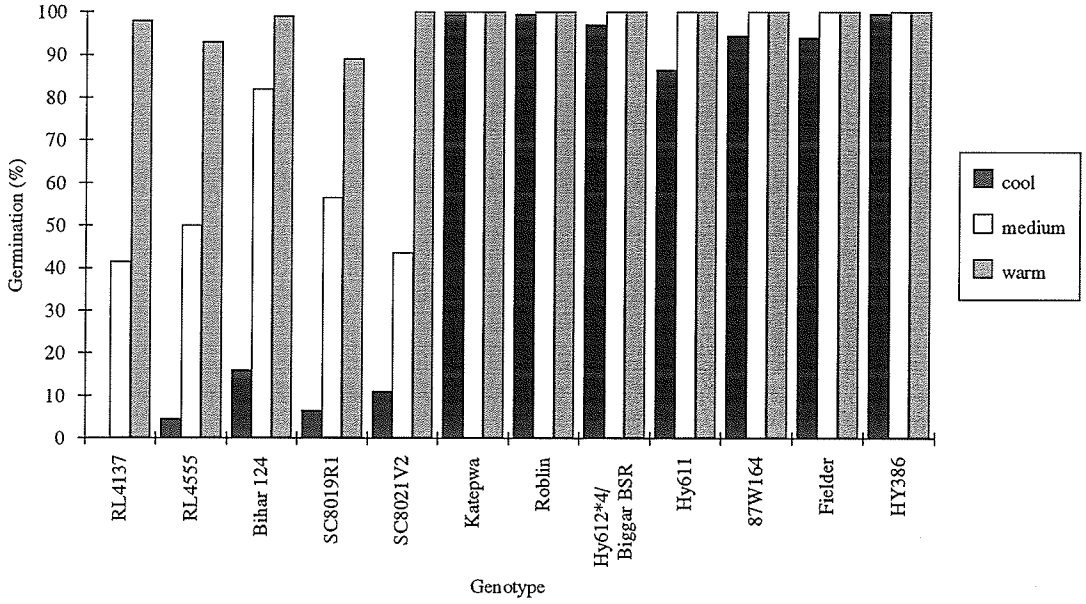

Figure 4.1.1 Germination percentages of twelve genotypes matured under three conditions recorded after eight days of testing at 15°C.

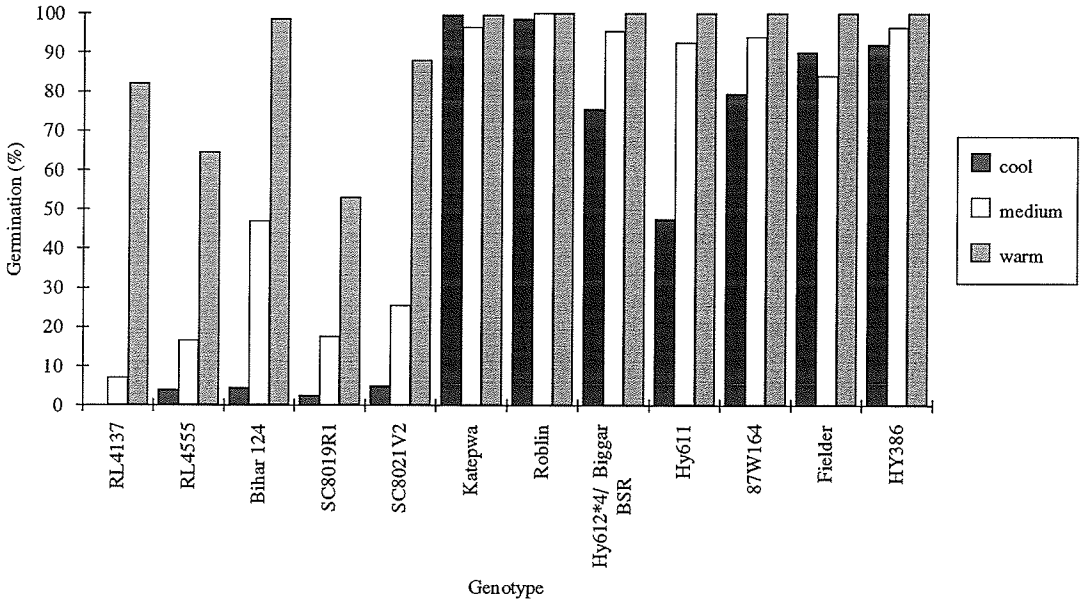

Figure 4.1.2 Germination percentages of twelve genotypes matured under three conditions recorded after eight days of testing at 20°C.

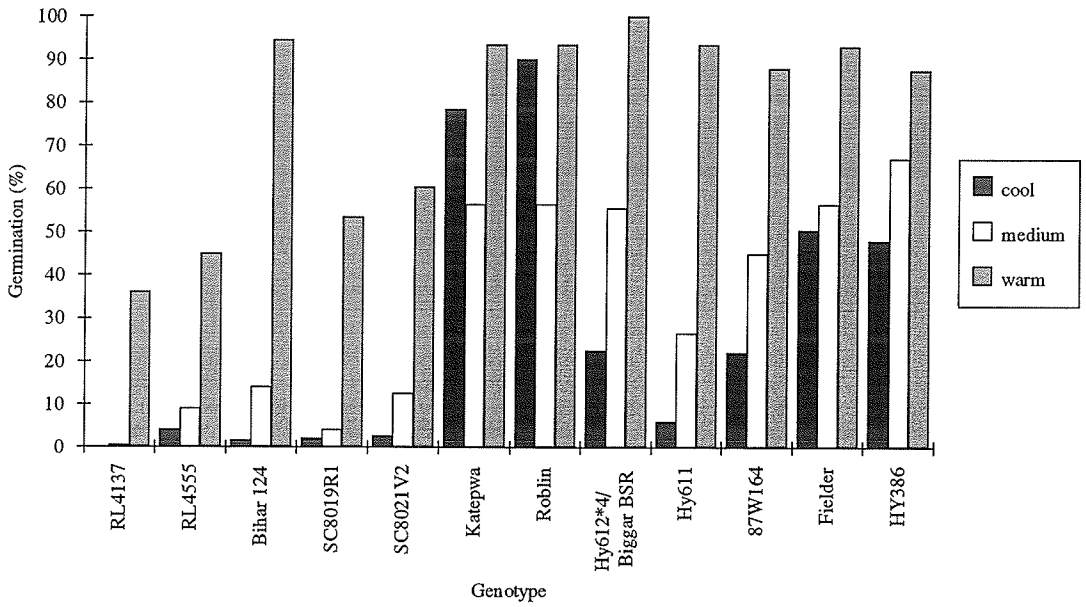

Figure 4.1.3 Germination percentages of twelve genotypes matured under three conditions recorded after eight days of testing at 25°C.

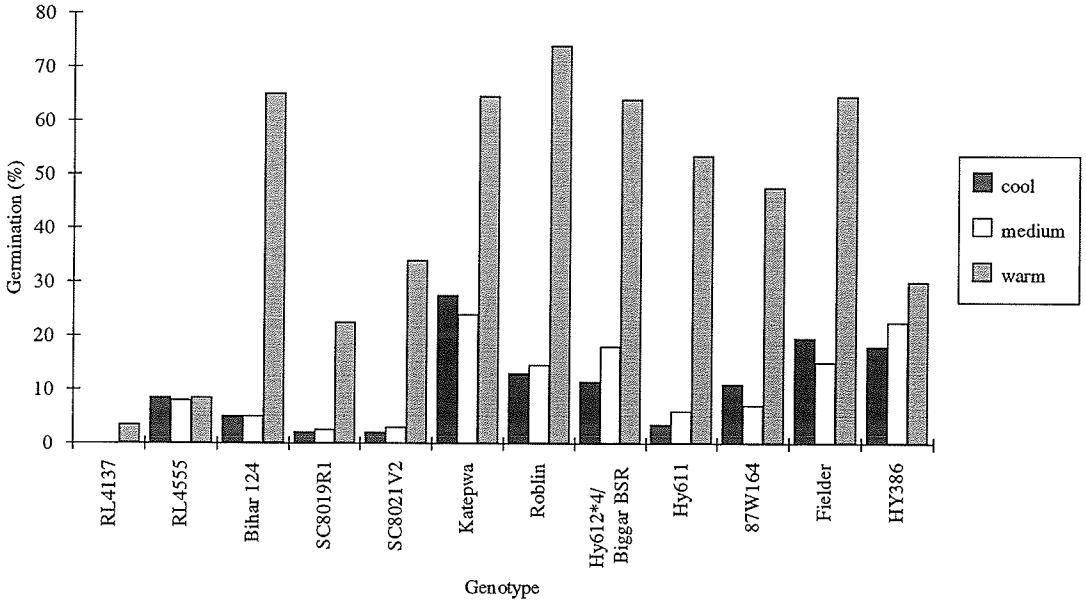

Figure 4.1.4 Germination percentages of twelve genotypes matured under three conditions recorded after eight days of testing at 30°C.

These same twelve genotypes all exhibit a higher range of germination percentages when ripened under a medium set of conditions. When the genotypes are ripened under a warm set of conditions all the germination percentages are higher than those of the medium maturation test. Buraas and Skinnes (1985) showed similar results in that the level of seed dormancy decreased with increased ripening temperatures. The range of maturation temperatures appears to be irrelevant for Katepwa, Roblin, Hy612*4/Biggar BSR, Hy611, 87W164, Fielder and Hy386 as no distinction can be made between genotypes at any temperature.

When the same samples are tested at germination temperatures of 20°C and 25°C, the same trend of increases in germination percentage as the maturation temperature increases (Figures 4.1.2, 4.1.3). In Figure 4.1.3, Katepwa and Roblin have higher germination percentages from material ripened at a cool temperature compared to material ripened at the medium set of conditions. This was not expected and goes against the general trends which can be seen in the other genotypes at all testing temperatures. The discrepancy found with Katepwa and Roblin could be due to the way in which the material was handled. For instance, both Katepwa and Roblin from the cool ripening regime could have been harvested slightly later and would lose some of its dormancy. In Figure 4.1.4. the relationship between maturation temperature and germination percentage still holds up even though the testing temperature was raised to 30°C.

4.1.1 Cool Temperature Maturation

The temperature at which the grain is tested plays a very important role in the expression of the dormancy of a genotype. Table 4.1.1.1 represents 12 genotypes which

have been ripened at a cool set of conditions and tested at 15°C, 20°C, 25°C, and 30°C. The germination percentages have been reported after four days and eight days of the germination test. RL4137, which is considered to be a very dormant genotype does not germinate after eight days of testing at 15°C. RL4555, SC8019R1 and SC8021V2 which are considered dormant, at four days have germination percentages of 0.5 %, 0.5% and 1.0% respectively, and these do not differ significantly from RL4137. DePauw et al. (1992) found that SC8019R1 and SC8021V2 both expressed high levels of sprouting resistance and were similar to the sprouting resistance found in RL4137.

Throughout this study SC8019R1 and SC8021V2 were also found to be very similar to RL4137 in terms of the level of dormancy. After eight days at 15°C there begins to be a separation of the germination percentages of these genotypes. RL4555 has a germination percentage of 4.5 which does not significantly differ from SC8019R1 whose germination percentage is 6.5. The germination percentage of SC8019R1 is not significantly different to that of SC8021V2 (11.0%). This illustrates that germination percentages increase differently the longer the test is conducted, although, there is a maximum time period that it is practical to run such tests. There is a period early in the germination test during which one cannot discriminate between genotypes just as there is a later period at which discrimination is difficult. If a germination test is run for an extended period of time then there is a possibility of confounding the results by making the majority of the genotypes appear non-dormant.

Table 4.1.1.1 Germination Percentages of twelve genotypes matured at 19 °C light/15 °C dark (cool temperature), tested at four germination temperatures recorded at four and eight days

	Germination Temperature							
	15°C		20°C		25°C		30°C	
Genotype	4d	8d	4d	8d	4d	8d	4d	8d
RL4137	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
RL4555	0.5 a	4.5 a	0.5 a	4.0 a	0.0 a	4.5 a	0.0 a	8.5 abc
Bihar 124	2.5 a	16.0 b	0.0 a	4.5 a	0.0 a	1.5 a	0.0 a	5.0 abc
SC8019R1	0.5 a	6.5 ab	0.0 a	2.5 a	0.0 a	2.0 a	0.0 a	2.0 ac
SC8021V2	1.0 a	11.0 bc	0.0 a	5.0 a	0.0 a	2.5 a	0.0 a	2.0 ab
Katepwa	65.5 c	100.0 d	78.0 c	99.5 d	24.0 b	78.5 d	0.5 a	27.5 ad
Roblin	77.0 cd	99.5 d	91.0 d	98.5 d	55.0 c	90.0 e	2.0 b	13.0abcd
Hy612*4/ Biggar BSR	44.5 b	97.0 d	5.0 a	75.5 c	4.5 a	22.5 b	0.0 a	11.5abcd
Hy611	40.5 b	86.5 c	0.5 a	47.5 b	0.0 a	6.0 a	0.0 a	3.5 abc
87W164	43.0 b	94.5 cd	2.5 a	79.5 c	0.0 a	22.0 b	0.0 a	11.0abcd
Fielder	35.0 b	94.0 cd	2.0 a	90.0 d	2.5 a	50.5 c	1.5 ab	19.5 cd
Hy386	83.0 d	99.5 d	13.0 b	92.0 d	4.5 a	48.0 c	0.0 a	18.0 bcd

Means followed by the same letter within columns are not significantly different as tested by Duncan's multiple range test at the 0.05 probability level

Bihar 124 and Katepwa are both considered to have intermediate dormancy although they appear highly different. Bihar 124 which is white-grained has germination percentages of 2.5 and 16% after four and eight days when tested at 15°C, while Katepwa which is red-grained has germination percentages of 65.5 and 100%. It appears that the level of dormancy in Bihar 124 is somewhat higher than that of Katepwa. Roblin, Hy612*4/Biggar BSR, Hy611, 87W164, Fielder and Hy386 are all classed as being non-dormant. At four days the germination percentages of these non-dormant genotypes range from 35-83% while at eight days these values range form 86.5-100%. The values at four days are all significantly different from the more dormant genotypes while at eight days the germination percentages of HY611, 87W164 and Fielder are similar to the germination percentage of Bihar 124.

The results of the 20°C testing temperature presented in Table 4.1.1.1 gives a better distinction between the dormant and the non-dormant genotypes. At four days of testing many of the non-dormant genotypes actually appear dormant. However, after eight days of testing there is a clear separation of dormant and non-dormant genotypes. The dormant genotypes have germination percentages which range from 0-5% while the values of the non-dormant genotypes range from 47.5-99.5%. There is no overlap at this testing temperature.

In order to separate the genotypes into two groups, RL4137, RL4555, Bihar 124, SC8019R1 and SC8021V2 are designated as the dormant genotypes while Katepwa, Roblin, Hy612*4/Biggar BSR, Hy611, 87W164, Fielder and Hy386 are the non-dormant genotypes. At a higher testing temperature of 25°C testing for only four days becomes meaningless as

all the genotypes appear dormant except for Katepwa and Roblin which are the two susceptible red wheats. There is still a problem after eight days of testing although it is less apparent as only one of the non-dormant genotypes falls into the dormant group.

A warm temperature of 30°C was also used as a testing temperature in this study. This temperature was not practical as can be seen after four days of testing all the genotypes had germination temperatures of 0-2% while at eight days of testing the germination percentages ranged from 0-27.5%. Adequate separation of dormant and non-dormant genotypes cannot be made at this temperature. George (1967) found that when using a testing temperature of 30°C on freshly harvested wheat there would be an induction of high temperature seed dormancy. This high temperature induced dormancy was also apparently present in this study which makes the use of high testing temperatures (ie. >30°C) unsatisfactory in dormancy studies.

Reddy et al. (1985) states that low temperatures in the range of 10°C during seed development induces a deep and prolonged dormancy. This is in agreement with the work in this study. However, at an extremely low temperature of 10°C would not be practical for growth cabinet dormancy studies due to the long time it would take for the material to mature.

4.1.2 Medium Temperature Maturation

Table 4.1.1.2 represents the same twelve genotypes tested at the same temperatures, but matured under a medium set of maturation conditions. In this set of germination percentages it appears that the data presented after four days of testing only starts to give an

Table 4.1.1.2 Germination Percentages of twelve genotypes matured at 24°C light/15°C dark (medium temperature), tested at four germination temperatures recorded at four and eight days.

	Germination Temperature							
	15°C		20°C		25°C		30°C	
Genotype	4d	8d	4d	8d	4d	8d	4d	8d
RL4137	7.5 a	41.5 a	1.5 a	7.0 a	0.0 a	0.5 a	0.0 a	0.0 a
RL4555	23.5 b	50.0 ab	0.0 a	16.5 b	0.0 a	9.0 ab	0.0 a	8.0 abc
Bihar 124	19.5 ab	82.0 c	3.5 a	47.0 c	0.5 a	14.0 b	0.0 a	5.0 abc
SC8019R1	17.0 ab	56.5 b	0.5 a	17.5 b	0.0 a	4.0 ab	0.0 a	2.5 ab
SC8021V2	18.5 ab	43.5 ab	4.5 a	25.5 b	2.5 a	12.5 b	0.0 a	3.0 ab
Katepwa	78.5 c	100.0 d	65.5 c	96.5 e	24.5 c	56.5 e	0.0 a	24.0 d
Roblin	78.0 c	100.0 d	74.5 c	100.0 e	24.5 c	56.5 e	0.0 a	14.5abcd
Hy612*4/ Biggar BSR	92.5 d	100.0 d	65.5 c	95.5 e	24.0 c	55.5 e	0.5 a	18.0 cd
Hy611	98.5 d	100.0 d	32.0 b	92.5 de	4.5 ab	26.5 c	0.0 a	6.0 abc
87W164	99.5 d	100.0 d	57.5 c	94.0 e	11.5 b	45.0 d	0.0 a	7.0 abc
Fielder	93.0 d	100.0 d	17.5 ab	84.0 d	5.5 ab	56.5 e	0.5 a	15.0 bcd
Hy386	98.5 d	100.0 d	70.5 c	96.5 e	32.0 d	67.0 f	0.5 a	22.5 d

Means followed by the same letter within columns are not significantly different as tested by Duncan's multiple range test at the 0.05 probability level

indication of the level of dormancy in the genotypes and one must look at germinations after eight days to assess their dormancy levels. After eight days at 15°C RL4137, RL4555, SC8019R1 and SC8021V2 appear to be dormant. Katepwa and Roblin are classed together with a moderately high sprouting percentages and the remainder of the genotypes are non-dormant after eight days of testing. Bihar 124 no longer falls in the dormant class, but falls into a more intermediate class.

At 20°C the separation of dormant and non-dormant genotypes again only begins to be seen at four days, while at eight days of testing the separation is more evident. RL4137, RL4555, SC8019R1 and SC8021V2 have germination percentages ranging form 7%-25.5% and appear dormant. Although germination percentages of 25% appear high one must bear in mind that these are relative values and in comparison to the other genotypes these are indeed dormant. Bihar 124 is in the centre of the germination percentages in this group with 47% germination. Katepwa, Roblin, Hy612*4/Biggar BSR, Hy611, 87W164, Fielder and Hy386 have germination percentages of 84%-100% and are clearly non-dormant. At the higher testing temperature of 25°C the separation between genotypes becomes less clear although still providing a separation between dormant and non-dormant genotypes. Bihar 124 is classed in with the dormant genotypes, however it has the highest germination percentage of these dormant genotypes.

At a testing temperature of 30°C, data after four days are meaningless. At eight days there is some germination evident, but definitely not enough to distinguish between dormant and non-dormant genotypes. This reduction of germination at high testing temperatures is the induction of secondary dormancy similar to that mentioned by George

(1967) who found a reduction in germination when a temperature of 30°C was used.

4.1.3 High Temperature Maturation

A high maturation temperature was used in conjunction with the four testing temperatures in Table 4.1.1.3. At 15°C both the four day and eight day germination percentages do not give any indication of the relative dormancies of the genotypes as the percentages are all very high and indicate almost no dormancy. At 20°C the shortened test of four days breaks down the dormant and non-dormant classes fairly reasonably. However, at this high maturation temperature elevated germination percentages are expected at most testing temperatures so the values for RL4137, RL4555, SC8019R1 and SC8021V2 which range from 64.5-88% are not surprising as high maturation temperatures reduce the expression of dormancy. These genotypes are still considered to have some dormancy as they are statistically better than the non-dormant genotypes.

Testing these high temperature matured genotypes at 25°C manages to separate the genotypes with slightly lower germination percentages. Bihar 124 is placed in the same class as the non-dormant genotypes while RL4137, RL4555, SC8019R1 and SC8021V2 are placed into three classes based on the mean comparison, but can be considered dormant. If testing is done at 30°C a four day test is meaningless as germination percentages range from 0-3.5%. An eight day test allows for germination to proceed further although the genotypes

Table 4.1.1.3 Germination Percentages of twelve genotypes matured at 31°C light/19°C dark (warm temperature), tested at four germination temperatures recorded at four and eight days.

	Germination Temperature							
	15°C		20°C		25°C		30°C	
Genotype	4d	8d	4d	8d	4d	8d	4d	8d
RL4137	96.0 c	98.0 b	55.5 c	82.0 c	6.5 a	36.0 a	0.0 a	3.5 a
RL4555	80.0 ab	93.0 ab	39.0 b	64.5 b	12.5 a	45.0 ab	0.0 a	8.5 ab
Bihar 124	89.5 bc	99.0 b	64.0 c	98.5 d	36.5 b	94.5 de	0.5 a	65.0 fg
SC8019R1	69.5 a	89.0 a	10.0 a	53.0 a	1.5 a	53.5 bc	0.0 a	22.5 bc
SC8021V2	92.0 bc	100.0 b	20.5 a	88.0 c	2.0 a	60.5 c	0.5 a	34.0 c
Katepwa	98.0 c	100.0 b	95.5 def	99.5 d	50.5 bc	93.5 de	1.5 ab	64.5 fg
Roblin	100.0 c	100.0 b	94.0 def	100.0 d	68.5 d	96.0 de	3.5 b	74.0 g
Hy612*4/ Biggar BSR	100.0 c	100.0 b	98.5 ef	100.0 d	48.5 bc	100.0 e	1.0 ab	64.0 fg
Hy611	100.0 c	100.0 b	80.5 d	100.0 d	10.0 a	93.5 de	1.5 ab	53.5 ef
87W164	100.0 c	100.0 b	83.0 de	100.0 d	13.0 a	88.0 de	2.0 ab	47.5 de
Fielder	100.0 c	100.0 b	84.0 de	100.0 d	16.5 a	93.0 de	2.0 ab	64.5 fg
Hy386	100.0 c	100.0 b	100.0 f	100.0 d	61.5 cd	87.5 d	0.5 a	30.0 c

Means followed by the same letter within columns are not significantly different as tested by Duncan's multiple range test at the 0.05 probability level

cannot be distinguished with any accuracy.

The results from the effect of germination temperature on the expression of dormancy found in this study appear to be in agreement with many other studies. Hagemann and Ciha (1987) tested three winter wheat genotypes grown under a wide range of field conditions and found that germination percentages were greater when seeds were germinated at 15°C compared to 30°C. Similar results were found by Hutchinson et al. (1948), as testing at low temperatures (15°C) assisted in breaking dormancy while high temperatures (23°C) accentuated dormancy, although they concluded that 23°C was more suitable than 15°C.

One other thing that should be mentioned is that upon first glance germination percentages of 40 or 50% may not indicate that a genotype is dormant or non-dormant. What must be taken into account is the relative germination percentages of the other genotypes that are used in the study.

If we simplify the categories of dormancy for the twelve genotypes into two very broad categories of dormant and non-dormant some trends start to come to light. In the dormant category RL4137 the sprouting resistant red seeded check and the white seeded strains RL4555, Bihar 124, SC8019R1 and SC8021V2 can be placed. In the non-dormant category are Katepwa and Roblin, the two sprouting susceptible red seeded checks and the white seeded strains Hy612*4/Biggar BSR, Hy611, 87W164, Fielder and Hy386. If a test is able to separate these two broad categories then it would be a useful test for dormancy. The 20°C testing temperature for the cool and medium maturation temperatures is able to separate the genotypes into these two categories. At the warm maturation temperature the 20°C test is barely able to separate the genotypes into two groups. It would be very rare to

have field conditions which would mimic the effects of the warm maturation temperature conditions. Constant 31°C temperatures in the day falling to 19°C at night for a time period extending from two weeks post anthesis to harvest would be most unlikely in Canada.

By looking at the data in two ways, from the perspective of the maturation temperature and the perspective of the testing temperature we begin to see certain trends. One must remember that each of these factors cannot be considered independently. It helps to control both maturation and testing temperature in a growth cabinet study in order to achieve adequate separation between genotypes for dormancy testing.

When we take the maturation temperature as well as the testing temperature into account the determination of dormancy becomes slightly more complicated. The first thing that must be decided is the level of harshness that one would like in the test. For a genetic study one would like a level of harshness which would allow a separation of the lines into dormant and non-dormant classes. As part of a breeding program only the elite would be desired as parents so a very harsh test may be desired. The sprouting resistance level in RL4137 is a target for varietal development. However, RL4137 is red seeded which is generally considered to be advantageous for dormancy. In this study it is usually very good, but some variation occurs in sprouting percent over the range of maturation and testing conditions used. By focusing on the 15°C germination testing temperature and at eight days it is easy to see that there is an decrease in dormancy of RL4137 as the maturation temperature increases. At the cool maturation condition there is no germination then at the medium maturation condition there is 41.5 % germination and finally at the warm maturation temperature there is 98 % germination. This shows that a test can be too harsh resulting in

no expression of dormancy.

For a growth cabinet evaluation of sprouting resistance the best maturation temperature would be an intermediate temperature of about 24°C/15°C (day/night). A cooler temperature similar to the one used in this study has the added problem of taking a very long time for the plant to ripen while a much warmer temperature has the added hazard of reduced seed set due to the elevated temperature.

The testing temperature which seems to be the best for separating dormant and non-dormant lines is 20°C. The selection of this germination temperature is in agreement with Reddy et al. (1985) who assert that a 20°C temperature allows different cultivars to express their degree of dormancy. So when both the maturation and testing temperatures are controlled at these conditions, the result is a very good test for sprouting resistance. In a study by Plett and Larter (1986) controlled conditions of 20/15°C day/night were used until one week post-anthesis then the plants were matured at 30/25°C, 25/20°C and 20/15°C and found that a 25°C germination temperature resulted in the most consistent ranking of the four genotypes used at the range of maturation temperatures. However, it should be mentioned that one of the genotypes was RL4137 and was always ranked as the most dormant and the other lines were triticales. In contrast with this study, Hagemann and Ciha (1987) states that 15°C is the optimum germination temperature for wheat. Their findings would agree with the cool maturation material in this study in which the 15°C test does give a good differentiation, but as stated previously maturation time is much longer.

4.1.4 Stage of Temperature Sensitivity

The timing of the period in which temperature has the most dramatic effect on the development of dormancy has not been precisely defined. Buraas and Skinnes (1985) state that the "mealy ripe stage" is sensitive to the change in temperature, while Belderok (1961) mentions the "dough stage". In this study the temperature was altered at two weeks post-anthesis and maintained until maturity. King (1993) reports that there may be a short period of four to ten days of sensitivity for dormancy imposition occurring in the late stages of grain development. Takahashi (1980) found that low levels of dormancy were expressed when the temperature was raised from 15°C to 25°C at 15 days post fertilization to harvest. It is obvious that altering the temperature at two weeks post-anthesis was sufficient in causing a difference in expression of dormancy. However, it may be that a period of time later in the stage of grain development is the critical time period for altering the expression of dormancy.

4.1.5 Storage of Grain

In order to test the material at the same time and for ease in handling the grain was stored at -15°C for a period of up to six months. In order to extend the testing time Noll and Czarnecki (1980) stored grain which was harvested at maturity for ten months and found them to react similarly to fresh samples. Mares (1983) also found that grain could be stored for up to nine months at -15°C and still remain similar to germination rates of freshly harvested material. The critical factor is the moisture content. For successful storage the moisture content should be in the range of 10-12%. At moisture contents greater than 12-

15% there is an increase risk of freezing injury (Mares, 1983).

4.2 Genetic Analysis of Field Study

In the 1992 material, in many of the crosses, the data from the four day test appears to be misleading as there are a very high number of lines which have germination percentages which are similar to the dormant parent. The means of the RL4555 and Bihar 124 were 2.00% and 5.00%, respectively (Table 4.2.1). On first observation this appears satisfactory . However, after four days the means of 87W164 and Hy611 are 51.00% and 30.00% (Table 4.2.2). The two non-dormant parents which are assumed not to have any genes for dormancy should exhibit higher germination percentages. Due to the fact that the non-dormant parental lines, those being Hy611 and 87W164, were very slow to germinate, the test had to be extended to eight days in order to get a greater separation between the dormant and non-dormant parents. The eight day test from 1992 is used instead of the four day test in the analysis of genetic ratios. The early and late 1993 material is presented for only a four day germination test (Table 4.2.5). After four days of the early test in 1993 the means for RL4555 and Bihar 124 were 13.73% and 26.91%, while the means for 87W164 and Hy611 were 84.00% and 91.71% (Tables 4.2.1 and 4.2.2). The means of the parents in the late seeded material in 1993 were 10.00% and 20.80% for RL4555 and Bihar 124 and 80.40% and 69.00% for 87W164 and Hy611, respectively. For four days of the germination test in 1993 there was an adequate separation of the dormant and non-dormant parents. Due to the fact that not all of the late seeded material was harvested because it never reached maturity, the population sizes are very much reduced and therefore this data is not used for

Table 4.2.1 Simple statistics and germination percentage cut off points from the germination tests for the dormant parents RL4555 and Bihar 124 from 1992 and 1993 field material.

Parent	Test	No. plots	Mean	S.D.†	S.E.‡	Cut off points
RL4555	1992 4 day	4	2.00	2.31	0.58	4.96
	1992 8 day	4	12.00	9.80	4.90	24.54
	1993 Early	15	13.73	8.51	2.20	24.62
	1993 Late	6	10.00	6.07	2.48	17.77
Bihar 124	1992 4 day	4	5.00	3.83	1.91	9.90
	1992 8 day	4	18.00	6.93	3.47	26.87
	1993 Early	11	26.91	9.93	3.00	39.62
	1993 Late	5	20.80	6.72	3.00	28.60

† Standard Deviation
‡ Standard Error of the mean

Table 4.2.2 Simple statistics from the germination tests for the non-dormant parents 87W164 and Hy611 from 1992 and 1993 field material.

Parent	Test	No. plots	Mean	S.D.†	S.E.‡
87W164	1992 4 day	4	51 .00	5.03	2.52
	1992 8 day	4	76.00	8.64	4.32
	1993 Early	9	84.00	15.81	5.27
	1993 Late	5	80.40	11.87	5.31
Hy611	1992 4 day	4	30.00	8.33	4.16
	1992 8 day	4	67.00	10.52	5.26
	1993 Early	7	91.71	6.05	2.29
	1993 Late	4	69.00	6.83	3.42

† Standard Deviation
‡ Standard Error of the mean

the genetic analysis.

The testing temperatures were also slightly different in 1992 (21°C) and 1993 (22.5°C). This study was done before the growth cabinet sprouting study was completed and these temperatures were chosen based on sprouting resistant and susceptible checks that were tested from the field. There was an adequate separation of dormant and non-dormant lines at these temperatures as the dormant checks had germination percentages less than 10% while the non-dormant checks had germination percentages which were greater than 75 %. Even though there was an adequate separation of check lines it is unknown as to whether this slight difference in germination temperatures had a substantial effect. From the growth cabinet sprouting study it was shown that differences of 5°C would cause dramatic differences in germination percentages however, a difference of 1.5°C may or may not be enough to cause a change in classification of dormant and non-dormant lines.

If we look at each the reciprocal crosses for each particular group of crosses there does not appear to be a significant difference in the proportion of dormant and non-dormant lines regardless of which genotype is used as the female parent. For the crosses with Bihar 124 as the dormant parent, with both 87W164 and Hy611 (Table 4.2.3), there are lines which are recovered which have germination percentages which are equivalent to Bihar 124 which is not dependant on the direction of the cross. When RL4555 is the dormant parent then the same effect can be seen (Table 4.2.4). When RL4555 is crossed to either 87W164 or Hy611, lines which have germination percentages that are similar to RL4555 are recovered when RL4555 is either the male or female parent. If there was a difference based on the maternal parent, then for the cross using the non-dormant parent as the female, none

Table 4.2.3 Simple statistics for populations including Bihar 124 as the dormant parent from the 1992 and 1993 field material.

Cross	Test	# Lines	Germination Percentage Mean	S.D.†	S.E.‡
Bihar 124/87W164	1992 4 day	117	10.67	13.20	1.22
	1992 8 day	117	32.48	22.38	2.07
	1993 Early	104	68.21	17.99	1.76
	1993 Late	62	29.10	17.06	2.17
87W164/Bihar 124	1992 4 day	112	24.00	23.40	2.21
	1992 8 day	112	45.14	26.88	2.53
	1993 Early	91	69.21	17.61	1.84
	1993 Late	47	24.72	15.84	2.31
Bihar 124/Hy611	1992 4 day	113	24.35	23.93	2.25
	1992 8 day	113	44.35	26.33	2.48
	1993 Early	99	60.77	17.30	1.74
	1993 Late	31	25.81	19.54	3.51
Hy611/Bihar 124	1992 4 day	111	21.55	20.81	1.98
	1992 8 day	111	39.78	23.28	2.21
	1993 Early	100	61.60	20.10	2.01
	1993 Late	26	24.08	21.96	4.31

† Standard Deviation
‡ Standard error of the mean

Table 4.2.4 Simple statistics for populations including RL4555 as the dormant parent from the 1992 and 1993 field material.

Cross	Test	# Lines	Germination Percentage Mean	S.D.†	S.E.‡
RL4555/87W164	1992 4 day	169	11.74	12.83	0.99
	1992 8 day	169	37.37	25.46	1.96
	1993 Early	159	59.03	25.52	2.02
	1993 Late	114	23.91	17.89	1.68
87W164/RL4555	1992 4 day	172	11.44	12.01	0.92
	1992 8 day	172	39.58	20.16	1.54
	1993 Early	149	64.56	23.44	1.92
	1993 Late	75	23.17	13.26	1.53
RL4555/Hy611	1992 4 day	165	25.01	18.38	1.43
	1992 8 day	165	51.56	20.79	1.62
	1993 Early	147	60.91	24.41	2.01
	1993 Late	66	20.45	14.60	1.80
Hy611/RL4555	1992 4 day	161	20.00	18.09	1.43
	1992 8 day	161	44.65	22.97	1.81
	1993 Early	132	64.30	18.98	1.65
	1993 Late	53	22.08	15.22	2.09

† Standard deviation
‡ Standard error of the mean

of the lines would have the resistance of the dormant male parent. As no differences were found the data from the reciprocal crosses were bulked in order to perform genetic analysis.

The results of the reciprocal crosses are contradictory to those found by Noll et al. (1982). In their study, crosses with the dormant red seeded genotype (RL4137) used as the maternal parent were always more dormant than crosses with the non-dormant line as the maternal genotype with the non-dormant lines being Neepawa, Garnet, a selection from an Era/Neepawa cross and CT932, a white grained genotype. However, one important difference is that this study utilizes only white seeded material and the study by Noll et al. (1982) uses both white and red seeded material. Since they studied F_1 seeds and seed coat colour is in the maternal tissue and also has a role in sprouting resistance this could be the contributing factor in a maternal difference in resistance. Mares (1992) found that when he used a single gene dormant red seeded line (AUS 1490) as the female parent crossed to a white non-dormant line (Janz) then a maternal effect was present. However, when a white seeded dormant line (AUS 1408) was crossed to a white seeded non-dormant line no maternal effect is seen.

An attempt was made to get a differential in maturation temperature in the field by seeding the 1993 material at an early and later date (two weeks after the early date). It was thought that the later seeded material would encounter maturation temperatures that would be cooler than the early seeded material. The harvest period was the same for both dates which was approximately September 3 to September 17. After this point the temperature dropped substantially and further efforts made to harvest the late seeded material were abandoned because of frost damage. When the material that was harvested from the late

seeded test is compared to the early seeded test, certain trends appear. In the crosses with RL4555 there appears to be a reduction in the germination percentage of the late seeded test (Table 4.2.4). Even though the population size is reduced it seems that there was a differential in the maturation temperatures. For the crosses with Bihar 124 there appears to be the same decrease in population means of the later seeded material (Table 4.2.3). The means of the dormant and non-dormant parents were also lower in the late seeded test with the most dramatic decrease of over 20% for Hy611. Even though the harvest period was the same for both of the tests, the bulk of the late seeded lines were collected in the latter part of the harvest period. Since cooler conditions are usually encountered in the latter part of the growing season there is the risk of selecting later maturing lines which may be considered dormant. In actuality, these lines may exhibit more dormancy because of the conditions they encounter as they are late maturing. However, in the late seeded test the lines that were harvested were obviously earlier maturing, but this maturation period was under somewhat cooler conditions. One must be careful in attempting to select dormant lines from a limited number of years of field data as the maturation conditions encountered have a dramatic effect on the outcome.

When comparing the material from 1992 and 1993, the maturation temperature that was encountered in these two years must be kept in mind. Comparing the dormant and non-dormant classes from the 1992 eight day test and the 1993 early test there are changes in the numbers of lines that fall into each of these classes (Table 4.2.5). There are many more dormant lines in the 1992 data as compared to the 1993 early data with this being true for all crosses, except RL4555/Hy611. Some of these differences can be attributed to the

Table 4.2.5 Dormant and non-dormant population frequencies for germination tests recorded at four days (4d) and eight days (8d) in 1992 and early and late seeded material tested for four days in 1993.

Cross	Class†	Frequency			
		1992		1993	
		4d	8d	early	late
Bihar 124/Hy611	d	43	32	9	19
	n	70	81	90	12
Hy611/Bihar 124	d	39	33	12	16
	n	72	78	88	10
Bihar 124/87W164	d	75	41	8	36
	n	42	76	96	26
87W164/Bihar 124	d	42	29	6	29
	n	70	83	85	18
RL4555/Hy611	d	23	16	20	33
	n	142	149	127	33
Hy611/RL4555	d	41	35	5	20
	n	120	126	127	33
RL4555/87W164	d	73	61	21	50
	n	96	108	138	64
87W164/RL4555	d	72	39	13	24
	n	100	133	136	51

† d = dormant, n = non-dormant

temperatures and amount of precipitation encountered in both of these years. Many studies (Weilenmann, 1989; Strand, 1989 and Reddy et al., 1985) have noted the general trend in which cooler conditions encountered during maturation tend to produce a deeper and prolonged dormancy while warmer conditions have the opposite effect. Figure 4.2.1 illustrates the minimum and maximum temperatures as well as the amount of rainfall encountered in 1992 and 1993 on a per week basis. Overall, 1993 appeared to have a smaller range between the maximum and minimum temperatures than 1992. During the mid part of the growing season up until the harvest period, 1993 had the higher maturation temperature. Also, 1993 had substantially more precipitation than 1992. The effect of the wetting and drying cycles may influence germination as Mares (1984) states that wetting and drying cycles may act to increase germination through changes in the seed coat or pericarp integrity. It may be that short periods of rainfall may not be sufficient to allow uptake of water, but longer periods may. In 1993 there were several very heavy rainfalls close to the harvest period, this coupled with the higher temperatures may explain the decreased dormancy of the material.

As already mentioned there was a considerable difference between the population means of the early and late seeded material in 1993. However, there is a much smaller difference in the population means of the late seeded 1993 material and the 1992 eight day test. The parental means from the 1992 eight day test are also more similar to the 1993 late seeded test than the early seeded test. It could be that if the late seeded 1993 lines were left to germinate for eight days that the data between these two tests may be more comparable than the early seeded data. The conditions encountered by this later seeded material may

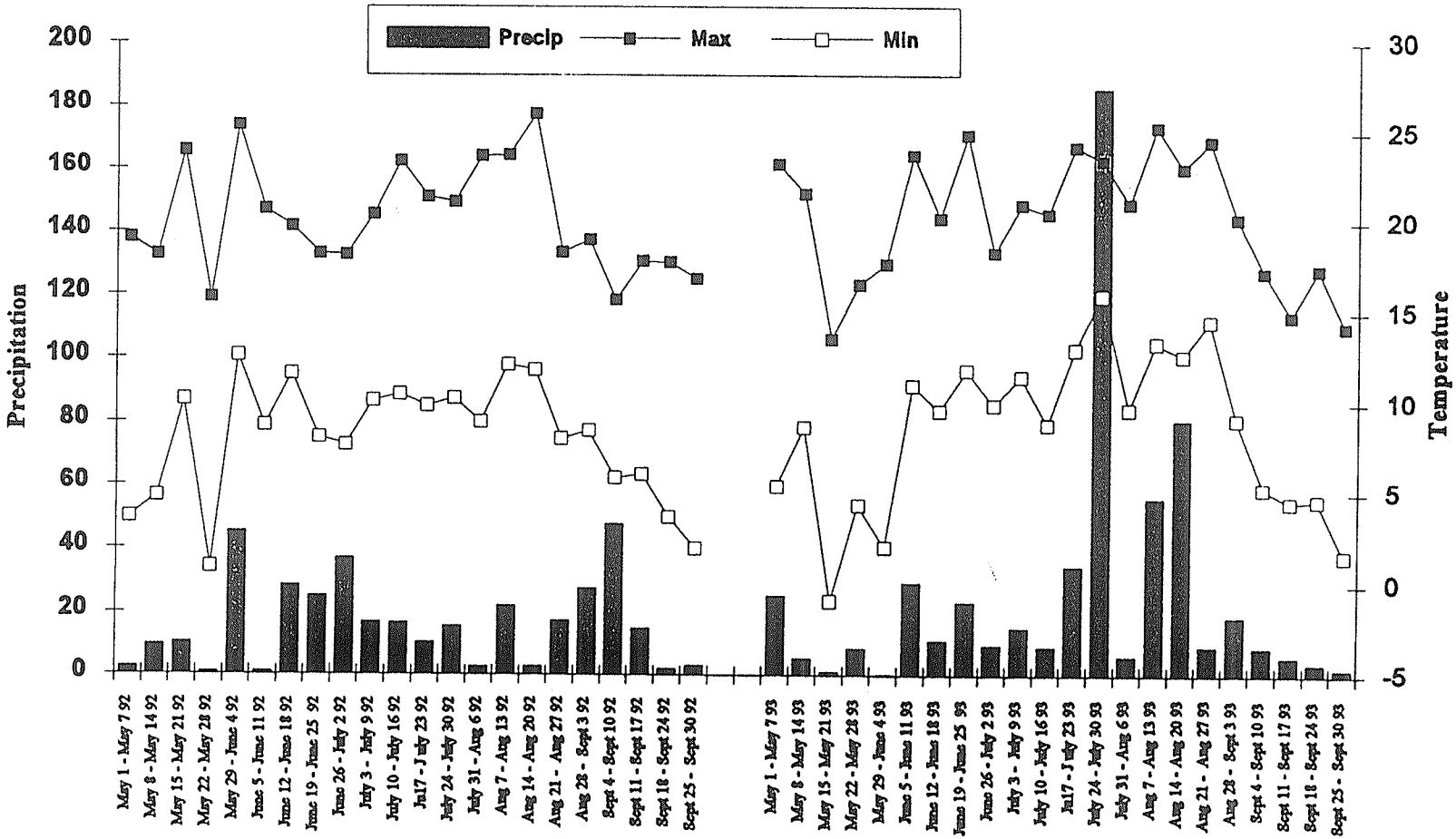

Figure 4.2.1. Weekly precipitation and mean minimum and maximum temperatures for the 1992 and 1993 growing seasons at Glenlea, Mb.

be more similar to those in 1992. This is only speculation as there is still the added problem of reduced population sizes in the late seeded test.

In 1992 the temperature dropped well below 0 °C on September 22 and frost damage occurred. In 1993 the same frost conditions were encountered at the end of September. Mares (1993) reported that cultivars which suffered moderate to severe frost damage had falling number values which were comparatively much lower than those plots which did not suffer frost damage. There may have been some altering of the germination percentages due to the presence of some frost damage although the extent of the damage is unknown.

The lines that were harvested were dried at ambient temperature for one week. This was so the heads would dry down to below 13.5 % moisture and then could be stored at -15°C. Pool and Paterson (1958a) have shown that there are varietal differences in the moisture loss of grain. Although one week at room temperature appeared most adequate for drying of the samples. However, if there was the slim chance that a few of the samples did not dry down enough then this difference in moisture loss may account for some of the variability in the germination tests. If the lines did not dry down to the critical moisture content for storage then there may have been some misclassification of dormant lines.

For inheritance studies the usual procedure is to analyse F_2 and back cross generations. Supposing that three genes were controlling pre-harvest sprouting resistance then 27 different genotypes would occur and dividing the data into these classes would be very difficult. However, due to the difficulty of measuring pre-harvest sprouting resistance and grouping of the data into various classes this study was performed using F_6 and F_7 generations (F_7 and F_8 seed). By using advanced generations which would be closer to

homozygosity for the gene pairs, the number of frequently occurring genotypes is reduced. As mentioned by Gfeller and Svejda (1960), if F_2 plants are carried through enough generations to reach homozygosity then for one gene pair there should be two homozygous genotypes in the ratio of 1:1. If two gene pairs are present then 1/4 would be as dormant as the dormant parent. With three gene pairs present there would be eight different homozygous genotypes with 1/8 as dormant as the dormant parent.

The 1992 material is an F_6 row derived from an F_5 plant. An F_5 generation may still have a small percentage (6.25%) of heterozygotes for each segregating locus. The F_6 may have 3.13% of the population being heteozygotes for one gene which may cause some misclassification of dormant and non-dormant lines. With two gens in an F_5 then 12.11% of the population is heterozygous and 6.15 % of an F_6 population is heterozygous. If three genes are considered then 17.6% of the F_5 population may be heterozygotes. In the $_6$F generation (1993 material) this proportion of heterozygotes is reduced 9.09% for three genes. Finally if four genes are considered then 22.75 and 11.93 of the population can be heterozygous in an F_5 and F_6 generation, respectively. These calculations are based on the origin of the F_6 row from an F_5 plant and the F_7 row from an F_6 plant.

Even though advanced generations are used for the field study as already shown there can still be a relatively high proportion of heterozygotes in the population. Due to this fact the genetic ratios of 1:1, 3:1, 7:1 and 15:1 which were calculated are only approximations. These ratios are true if the population contains only homozygotes as in the case of the doubled haploid populations. For example if three genes are controlling dormancy than in the F_5 the genetic ratio would be 5.77:1 and in the F_6 the genetic ratio would be 6.36:1.

However, for these corrected genetic ratios there are many assumptions which have to be made regarding dominance and epistasis. For this reason the approximated ratios are used for the determination of the number of genes controlling dormancy.

4.2.1 Genetic Ratios

From the sprouting study Bihar 124 has been shown to have an intermediate level of dormancy. Mares (1986) mentions Bihar 124 as a new source of sprouting tolerance and places it at a similar level of tolerance with Kenya 321. RL4555 was shown to be very dormant in the growth cabinet sprouting study. Hy611 and 87W164 which are derived from the same cross are considered non-dormant, but there are other genotypes which are much less dormant (i.e. Hy386 or Hy612*4/Biggar BSR). When selecting parents for a genetic study of this nature it is important to select one parent with all possible genes for the trait and the other parent should not have any genes for the trait in question. In this study the dormant parent should have all possible genes for dormancy while the non-dormant parent should not have any genes for dormancy. If either Hy611 or 87W164 has a dormancy gene then the genetic ratio would be affected.

The 1992 eight day test had a lower cut off point (26.87%) than the 1993 early test (39.62%) (Table 4.2.1.). With a much lower cut off point in 1992 one would assume that there would be fewer dormant lines, but this was not the case, as the population mean is also lower in 1992. The combined data from the 1992 eight day test for the cross of Bihar 124/Hy611 fits a 3:1 ratio, indicating two genes are present. However, the 1993 early material fits a 7:1 ratio which indicates that three genes are present. The data for the cross

of Bihar 124/87W164 also produces similar ratios for the eight day test. The 1992 eight

Table 4.2.1.1 Chi-square values and probabilities for testing genetic ratios on grouped data from 1992 8 day test (8d) and 1993 (early) field material.

Cross	Test	Frequency		Ratio	χ^2	Probability
		d	n			
Bihar 124/Hy611	1992 8 d	65	159	3:1	1.93	0.10-0.20
	1993 early	21	178	7:1	0.70	0.30-0.50
Bihar 124/87W164	1992 8 d	70	159	3:1	3.79	0.05-0.10
	1993 early	14	181	7:1	5.05	0.02-0.05
				15:1	0.29	0.50-0.70
RL4555/Hy611	1992 8 d	51	275	7 :1	2.94	0.05-0.10
	1993 early	25	254	7 :1	3.20	0.05-0.10
				15.1	3.54	0.05-0.10
RL4555/87W164	1992 8d	100	241	3:1	3.40	0.05-0.10
	1993 early	34	274	7:1	0.61	0.30-0.50

day test fits a 3:1 ratio and the 1993 early data fits a 15:1 ratio (Table 4.2.1.1). These tests indicate that two, three or four genes are present in these Bihar 124 crosses.

In the population of RL4555/Hy611 the 1992 eight day test as well as the 1993 early test suggests that three genes are controlling dormancy as a 7:1 ratio exits with a probability ranging from 0.05-0.10. The data from the 1993 early test also fits a 15:1 ratio with a probability of 0.05-0.10 which indicates 4 genes. The data for the 1993 early test gives a good fit to both a 7:1 ratio and a 15:1 ratio. The population of RL4555/87W164 provides a ratio of 3:1 for two genes from the 1992 data while the 1993 data provides a good fit to a genetic ratio of 7:1, indicating that there are three genes controlling dormancy. Therefore, based on these tests it is difficult to say for certain whether there are two, three or four genes which convey dormancy in RL4555.

If the same mechanisms are controlling dormancy in both Bihar 124 and RL4555 then it may be that RL4555 possesses more genes for dormancy than Bihar 124. Since Bihar 124 exhibits higher germination percentages than RL4555 and has been shown in the growth cabinet study to have an intermediate rather than a high level of dormancy it may have fewer genes for dormancy. It could be that there may be another gene in RL4555 which results in a lower germination percentage or perhaps there is a quite different set of genes in the two cultivars.

In determining which population may be better suited for the genetic ratio determination one must bear in mind that these are different generations. There is still a substantial number of heterozygotes in the population even in these advanced generations.

The 1993 data is one generation further than the 1992 data and therefore, there is a higher percentage of possible heterozygotes in the 1992 material.

4.2.2 Agronomic Characters and Pre-harvest Sprouting

Pool and Patterson (1958b) noted the effects of awns as acting to increase moisture contents that in turn would lead to increased germination percentages. In this study each population was examined to see whether there was any effect of awns. The germination percentages were not correlated to whether or not the plants had awns and in this study moisture content of grain was not determined. Therefore there is insufficient evidence to determine whether awns had any effect on germination.

In 1992 the incidence of stem rust was quite severe and there was concern as to the level of rust affecting the germination test. It was thought that a high incidence of rust on a line would inhibit the grain fill or grain maturation process. For each line a score ranging from one to nine (one being less, nine being most) was given for the amount of rust on the plant. There was no correlation with the visual scoring for rust and the germination percentage.

Waxyness was also given a visual score of one to nine based on how blue the glumes appeared. If the glumes were a bright green then they were given a score of one, if they had a dark blueish appearance then they were given a score of nine with gradations between these two extremes. Pool and Paterson (1958b) have shown that waxy glumed lines tend to reduce grain moisture uptake during rains they also dried much slower and overall were considered undesirable. There was no correlation between germination percentage and

waxyness. However, an arbitrary scoring system was used and errors could have occurred. There is insufficient evidence as to whether waxyness had an effect on germination in these crosses.

4.2.3 Indicator of Maturity

In this study it was important to have a visual indicator of maturity that could be readily utilized for harvesting spikes. Hanft and Wych (1982) looked at the complete loss of green colour from the glumes and found that this particular characteristic occurred 1.6 days prior to physiological maturity which is in agreement with Singh et al. (1984) who also state that the complete loss of green colour from the glumes occurs close to physiological maturity and is a useful indicator. Complete loss of green colour from the glumes is easy to determine and was chosen as the criteria for harvesting. The date of harvest was recorded in order to determine if there was any effect of the harvest date on the germination test. There was no significant correlations between the date at which the lines were harvested and their subsequent germination percentages.

Although there was no effect on date of harvest and dormancy in this study there is still the risk of selecting later maturing genotypes with improved dormancy. This can occur as cooler temperatures are usually encountered in the latter part of the growing season and as already mentioned cooler temperatures allow for a greater induction of dormancy. This in turn would lead to selecting material that is late ripening.

4.3 Doubled Haploid Study

The doubled haploid populations produced for this study have been matured in the growth cabinet under two conditions, a cool (19/17°C) and medium (25/17°C) temperature with the harvested seed tested at two temperatures (15°C and 20°C). As already noted the cool maturation temperature should provide an environment conducive to developing dormancy while the warm maturation temperature should reduce dormancy. Also, cooler testing temperatures cause genotypes that would normally be considered dormant to appear non-dormant. If the harshness of these tests were to be ranked in order of gentle to harsh then the cool maturation temperature with a 20°C test followed by 15°C, next is the warm maturation temperature 20°C test and finally the 15°C test would be the harshest. The relative numbers of doubled haploids in the dormant and non-dormant classes reflect the harshness of these tests. The SC8019R1 population is small. The numbers for the dormant and non-dormant classes of the SC8021V2 population clearly displays the trend in the lowering of the expression of dormancy as the test becomes more harsh. The highest number of dormant lines can be seen at the cool maturation, 20°C test while the fewest number of dormant lines are found in the warm maturation, 15°C test (Table 4.3.1).

Usually, in a inheritance study a Chi-square test is used to determine if the hypothesis is to be rejected or not. The major limitation to the chi-square test is the population size. Due to the small doubled haploid population sizes and low observed frequencies other means of testing the hypothesis have been utilized. In this study the genetic ratios have been tested using binomial distributions.

The ratios that have been tested are the same as that for the genetic study as doubled

haploids are totally homozygous for each gene pair. A 1:1 ratio indicates one gene, 3:1 indicates two genes, and 7:1 indicates three genes. The difference between a doubled haploid population and a population that is a F_7 generation, is that there is no chance for heterozygotes for any genes pairs in the doubled haploid population. To conduct a genetic study with a doubled haploid population a minimum population size must be met. In order to select for specific homozygous genotypes for unlinked loci at a probability level of 0.90 certain population sizes are required. For example four plants are required for one gene, nine for two genes, and 18 plants are required for three genes to guarantee that a specific homozygote is retained by at least one plant (Jansen, 1991).

In order to determine the number of genes for each of the doubled haploid populations the germination test which combined a warm maturation temperature and a testing temperature of 20°C were utilized to determine the frequencies for the dormant and non-dormant classes. In accordance with the sprouting study in which it was found that 20°C is the most reliable to distinguish between dormant and non-dormant lines at a warmer maturation temperature. For the RL4555/3/Alpha*2//Hy612*4/Biggar BSR doubled haploid population the genetic ratios of 0.75 and 0.25 which designates two genes and 0.5 and 0.5 which designates one gene are the ratios that fall into the confidence limits, all other hypotheses relating to the number of genes are rejected at the five percent level of significance (Table 4.3.2). Three genetic ratios those relating to one, two or three genes fall into the upper and lower confidence limits for the SC8019R1 population. The population of SC8021V2 could have either one or two genes controlling dormancy. If the harshest of the tests were used, that being the warm maturation and 15°C testing temperature then some

Table 4.3.1 Frequencies of dormant and non-dormant lines grown at two maturation temperatures and tested at two temperatures for three doubled haploid populations.

	Cool Maturation				Warm Maturation			
	15°C		20°C		15°C		20°C	
Population†	n‡	d	n	d	n	d	n	d
RL4555	13	7	2	16	19	0	13	6
SC8019R1	4	6	5	5	7	3	7	3
SC8021V2	7	24	5	26	26	5	19	12

† RL4555 = RL4555/3/Alpha*2//Hy612*4/Biggar BSR
SC8019R1 = SC8019R1/3/Alpha*2//Hy612*4/Biggar BSR
SC8021V2 = SC8021V2/3/Alpha*2//Hy612*4/Biggar BSR
‡ n = non-dormant, d = dormant

slight differences occur. In the SC8021V2 population the genetic ratios for both two and three genes now fit. There is no change for the SC8019R1 population. For the RL4555 population no dormant lines were found in this test. At a probability level of 0.90 a population size of 18 is required so that a specific homozygote is found if three genes are involved. It could be that the population size is too small to find that homozygote, which indicates three genes could be involved in the dormancy associated with RL4555. While the probability of having one gene for dormancy is applicable for all the doubled haploid populations it would be quite unlikely if it were so. Based on one gene it would be very easy to breed for improved resistance to pre-harvest sprouting. As can be seen from the literature improved genotypes have been very slow in coming.

The parentage of SC8019R1 and SC8021V2 are similar in that they both have Kenya 321 in their background. Kenya 321 has been described as having moderate sprouting resistance and moderate length of sprouting resistant period (DePauw and McCaig, 1986). In an inheritance study by Bhatt et al. (1983), Kenya 321 sib., when crossed to a non-dormant line (Gamut or Shortim) produced an F_2 which segregated into a 15:1 ratio indicating two genes for dormancy. If the dormancy is coming solely from that genotype than we may assume that both lines would have the same number of genes controlling dormancy. However, other studies using Takahe which is a one gene red cultivar show that it has a high degree of dormancy at harvest time (Noll and Czarnecki, 1980). Due to the fact that other sprouting resistance genes are introduced from Peck and Takahe then we cannot conclude that SC8019R1 and SC8021V2 have the an equal number of sprouting resistance genes or even the same sprouting resistance genes. However, at least two genes in each of

Table 4.3.2 Confidence intervals (0.95) for binomial distributions for three doubled haploid populations.

Population†	frequency‡		upper and lower confidence limits	genetic ratios			
				1 gene	2 genes	3 genes	4 genes
RL4555	d	6	0.13 - 0.53	0.50 *	0.25 *	0.12	0.06
	n	13	0.47 - 0.77	0.50 *	0.75 *	0.88	0.94
SC8019R1	d	3	0.07 - 0.65	0.50 *	0.25 *	0.12 *	0.06
	n	7	0.35 - 0.93	0.50 *	0.75 *	0.88 *	0.94
SC8021V2	d	12	0.22 - 0.58	0.50 *	0.25 *	0.12	0.06
	n	19	0.42 - 0.77	0.50 *	0.75 *	0.88	0.94

† RL4555 = RL4555/3/Alpha*2//Hy612*4/Biggar BSR
SC8019R1 = SC8019R1/3/Alpha*2//Hy612*4/Biggar BSR
SC8021V2 = SC8021V2/3/Alpha*2//Hy612*4/Biggar BSR
‡ n = non-dormant, d = dormant
* Denotes genetic ratio fit upper and lower confidence limits for designated number of genes.

these lines would seem very likely.

The data from the genetic study of field material involving RL4555 indicate that there are either two or three genes conveying pre-harvest sprouting resistance. This study using doubled haploids indicates either one, two or three genes. One of the parents of these doubled haploids is Alpha*2//Hy612*4/Biggar BSR. It has been tested in the study and found to be very susceptible to pre-harvest sprouting so it might be assumed that there are no dormancy genes coming from it. If there were any misclassed lines in the doubled haploid study they would be put into the dormant class. There was some uncertainty as to the susceptible parent having any genes for dormancy in the field genetic study. Taking this into account the genetic ratios favouring two or more genes would be most likely for RL4555.

5.0 General Discussion

The data from the genetic analysis of the field material provided genetic ratios which indicate two, three of four genes for dormancy in Bihar 124. RL4555 was also shown to have two, three or four genes from the field study. These genetic ratios were determined using advanced generations. With these advanced generations no determination of dominance or any epistatic relationships could be made. Noll et al. (1982) determined that at least part of the genetic component is dominant. Sharma et al. (1994) also found that dormancy is controlled by a dominant gene. This is in agreement with Paterson and Sorrells (1990) who also state that the control of dormancy is dominant. In contrast, Mares and Ellison (1989) found that dormancy was recessive and first expressed in grain on F_2 plants. If control of dormancy is due to a dominant gene or genes then selecting for sprouting resistance in a breeding program should be relatively easy. If control was primarily recessive then it would be much more difficult to select for sprouting resistance.

There is not much known as to the number of genes which control dormancy. With all of the diverse genotypes there is a range in the number of genes controlling dormancy. Of course the more genes that control dormancy the more difficult it is to breed for sprouting resistance. This may be one of the reasons why the development of sprouting resistant varieties has been so slow.

The analysis of the doubled haploid population which had RL4555 as the dormant parent showed that it may have two, three or four genes for dormancy. This study of the doubled haploids is in agreement with the field study. The Swift Current lines SC8019R1 and SC8021V2 both possess either two or three genes for dormancy. Bhatt et al. (1983)

have determined that the dormancy of Kenya 321 is controlled by two recessive genes. Both SC8019R1 and SC8021V2 have Kenya 321 in their pedigree so it would be reasonable to say that these lines have at least two genes for dormancy. SC8019R1 contains Takahe while SC8021V2 has Peck in its parentage. It is not known whether these genotypes convey any genes for dormancy, but if the assumption of three genes for dormancy is true for either SC8019R1 or SC8021V2 then Peck or Takahe would be a contributor of the third gene. The assumption here is that a genotype with three genes for dormancy would be more dormant than a genotype with two genes for dormancy. This is true if the genes for dormancy are additive.

The genes for the control of dormancy could also be located in different areas of the seed, for example in the seed coat and in the embryo. DePauw and McCaig (1983) have stated that RL4137 a red-seeded genotype has two mechanisms which control dormancy, one that is associated with seed coat colour and one that is not. Reitan (1980) also found two mechanisms which control dormancy and the mechanism that is not associated with seed coat colour is controlled by recessive genes. Under certain conditions one mechanism may be more effective than another mechanism. It would stand to reason that if two mechanisms are controlling dormancy in red-seeded genotypes and if only one mechanism for dormancy is found in white-seeded genotypes then it would be very difficult for a white genotype to achieve the same level of dormancy as that of a red-seeded genotype. There is a possibility that there could be genes controlling dormancy in the seed coat which may come into play even in a white-seeded genotype.

There are many hypothesis which could explain the genetic control of dormancy. For

instance it could be that there are a few major genes for dormancy and then minor genes which come into effect under certain conditions. There could also be modifying genes which could affect the expression of dormancy genes under certain environmental conditions. If there are different mechanisms or a number of genes then it would be best to incorporate the different mechanism or the most genes possible into a genotype to endure a deep and prolonged dormancy. There is still much to be learned about the control of dormancy which would make it much easier for breeders to produce sprouting resistant varieties.

6.0 Conclusions

Pre-harvest sprouting resistance is a very important trait for any crop to possess, but is especially true in white wheats, particularly if grown under conditions of high humidity and rainfall. In this study, utilizing a range of red and white seeded genotypes varying in resistance to pre-harvest sprouting, conditions which provide a good separation of resistant and susceptible lines have been determined. This testing methodology takes into account both maturation and testing temperatures. Cooler conditions will lead to a deeper dormancy and warm maturation conditions will reduce dormancy while cool testing temperatures will reduce the expression of dormancy and warm testing temperatures will increase the expression of dormancy. When taking into consideration both of these factors, it was found that the conditions that provide the best separation between dormant and non-dormant lines are a maturation temperature of 24°C/15°C (light/dark) and a 20°C testing temperature.

The inheritance study conducted on two dormant white seeded genotypes (RL4555 and Bihar 124) crossed to two non-dormant white seeded genotypes (87W164 and Hy611) with the hybrid populations grown in the field for two years produced some interesting results. In 1992, crosses involving both the dormant parents gave a good fit to genetic ratios which indicate two genes are present. The 1993 field data indicates that three genes are present in both of the dormant parents. These may be the same genes in the two parents, or they could be totally different genes. The difference in the results for the two years may indicate that there is a gene which varies in its expression based on the weather conditions encountered. Due to the variability of weather conditions, it is very difficult to perform dormancy studies on field grown material. The testing methodology that has been described

in the growth cabinet study could be used to test experimental lines for pre-harvest sprouting resistance in a breeding program rather than relying on field conditions.

The significance of doubled haploids in genetics studies has long been known. The only problem in the past is that they could not be easily or reliably produced. Since the inception of the maize pollination technique coupled with embryo culture, doubled haploids can now be produced quite easily. With doubled haploids one can be sure that the population under study is completely homozygous for all genes, this in turn reduces the number of genotypic classes in the analysis and also reduces the population size required for analysis. In this study, RL4555, the dormant parent most likely has two genes for pre-harvest sprouting resistance which is in agreement with the 1992 field study. However, there is some evidence which may suggest that three genes for dormancy are in RL4555, which is in agreement with the 1993 field study. This one gene difference may be due to a variation in expression based on the maturation temperature encountered. SC8021V2, in this study appears to have two genes while SC8019R1 may have either two or three genes for resistance. This study utilizes the growth cabinet methodology in order to determine dormancy. For the determination of the number of dormancy genes present there is a good agreement with the field study for RL4555. Once again this proves that it is much easier to conduct a growth cabinet study for dormancy rather than a field study.

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