THE ASSOCIATION OF SEED COAT COLOUR AND OTHER FACTORS WITH PRE-HARVEST SPROUTING IN WHEAT

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©Brian Albert Ludwig

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ABSTRACT

Ludwig, Brian Albert, M.Sc., The University of Manitoba, December, 1988. The Association of Seed Coat Colour and Other Factors with Pre-Harvest Sprouting in Wheat.

Major Professor: Dr. R.D. Hill.

The association of seed coat colour, afterripening, and α-amylase inhibitor content with pre-harvest sprouting in wheat was studied. These studies involved characterizing mature, dry seeds for α-amylase activity and α-amylase inhibitor content, germination capacity, and the ability to stimulate embryoless quarter-seeds with GA_3 and ABA. Germinability, and more specifically, the rate of germination, was strongly associated with seed coat colour. The white wheats tended to have a higher rate of germination than the red wheats. Another weakly associated parameter to seed coat colour was α-amylase activity of mature, whole, dry seeds. The white wheats tended to have a higher level of α-amylase activity than the red wheats. None of the other parameters used in this study indicated an association with seed coat colour. In addition to being associated with seed coat colour, the rate of germination was also strongly associated with the afterripening period.

During afterripening, there was an increase in the rate of germination. Furthermore, during the afterripening period, there was an apparent increase in the ability to synthesize α-amylase and other (N-tris{Hydroxymethyl) methylamino}-2-hydroxypropanesulfonic acid soluble proteins and a decrease in the tissue sensitivity to ABA. Finally, there was no apparent relationship between the α-amylase inhibitor content and any of the parameters used to characterize pre-harvest sprouting resistance.

1. INTRODUCTION

Pre-harvest sprouting may be defined as the precocious germination of grain which is usually accompanied by the production of hydrolytic enzymes, most notably ∝-amylase. Production of these hydrolytic enzymes, especially ∝-amylase, in excessive amounts results in flour that produces breads with sticky doughs and crumbs and poor eating quality (Meredith and Pomeranz, 1985). Thus, sprouted grain samples are usually downgraded in terms of quality. In Canada, grain samples are graded on a visual basis. Thus, in grading for sprouting damage, percent sprouted kernels is used as the basis for grading. In order to qualify for the grade of No. 1 C.W. Red Spring Wheat, the maximum limits for the presence of sprouted kernels are 0.1% severely sprouted kernels and 0.5% total sprouted kernels (Canadian Grain Commission, 1987). For No. 1 C.W. Soft White Spring wheat, the maximum limit is 1.0% sprouted kernels.

In wheat, pre-harvest sprouting resistance in terms of dormancy has been thought to be closely related to the seed coat colour. White wheats were commonly regarded as being more susceptible to sprouting than red wheats.

Studies conducted by Wellington and Durham (1958), Gfeller and Svejda (1960), Freed et al. (1976), Khan and Strand (1977), and McEwan (1980) have supported this view by showing that white wheats tend to lack dormancy, and thus, lack the sprouting resistance that the red wheats possess. However, in recent studies by Gordon (1983) and Clarke et al. (1984), tolerance to pre-harvest sprouting ranged from susceptible to resistant for both red and white wheat lines. Furthermore, DePauw et al. (1985) and Mares (1987) both have developed or identified sprouting-resistant white wheat lines that were rated similarly to highly tolerant red wheat lines. Thus mechanisms associated with seed coat colour and mechanisms that are not associated with seed coat colour may exist to provide resistance to pre-harvest sprouting (DePauw and McCaig, 1983).

The objectives of this study were firstly to investigate the potential association between seed coat colour and pre-harvest sprouting, secondly, to determine what influence seed afterripening may have on pre-harvest sprouting resistance and, thirdly, to look at the association of the α-amylase inhibitor with pre-harvest sprouting. A series of red and white cultivars, lines, and isolines were characterized for their pre-harvest

sprouting resistance. The parameters used in this characterization included dormancy and germinability, production of germinative enzymes, notably α-amylase, and soluble protein content. The effects of afterripening on these parameters were also studied.

2. LITERATURE REVIEW

2.1 Factors Determining Pre-Harvest Sprouting Resistance

2.1.1 Penetration of water into the grain

The first step in the sequence of pre-harvest sprouting is the penetration of water into the grain. Without adequate penetration of moisture, neither germination of the embryo nor activation of the aleurone layer can occur. The threshold water requirement for germination has been estimated as 40 percent (Owen, 1951; Lush et al., 1981). It would thus be ideal to select for cultivar characteristics that are able to limit the rate of water uptake into the grain. There are numerous diverse mechanisms involved in this aspect of pre-harvest sprouting resistance. These include head morphological characteristics that influence the catching and trapping of water, and grain and chaff characteristics that influence the rate of water uptake.

The presence or absence of awns is a common head characteristic which may influence the catching and trapping of water. King (1984) reported that a

collection of wheat cultivars, consisting of several isogenic awned/awnless cultivars, differed widely in their grain water uptake with awns accounting for some of these differences (King and Richards, 1984). The awned cultivars had a higher rate of water uptake and enhanced in-head sprouting. The increased rate of water uptake induced the initiation of germination 10 to 15 hours earlier in the awned lines. Despite this potential involvement of awns in increasing water uptake, Duffus (1987) showed that by physically removing the awns from wheat heads, there was no difference in the rates of head wetting between the awned and awnless heads. Thus, some other structural characteristic in awned cultivars may have accounted for the increased water uptake. Awns have also been implicated in other studies (Pool and Patterson, 1958; Clarke, 1982) as having an effect on grain drying rates within the field.

Pool and Patterson (1958) have suggested that waxiness may have an effect on grain water uptake. King (1984), however, showed that within the collection of evaluated cultivars, the presence of either hairs or wax contributed little to the differences in water uptake. The club head characteristic was another feature that enhanced water uptake. Head nodding angle also

influenced grain water uptake, and subsequently, the occurrence of sprouting damage (Brinkman and Luk, 1979). Other characteristics such as seed coat colour (Wellington, 1956) and grain hardness (Butcher and Stenvert, 1973; Moss, 1973) have also been implicated in affecting water uptake, however, King (1984) using isogenic cultivars for these characteristics, showed that these factors were not involved. Grain protein content and testa thickness were also shown to be not involved with grain water uptake.

2.1.2 Dormancy

Pre-harvest sprouting is defined as germination of the grains within the head prior to harvest. With this definition in mind, it is obvious that the one major factor that will influence the occurrence of pre-harvest sprouting is the dormancy of the cultivar. Dormancy is simply the inability of grains to germinate when exposed to favourable environmental conditons.

The dormancy of developing and maturing grains is important with respect to minimizing losses due to pre-harvest sprouting (Black et al., 1987). Dormancy can develop early during grain development and then may

disappear later during maturation (King, 1976). One of the major environmental factors influencing the level of dormancy during development and maturation is temperature (Sawhney and Naylor, 1979; Peters, 1982; Rauber, 1984; Black et al., 1987). Lower temperatures during grain development and maturation encouraged the production of more highly dormant grains as compared to higher temperatures.

Another aspect of the effect of temperature on dormancy is the termination of dormancy after grain maturation and drying. Mature, dry seeds lose their dormancy through a period of afterripening. As storage temperature increases, the loss of dormancy through afterripening was generally accelerated (Brown et al., 1948; Ching and Foote, 1961; Belderok, 1961; Derrie et al., 1979). If the seed was stored at low or sub-zero temperatures, the afterripening was either retarded or prevented. This aspect has been used to advantage in sprouting research. By storing dry (<13 percent moisture), mature seeds at -20° C, dormancy can be preserved within the grain samples (Noll and Czarnecki, 1979; Mares, 1983a). This has enabled researchers to considerably extend the time period in which material can be tested. Another aspect of temperature and its effects

on afterripening was that cooler temperatures during grain development and maturation generally caused an increase in the rate of dormancy loss during the afterripening period (Hagemann and Ciha, 1987).

If the grains have a water content above 25%, low temperatures had the reverse effect, terminating the dormancy of the grain samples (Black et al., 1987). This phenomenon primarily occurred on grains that were dried, reimbibed, and then chilled. Low temperatures have had little effect on terminating dormancy when immature, moist seeds that had not been dried were used.

Plant growth substances, especially abscisic acid (ABA), are thought to be involved in the development of dormancy in cereals. Walker-Simmons (1987) demonstrated in a study involving one sprouting-susceptible and one sprouting-resistant wheat cultivar that ABA levels during early development were initially higher in the sprouting-resistant cultivar but towards the end of seed development, ABA levels decreased in both cultivars. At maturity, there were no differences in ABA levels. Walker-Simmons further demonstrated that the embryos from the sprouting-resistant cultivar were more sensitive to ABA with respect to the inhibition of germination after

maturity. King (1976) also demonstrated a negative correlation of extractable ABA with germination in various cultivars. Higher ABA levels were correlated with lower germination levels. Slominski et al. (1979) further showed a similar correlation of ABA with germination at times after maturity. However, other studies by Radley (1979) and King et al. (1979) did not show this correlation and suggest that other factors besides ABA are involved.

2.1.3 Production of germinative enzymes

Following imbibition, enzyme activity may arise from either one of two sources (Cardwell, 1984). The first source of enzyme activity is from those enzymes that were synthesized prior to maturation. The only requirement for enzyme activity in this group is the need for activation. They may either be activated by simply being hydrated or through the action of plant growth substances and other enzymes. Adenylate cyclase is an example of an enzyme that simply require hydration for activity (Ching and Kronstad, 1972). ß-amylase is an example of an enzyme that is preformed but requires processing following imbibition (Tronier and Ory, 1970). A

gibberellic acid (GA) induced proteinase is thought to cleave ß-amylase protein releasing active ß-amylase.

The second source of enzymes arises from de novo synthesis (Cardwell, 1984) and can be subdivided into two groups. The first group is synthesized based on mRNA that is formed prior to maturation. Activity from these enzymes usually appears after 2-4 hours. The second group of enzymes is synthesized from newly synthesized mRNA. α-amylase is considered to be in this category.

After the onset of the first phases of germination, GA produced by the embryo, or possibly produced within the aleurone layer itself, stimulates the aleurone layer to synthesize and secrete numerous germinative enzymes. These enzymes include α- and ß-amylases, oxidases, and proteases. The prime function of these enzymes is to break down the endosperm reserves into simple molecules which can be mobilized and utilized by the developing embryo. The majority of these enzymes are derived primarily from the aleurone layer. Furthermore, several of these enzymes require GA stimulation. Of these enzymes, the potentially most devastating enzyme with respect to grain quality is α-amylase (Kruger, 1976). Consequently, α-amylase has been universally used as a

indicator for quality and the presence of pre-harvest sprouting damage.

2.1.3.1 Characteristics of α-amylase

α-amylase (alpha, 1->4 glucan 4-glucano hydrolase, E. C. 3.2.1.1.) is an endohydrolase that cleaves the α-1,4 glycosidic links within the glucose polymers, amylose, and amylopectin. The cereal α-amylases are monomeric enzymes with reported molecular weights ranging from 40,000 to 58,000 (Greenwood and Milne, 1968; MacGregor et al., 1974; Tkachuk and Kruger, 1974; Marchylo et al., 1976; Silvanovich and Hill, 1977; MacGregor, 1978). Based on isoelectric points, Olered and Jonsson (1970) showed the presence of two distinct groups of α-amylase isozymes. These two groups were designated as alpha-I and alpha-II. Furthermore, each isozyme group consists of a collection of isozymes with similar isoelectric points (Nishikawa and Nobuhara, 1971; MacGregor, 1976). In triticale, Silvanovich and Hill (1977) demonstrated a range of isoelectric points of 4.6 - 5 for the alpha-I isozyme group and isoelectric points near 6.2 for the alpha-II group. In barley, Jacobsen and Higgins (1982) demonstrated a range of 4.5 - 4.85 for the alpha-I group and a range of 5.9 - 6.3 for the alpha-II group.

Additional differences between these two isozyme groups have been characterized. The alpha-I isozymes can be secreted by the aleurone layer with no added Ca, are insensitive to EDTA, are stable at low pH's, are sensitive to sulphydryl reagents, and are inhibited by heavy metal ions (Jacobsen and Knox, 1973). The alpha-II isozyme group is not secreted by the aleurone layer without added Ca, is inhibited by EDTA, is unstable at low pH's is insensitive to sulphydryl reagents, and is not inhibited by heavy metal ions. Furthermore, the alpha-I isozymes tend to bind Ca relatively more tightly than the alpha-II isozymes (Jacobsen et al. 1970). The alpha-I isozymes were less heat stable than the alpha-II isozymes (Silvanovich and Hill, 1977; MacGregor, 1978). In wheat, the genes for the alpha-I isozymes are located on chromosome 7 while the genes for the alpha-II isozymes are located on chromosome 6 (Gale and Spencer, 1977). Only the alpha-I isozyme group was found to be present throughout cereal kernel development (Olered and Jonsson, 1970). During germination, both isozyme groups were found. However, the alpha-II isozymes were the predominant group.

2.1.3.2 Characteristics of the α-amylase inhibitor

Weselake et al. (1983a, 1983b) and Mundy et al. (1983) both identified an endogenous cereal protein that specifically inhibits the cereal alpha-II amylases (Weselake et al., 1983b). This α-amylase inhibitor has no effect on the alpha-I amylases. The -amylase inhibitor has a molecular weight of 20,000, an isoelectric point of 7.3, and a pH optimum for inhibitory activity of approximately 7. Halayko et al. (1986) have determined the inhibitor:enzyme binding ratio to be 2:1.

The α-amylase inhibitor protein has been found in barley, tetraploid and hexaploid wheat, rye, and hexaploid triticale (Weselake et al., 1985). In barley grains, the α-amylase inhibitor protein was distributed throughout the endosperm, the aleurone layer, and the embryo. The α-amylase inhibitor has not been detected in either sorghum, oat, millet, rice, or maize.

2.1.3.3 The role of plant growth substances on enzyme synthesis

The two plant growth substances that are primarily involved in enzyme synthesis are ABA and GA. In developing grains, the levels of both GA and ABA

increased as fresh weight of developing wheat grains increased (Radley, 1976; Mounla, 1978; Ranki and Sopanen, 1984). Peak levels of GA were attained prior to maximum dry weight while peak levels of ABA were attained after GA reached its peak values and prior to dehydration. As maturation progressed, both growth substances decreased in concentration possibly as a consequence of dehydration (King, 1979).

Duffus (1969) suggested that α-amylase present in the endosperm and aleurone of developing barley grains was produced in response to locally synthesized GA. When CCC was applied to these developing grains, there was a reduction in the levels of α-amylase. Even though locally synthesized GA was associated with production of α-amylase in developing seeds by Duffus (1969), and thus, may have some importance during seed development, Jacobsen and Chandler (1987) associated GA with only a minor or negligible role in the overall process of seed development. ABA was considered to be the predominant plant growth substance involved in seed development.

Triplett and Quatrano (1982) suggested a two fold function for ABA in developing grains, namely, the promotion of storage protein synthesis and the prevention

of premature germination. Ho and Varner (1976) demonstrated that ABA caused the synthesis of a unique pattern of uncharacterized proteins, and more specifically, Mundy (1984) demonstrated that ABA increased the synthesis of an ∝-amylase inhibitor. Quatrano et al. (1983) further identified a series of acid soluble proteins in wheat whose synthesis could be promoted by applying ABA. ABA has also been shown to reverse or inhibit the stimulation response of GA at both the transcriptional and translational levels (Baulcombe et al., 1984; Chandler et al., 1984). The effects of ABA on developing wheat grains, whether stimulatory or inhibitory, were primarily associated with responses at the transcriptional level (Quatrano, 1986; Williamson, 1985).

Based on this information, Quatrano (1987) suggested a hypothesis concerning the involvement of GA and protein synthesis during development. Two gene sets, one maturation and the other germination, might exist. After fertilization, both sets are open for expression, however, increasing levels of endogenous ABA levels promotes the expression of the maturation gene set and inhibits the expression of the germination gene set. During the latter stages of development, desiccation of

the seed becomes the preventive factor for germination. Desiccation, however, also terminates the maturation gene set permanently and only the germination gene set is left open for expression following hydration. Recent studies by Berge and Quatrano (unpublished) has shown that the terminating effects of dessication are not absolute. Some of the proteins expressed during maturation can be synthesized upon stimulation by ABA 120 hours after germination. These proteins have been suggested to have more of a general function by being required during vegetative growth as well as during embryogenesis.

During germination, the production of GA is initiated in the scutellum region with GA_1 being the predominant GA present (Radley, 1967; Gaskin et al., 1985). After 48 hours, the embryonic axis may become the predominant source of GA. Recently, new evidence using immunoassays (Atzorn and Weiler 1983) has suggested that synthesis of GA_4 in the aleurone layer can occur in response to some signal from the embryo. This synthesized GA_4 then stimulates α-amylase synthesis in the scutellum and aleurone layers. However, other researchers have not been able to confirm the presence of GA_4 in germinating barley caryopsis (Yamada, 1982; Gaskin et al., 1984) or

confirm the sensitivity of the aleurone layers to inhibitors of GA synthesis (Gilmour and MacMillan, 1984).

Evidence based on scanning electron microscopy (Gibbons, 1981; MacGregor and Matsuo, 1982) indicated that initial starch breakdown occurred adjacent to the scutellum. Ranki and Sopanen (1984), using excised scutellum and aleurone layers, further indicated that alpha-amylase synthesis by the scutellum peaked at 1 day after germination compared to 3-4 days for the aleurone layers. This evidence suggested that the scutellum is the initial predominant site of α-amylase synthesis, however, once the aleurone layer initiated α-amylase synthesis, the aleurone layer became the predominant source of α-amylase. Ranki and Sopanen (1984) calculated that the scutellum only accounted for 5 - 10 percent of the total α-amylase activity while the remainder of the activity was associated with the aleurone layer. Earlier studies by Palmer (1982) and MacGregor and Matsuo (1982) further indicated this relative importance of the aleurone layer over the scutellum with respect to the production of α-amylase. Thus, aleurone layers have been studied extensively for the effect of GA and ABA on the production of hydrolases and other proteins (Jacobsen and Chandler, 1987).

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2.2 Methods for Detecting Pre-Harvest Sprouting Resistance

2.2.1 Storage of experimental material

In sprouting research, it is desireable to preserve material in order to maintain its dormancy and sprouting resistance characteristics. This enables ample time for analysis especially in the case of large breeding programs. Grain samples can be preserved by deep freeze storage (Noll and Czarnecki, 1979; Mares, 1983a). In order to preserve the grain samples, they must be dried down to below 13 percent moisture and then placed in storage at -20° C.

2.2.2 Determination of water movement into the heads and grains

By determining the water movement into the heads and grains, an estimation of differences in water uptake between cultivars can be determined. This approach has been taken by several researchers including Mares (1983b) and King (1987). Water uptake can be either expressed as change in weight over an initial weight or as a percentage increase (King, 1987). The difficulty with the first expression is that cultivar differences in head

and grain weights are not taken into account. The latter expression is preferred as less emphasis is placed on these cultivar differences in head and grain weights (King and Richards, 1984).

2.2.3 Rain simulation tests

Rain simulators are currently the most common tool for assessing sprouting resistance in wheat cultivars and lines. McMaster and Derera (1976) were the first to describe and utilize the rain simulator, and since then, rain simulators have been widely adopted. By using rain simulators, all three components of sprouting resistance are rated as a whole. The definite advantage of rain simulation tests is that the head characteristics which can have a large influence on the ease of water penetration to the grain are accounted for in the resistance determinations.

The sample material used in rain simulation tests consists of the wheat head along with 10 cm or more length of stem (Czarnecki, 1987). Sample sizes are usually a minimum of 10 heads for visual sprouting assessment and 15-20 heads for falling number determinations. The samples within the rain simulator

are subjected to a pre-determined amount of water during an initial three hour duration and then maintained at 100% relative humidity and a temperature of 15-20° C for a pre-determined time. At the conclusion of the rain simulation test, the heads are assessed for visual sprouting and may be assessed for the falling number value and/or α-amylase activity.

With the widespread use of rain simulators, a few problems have arisen. Due to a lack of uniform testing conditons between researchers, problems with inconsistent results may occur. For example, the awned/awnless lines which showed different water uptakes when examined by King and Richards (1984) failed to show any differences (King, unpublished data) when examined in the rain simulator used by Mares (1983).

2.2.4 Germination tests

By using germination tests, the dormancy of the cultivars and the response of the aleurone layer to germination can be determined. Germination tests are similar to the rain simulation tests except that germination tests do not account for any potential

advantages or disadvantages provided by head characteristics.

2.2.4 Determination of -amylase activity

α-amylase is involved in the mobilization of starch reserves within the endosperm in germinating cereal kernels. Thus, much attention has been focused on utilizing levels of α-amylase activity as an indication of sprouting resistance. The definite advantage of basing sprouting resistance on α-amylase levels is that most quality losses due to sprouting are a result of excessive levels of α-amylase activity within the flour.

There are numerous methods for detecting α-amylase activity. A brief survey and description of the various detection methods is as follows:

a) Briggs' Assay - This technique was described by Briggs (1961). The Briggs' assay measures α-amylase activity by determining the rate of substrate degradation over a period of time. This assay utilizes the starch-iodine colur reaction. The substrate used can be either starch which is used to measure both α- and ß-amylase activities or ß-limit dextrin which is used to measure only α-amylase activity. Substrate quantities are

determined by measuring the colour intensity of the iodine complexed starch/ß-limit dextrin at a wavelength of 540 nm.

b) Fluorometric Method - An automated fluorometric assay was described by Marchylo and Kruger (1978). The fluorometric method uses ß-limit dextrin anthranilate as a substrate which is acted upon by α-amylase. The formation of dialyzable fluorescent products increases linearly with the level of α-amylase activity present.

c) Gel-Diffusion Technique - This technique was described by Hejgaard and Gibbons (1979). The gel-diffusion technique employs the use of starch-embedded agar plates on which seeds or half-seeds are placed on the surface of the agar. α-amylase activity is measured by staining the plates with iodine and measuring the size of the areas that are not stained darkly. The size of the clear areas are directly poroportional to the level of enzyme activity.

d) Hagberg Falling Number - This technique is described by the AACC (1976). This technique measures the rate of decrease in substrate viscosity. The falling number is defined as the time in seconds required to

allow the stirrer to fall a measured distance through a hot aqueous flour gel undergoing liquefaction. The falling number value and enzymatic activity are inversely related. Falling number is a more consistent characteristic of cultivars as compared to the direct α-amylase assays (McCrate et al., 1981). This is due to the falling number value being dependent on other grain characteristics including protein, amylose, and fiber content as well as α-amylase activity (Moss and Kirby, 1976; Moss, 1980). Despite the involvement of other grain characteristics in falling number, McCrate et al. (1981) obtained a high correlation between the Phadebas blue α-amylase method (section f) and the falling number methods.

e) Nephelometric Method - A nephelometric determination of α-amylase activity using a Perkin-Elmer Model 191 Grain Amylase Analyzer was described by Campbell (1980). This method directly measures α-amylase activity based on the rate of change of light scattered from the substrate suspension.

f) Phadebas Blue Method - This technique was described by Mathewson and Pomeranz (1977) in which Phadebas tablets are used as the dye-labeled starch

substrate. As amylase enzymatic activity increases, a blue colour is developed. This colour development is measured using a spectrophotometer at a wavelength of 620 nm.

α-amylase activity can be monitored to test for sprouting resistance throughout grain development, maturation, and germination. At maturity, non-weathered grain samples can be analyzed to determine the level of amylase within the whole seed. Head or seed samples could also be subjected to rain simulation or germination tests and α-amylase tests can be employed to monitor the production of α-amylase as a measure of potential sprouting damage. Henry and McLean (1987) stress this fact as a potential monitor when making their case for using α-amylase activities to determine sprouting losses.

2.2.6 Observation of endosperm modification

The use of endosperm modification observations has been suggested as a tool for detecting sprouting resistance by Jensen and Law (1983) and Jensen et al. (1984). Assessment of endosperm modification is accomplished by staining with fluorescein dibutyrate (Jensen and Heltved, 1982; Heltved et al., 1982),

counterstaining with Fast Green, and finally examining with a Carlsberg macrofluorescence microscope. The theory behind this technique is that fluorescein dibutyrate is sensitive to the presence of lipases or esterases that are present in those parts of the endosperm modified by sprouting. As the detection of fluorescein increases, this signifies the presence of a higher level of sprouting damage due to starch breakdown by amylase as well as the presence of increased protein and cell wall degradation due to the action of other hydrolytic enzymes besides amylase.

2.3 Interrelationships of the Resistance Detection Methods

DePauw and McCaig (1987) conducted an assessment of several techniques for detecting sprouting resistance and then determined any correlative interrelationships between the various methods. The parameters measured were determinations of α-amylase activity on non-weathered and rain stimulated grain samples, measurements of visual head sprouting, and individual grain germination.

There were no correlations between the α-amylase activity of the non-weathered grain samples and the α-

amylase activity of the rain simulated grain samples. The simulated rain treatment, therefore, prompted the genotypes to respond to the favourable germination conditions, and thus, to fully express their true genetic response to pre-harvest sprouting. This response to the favourable germination conditions was used to determine the differential sprouting responses of the individual genotypes.

In addition to the lack of correlation between α-amylase activity between non-weathered and rain simulated grain samples, DePauw and McCaig (1987) demonstrated other correlations between the various parameters measured. There was a significantly high correlation between the various measurements conducted on the rain simulation tests. Furthermore, the number of spikes with visible evidence of sprouting, percentage of kernels germinated, and α-amylase activity were all significantly related to each other.

In a similar study by Henry and McLean (1987), measurements based on visual sprouting, α-amylase activity and endosperm modification were taken and correlated to each other. All correlations were significant suggesting that as sprouting damage

increases, visual sprouting increases, α-amylase activity rises, and endosperm modification is greater. However, there were deviations from this correlation due to both environments and cultivars. Based on these observations, Henry and McLean (1987) concluded that the level of sprouting damage due to increased α-amylase levels cannot be reliably determined based on the level of visual sprouting and the degree of endosperm modification. Cultivar differences for α-amylase production will confound sprouting damage predictions based only on visual sprouting. Cultivar differences for production of the other hydrolytic enzymes will also confound damage predictions based only on measurements of endosperm modification.

Another relationship which could be used to obtain an indication of the importance of head characteristics in conferring resistance is the rate of head sprouting relative to the rate of germination of threshed grains. This association was discussed by Mares (1987). By comparing rates of head sprouting with rates of germination of threshed grains under identical conditions for a series of cultivars, there should be a linear relationship between the two measurements. Any significant deviation from this line should indicate the

presence of additional factors associated with head characteristics.

2.4 Pre-Harvest Sprouting Resistance in Wheat

2.4.1 Sources of resistance

During 1970 - 1975, Czarnecki (1987) evaluated a wide array of wheat lines from a diverse collection consisting of cultivars and lines from several countries. More recently, Czarnecki, in co-operation with Noll, further determined the falling number values from rain simulation treated samples. From these tests, RL4137 was established as a premier source for sprouting resistance. RL4137 possessed excellent dormancy, maintained low levels of α-amylase, and possessed a high falling number value. Few cultivars equalled the resistance of RL4137. Other cultivars or lines which also possessed good resistance included Chris, Frontana, Exchange, Sommerweizen 8793, Chinese Spring, Park, Columbus, Leader, Kenya 321, Kite, Spica, Suneca, and Sunelg (Czarnecki, 1987; Mares, 1987).

During 1983 - 1985, Mares (1987) further evaluated 3000 white-grained bread wheats from the Australian Winter Cereals Collection. After three years of

intensive evaluation, 14 distinct genotypes were rated for sprouting tolerance as equal to or better than Kite and Kenya 321. The best of these 14 lines had a sprouting tolerance similar to that of RL4137. These new sources of spouting tolerance were Aus 109 (Chile 59), Aus 320 (Hellas), Aus 471 (Lerma 52), Aus 633 (Mexican 852), Aus 758 (Pakistan C228), Aus 1293 (South African 1166), Aus 1305 (Spoetnik), Aus 1408 (Transvaal), Aus 1490 (Unknown), Aus 5820 (Kenya 59), Aus 7122 (Bihar 124), Aus 13054 (Egypt 1167-109), Aus 16115 (Saberbeg), and Aus 16116 (Homera).

Wild diploid wheats also possessed a level of dormancy (MacKey, 1975) which may be utilized in breeding programs.

Due to its excellent sprouting resistance, RL4137 has been used in several breeding programs (Czarnecki, 1987). The first and only licensed Canadian cultivar utilizing the sprouting resistance of RL4137 was Columbus. The sprouting resistance of RL4137 has also been incorporated into another potential new cultivar, namely, HY355 (DePauw, personel communication). In Europe, Frontana has also been used as a source for sprouting resistance (Olsson, 1975). Frontana is a

component of RL4137's pedigree (DePauw and McCaig, 1983). The dormancy characteristics from Frontana may have been transferred to RL4137 during attempts to transfer leaf rust resistance possessed by Frontana.

2.4.2 Inheritance of resistance

In earlier research, sprouting resistance and post-harvest dormancy have often been closely associated with seed coat colour in wheat with the red wheats being more resistant than the white wheats (Wellington and Durham, 1958; Gfeller and Svejda, 1960; Freed et al, 1976; Khan and Strand, 1977). However, since this earlier research, evidence has been compiled to dispute this generalization. Gordon (1983) determined that the relationship between seed coat colour and sprouting resistance in 97 genotypes of several Triticum species was only moderate. Clarke et al. (1984) further compared 14 cultivars based on falling numbers and concluded that a range of genotypic expression of sprouting resistance existed within both red and white wheats. Furthermore, numerous examples of resistant white wheats have been discovered or developed over the past years (DePauw et al, 1985; Mares, 1987).

In wheat, there are 3 gene loci involved with red seed coat colour. It was reported that the most resistant cultivars possessed all 3 red genes and that these genes were acting in an additive manner. This association could be due to a tight link between the genes controlling sprouting resistance and the genes for red seed coat colour. At present, it is not known if the genes for sprouting resistance and dormancy and the genes for seed coat colour are either pleiotrophic or linked (Czarnecki, 1987). There may be a link between sprouting resistance and a chemical precursor of the seed coat pigment (Gordon, 1978).

Baker (1981) reported that the cultivars RL4137, Park, and Neepawa all possessed 3 genes for seed coat colour, however, Czarnecki (1987) has shown that these three cultivars had different levels of sprouting resistance. These differences could be either due to allelic differences at the seed coat colour gene loci or due to mechanisms that are not associated at all with seed coat colour.

DePauw and McCaig (1983) reported that RL4137 had two or more genes controlling seed dormancy and sprouting resistance. One gene was associated with seed coat

colour and one or more genes were not associated with seed coat colour. This conclusion was also reported earlier by Reitan (1980). Reitan analyzed an 8 X 8 diallele cross for seed coat colour and detected both a mechanism that was associatied with seed coat colour and a mechanism that was not. Dyck et al. (Czarnecki, 1987) conducted an inheritance study for sprouting resistance using a RL4137/Neepawa cross. The heritability estimates were 0.78 suggesting that there was a high inheritance of sprouting resistance expressed in the progeny. Further analysis indicated that there was at least a two gene difference independent of the red seed coat colour. Thus, the seed coat colour genes are not the only factors determining sprouting resistance.

The inheritance of simple qualitative traits such as awns, head erectness, and other characteristics may also improve sprouting resistance. King (1984) has reported that cultivars differed widely in water uptake by the grain. The presence or absence of awns accounted for some of the differences in grain water uptake rate (King and Richards, 1984). These simple, qualitative traits usually have a heritability value.

3. METHODS AND MATERIALS

3.1 Biological Material

3.1.1 Description of wheat cultivars

In these studies, a series of wheat (Triticum aestivum) genotypes including licensed Canadian cultivars, breeding lines, and isolines were used. These genotypes were:

1) Columbus: Columbus is a licensed Canadian 3 gene red spring wheat. Columbus usually has a high falling number value and a long afterripening period. The pedigree of Columbus is Neepawa*6/RL4137.

2) Cypress: Cypress is a delicensed sawfly-resistant Canadian hard 3 gene red spring wheat. Cypress tends to have a low falling number value.

3) Era: Era is an American red spring wheat.

4) Era, white: This line is a white isoline of Era derived from CT 932. The pedigree is Era*4/CT 932. CT 932 is a sprouting susceptible white wheat. This line

was developed by E. Czarnecki (Agriculture Canada Research Station, Winnipeg).

5) Fielder: Fielder is a licensed Canadian soft white spring wheat.

6) Garnet: Garnet is a 3 gene red spring wheat. Garnet has a short afterripening period and is considered to be a susceptible red wheat.

7) Glenlea: Glenlea is a registered Canadian 2 gene red utility spring wheat. The pedigree for Glenlea is UM530/CB100.

8) Glenlea, white: This line is a white isoline of Glenlea dervied from CT 932. The pedigree is Glenlea*6/CT 932. This line was developed by E. Czarnecki.

9) Neepawa: Neepawa is a licensed Canadian hard 3 gene red spring wheat. Neepawa has a falling number value intermediate to RL4137 and Cypress. Neepawa tends to have a fairly short but deep or pronounced afterripening period.

10) Norquay: Norquay is a delicensed Canadian soft white spring wheat.

11) Pitic: Pitic is a Mexican high yielding 1 gene red spring wheat. Pitic is considered to be sprouting susceptible.

12) RL4137: RL4137 is a hard 3 gene red spring wheat breeding line. RL4137 has a high falling number value and a deep, long afterripening period.

13) RL4137, white: This line is a white isoline of RL4137 derived from Thatcher/Poso 49. The pedigree is RL4137*4//Thatcher/Poso 49. This line was developed by D. Zuzens (University of Manitoba) and maintained by E. Czarnecki.

14) RL4555: RL4555 is a white spring wheat breeding line. The pedigree for this breeding line is Kenya Farmer*2/Kenya 321.BT.1.B.1.

15) Sonora 64: Sonora 64 is a Mexican 1 gene red spring wheat. Sonora 64 is considered to be a sprouting susceptible wheat. The pedigree for Sonora 64 is YT54/N10B//2*Y54.

16) Sonora 64A: This is a 1 gene red isoline of Sonora 64.

17) Takahe: Takahe is a 1 gene red spring wheat. Takahe has a high falling number value and a deep long afterripening period. Takahe has a moderate vernalization requirement.

3.1.2 Source of seed samples

In the preliminary studies, non-dormant bulk seed samples of Columbus, Neepawa, Fielder, and Norquay were used. These samples were grown either in Manitoba or in Saskatchewan.

In the main wheat line characterization studies, groups of selected wheat lines were grown at 3 locations during 1987. The first two locations, W1 and W2, were located at the University of Manitoba, Winnipeg, Manitoba. The wheat lines at the W1 site included Columbus, Neepawa, Fielder, Norquay, RL4555, Era, Era (white), Glenlea, and Glenlea (white). These lines were selected based on either their importance in Western Canadian wheat production or their unique sprouting and/or kernel colour characteristics. The wheat lines at

the W2 site included Cypress, Fielder, Garnet, Neepawa, Pitic, RL4137, Sonora 64, Sonora 64A, and Takahe. These lines were selected based on their number of genes for red kernel colour. The growing conditions for both these sites could be described as ideal for crop growth. There was both adequate moisture and desireable temperatures. Crop growth was excellent. The cultivars at the W1 site was exposed to relatively longer period of weathering as compared to the W2 site. The third location, L1, was located at Lampman, Saskatchewan. The wheat lines at the L1 site included Columbus, Neepawa, Fielder, Norquay, RL4555, Era, Era (white), Glenlea, Glenlea (white), RL4137, and RL4137 (white). These lines were selected based on the selection criteria for the W1 site. The growing conditions at the L1 site could be described as devastating for crop growth. Initially, there was considerable heat stress for approximately 3 weeks after 1 week of seedling emergence, followed by drought stress for the remaining of the growing season. Poor soil structure further enhanced the effects of the heat and drought stress. Crop growth ranged from poor to non-existant.

At both the W1 and W2 sites, there was a moderate to heavy incidence of foliar diseases. Furthermore, at the

W1 site, RL4555 had severe lodging problems and was subjected to relatively more weathering as compared to the other lines. Takahe, at the W2 site, requires a slight vernalization treatment for proper seed set. Early spring growing conditions were not adequate enough to supply this requirement and, as a consequence, there was poor seed set. Due to this poor seed set, Takahe had to be excluded from the majority of the characterization studies. At the L1 site, RL4555 was completely devastated during the heat and drought stress periods. The red and white Era isolines were also affected by these stresses. As a consequence, there was minimal seed set. These three lines were not included in the majority of the characterization studies. The remaining lines, although they all had some seed set, were only able to be included in a portion of the characterization studies.

3.1.3 Storage of the wheat samples

All wheat lines were harvested at maturity. These wheat samples were dried down to <13% moisture and stored at room temperature during the afterripening period. At specific afterripening intervals of 0, 45, and 90 days, sub-samples were taken and these sub-samples were stored at -20° C. Noll and Czarnecki (1979) and Mares (1983)

have demonstrated that storage at -20° C preserved the dormancy characteristics of the grain samples. This preservation allowed a dramatic increase in the period during which the samples can be evaluated for their sprouting characteristics.

3.2 Protein Extraction

Throughout the study, the main extraction buffer was 20 mM (N-tris{Hydroxymethyl)methylamino}-2-hydroxypropanesulfonic acid, 0.5 M NaCl, pH 8.0 (TES) buffer. Extraction with this buffer primarily removes the globulin proteins (Osborne, 1907) which includes both α-amylase and α-amylase inhibitor. The globulin fractions constitute approximately 5% of the total wheat proteins (Chen and Bushuk, 1970). These proteins are located primarily in the embryo and aleurone layer of the wheat kernel (Payne et al., 1982). In addition to the TES buffer, a sodium acetate buffer (200 mM, 1 mM $CaCl_2$, pH 5.5) was used for the α-amylase inhibitor studies involving the mature, dry seeds.

All samples were either milled or macerated with a polytron prior to extraction. Throughout the extraction process, the samples were kept either on ice or in the

cold (0-4° C). For the α-amylase activity measurements of germinated and non-germinated seeds utilizing the TES buffer, the samples were extracted with 10 mL buffer. A 30 minute extraction time was used. For the α-amylase inhibitor extractions utilizing the sodium acetate buffer, the samples were extracted at a ratio of 1 g sample/10 mL buffer. A 48 hour extraction time was used. At the end of the extraction period, the samples were centrifuged at 13000 x g for 20 minutes. The supernatant was then removed and stored at -20° C immediately.

3.3 Measurement of Soluble Protein

Protein measurements were conducted based on the Biorad protein assay utilizing 96-well microtitre plates. Ten uL of samples were added to 200 uL of diluted (1:4) dye reagent for each well. The protein standard was α-globulin. The absorbance at 590 nm was measured with an ELISA plate reader 5-60 minutes, the period of color stability, after the addition of the reagent.

3.4 Determination of α-Amylase Activity

A modified procedure based on the method of Briggs (1961) was used. ß-limit dextrin at a concentration of

0.05% was used as the substrate. The use of β-limit dextrin prevents the expression of α-amylase activity. Thus, only α-amylase activity is measured. Furthermore, the assay was performed using only half the volume of the various solutions involved. The buffer solution used was 200 mM sodium acetate (pH 5.5, 1 mM $CaCl_2$). The assay was conducted at 35° C. Absorbances were measured at 540 nm using either a Zeiss PM QII spectrophotometer or a Hewlett-Packard 8452A diode array spectrophotometer. α-amylase activity, based on the Briggs' assay is expressed in IDC units with 1 IDC unit being defined as the amount of enzyme required to change the absorbance of an iodine complexed-β-limit dextrin solution from 0.6 to 0.4 in 100 minutes.

3.5 Measurement of α-amylase Inhibitor Content

α-amylase inhibitor contents were determined with an ELISA protocol utilizing the biotin-streptavidin system. This procedure was developed by D. Audette (University of Manitoba). The buffers used were carbonate buffer (0.2 M, pH 9.6), phosphate buffered saline (PBS) (0.05 M, pH 7.2), PBS-Tween (0.05% Tween), and o-phenylene diamine (4 mM in 0.004% v/v hydrogen peroxide and citric acid (0.02 M) sodium phosphate (0.05 M) buffer, pH 5.0). The

procedure is illustrated in Figure 1. An affinity-purified anti-rabbit Ig α-amylase inhibitor antibody was used for this assay.

3.6 Seed Surface Sterilization Procedure

Due to disease infections in the field, all the samples from the W1, W2, and L1 sites had to be surface sterilized prior to use in the germinability or the quarter-seed studies. The samples were soaked in diluted Javex (0.1% available chlorine) for 30 min. (Goudy et al., 1987), rinsed 4X in ddH_2O, soaked in 0.1 N HCl for 10 min, and finally rinsed 4X in ddH_2O.

3.7 Characterization of the Mature, Dry Seed

Seed samples from the 0 days afterripening samples for the W1, W2, and L1 sites were used. Only the samples from the W1 and W2 sites were used for the α-amylase activity assays while the samples from all 3 sites were used for the α-amylase inhibitor assays. For the α-amylase activity and α-amylase inhibitor assays, three replicates of 100 seeds were grounded in a Wiley mill utilizing a #20 sieve screen. The α-amylase activity samples were extracted with TES buffer. The α-amylase

Figure 1. Outline of the α-amylase inhibitor ELISA protocol.

α-AMYLASE INHIBITOR ELISA PROTOCOL

ANTIGEN COATING
(500-1000 ng protein/100 uL carbonate buffer, overnight @ 20°C)

↓

Wash 6X with PBS-Tween (200 uL/well)

↓

BLOCK NON-SPECIFIC SITES
(1% BSA in PBS, 100 uL/well, 30 min. @ 37° C)

↓

PRIMARY ANTIBODY INCUBATION
(1:500 in PBS (0.1% BSA), 100 uL/well, overnight @ 20°C)

↓

Wash 3X with PBS-Tween (200 uL/well)

↓

BIOTINYLATED SECOND ANTIBODY INCUBATION
(1:750 in PBS (0.1% BSA), 100 uL/well, 2-3 hours @ 37° C)

↓

Wash 3X with PBS-Tween (200 uL/well)

↓

STREPTAVIDIN-BIOTINYLATED HORSERADISH PEROXIDASE COMPLEX INCUBATION
(1:750 in PBS (0.1% BSA), 100 uL/well, 30 min. @ 37° C)

↓

Wash 2X PBS, Wash 2X ddH_2O (200 uL/well)

↓

SUBSTRATE INCUBATION
(4 mM o-Phenylene diamine, 150 uL/well, 30-90 min.)

↓

MEASURE ABSORBANCE (495 nm)

inhibitor samples were extracted with sodium acetate buffer. Selected seed samples were further characterized by determining the inhibitor contents of embryo-rich and endosperm-rich samples. 0.5-1.5 g samples of isolated embryos with some adhering endosperm and embryoless half-seeds were macerated in 10 mL of sodium acetate buffer for 1.5 min.

3.8 Germination Studies

Germination studies were conducted on all seed samples. Seeds were placed in a 9 cm diameter petri dish that was lined with a double layer of #3 qualitative filter paper. Treatment solution was added to these plates. The seeds were then placed in the dark and exposed to light only during measurements. Germination was measured at periodic intervals for 3 days.

A germination index was calculated based on the formula used by Kendrick and Frankland (1969). The formula is as follows:

$$\text{Germination Index (GI)} = 1/T \times P$$

where P = % germination at the final time interval and T = time at which 1/2 P occurs.

This formula accounts for both the amount and the rate of germination.

In the germination studies, three replicates of 50 seeds were plated followed by the addition of 8 mL of a 30 uM chloramphenicol solution. A seed was considered to be germinated when the radicle was approximately 2-3 mm long. Once a seed was considered germinated, it was removed from the dish only if it showed evidence of fungal contamination. Germination was allowed to continue for 3 days after which the experiment was terminated. The time was expressed in days with respect to use in the germination index calculations. From each plate, 15 seeds were selected. A representative sample was selected depending on the proportion of germinated:non-germinated seeds. A protein extract using TES buffer was conducted. This extract was prepared by grinding the seeds in a polytron at a speed setting of 3.4 for 1.5 min.. The sample extracts from all the germination experiments were analyzed for -amylase activity.

3.9 Characterization of Aleurone Layer Responsiveness

3.9.1 Half-seed experimental system

This system was primarily used in the preliminary studies. The procedure is a modified version of the procedure described by Cornford et al. (1986). The seed samples are cut in half transversely and the embryoless half is retained. The seeds were surface sterilized. Twenty half-seeds were placed in each 25 mL Erlenmeyer flask, after which 3 mL of solution was added. The solution consisted of 2 mM sodium acetate buffer (pH 5.5), 20 mM $CaCl_2$, 30 uM chloramphenicol, and the appropriate treatment. The flasks were then incubated for 3 days in the dark at room temperature on a shaking water bath oscillating at 120 cycles/minute. After incubation, the media and half-seeds were extracted with 8 mL of TES buffer using a polytron at a speed setting of 3.4 for 1.5 minutes. The extract samples were then analyzed for α-amylase activity.

3.9.2 Quarter-seed experimental system

This procedure was primarily used for the final characterization studies involving the cultivars of the

W1 site at 0 and 90 days afterripening. This procedure is a modified version of the procedure described by Chrispeels and Varner (1967). The seed samples were bissected transversely and the embryoless half was retained and further bissected logitudinally into quarter-seeds. These quarter-seeds were surface sterilized. The surface sterilization steps and all subsequent steps were conducted using sterile techniques. The quarter-seeds were then imbibed on a double layer of #3 qualitative filter paper within a 9 cm petri dish for 3 days. Four mL of 30 uM chloramphenicol was added to the petri dish. During this imbibition step, any quarter-seeds that displayed signs of fungal contamination were removed. At the end of the imbibition step, samples of 30 quarter-seeds were transferred to 25 mL silinized erlenmeyer flasks. All flasks were covered with aluminum foil. Two mL of the appropriate treatment solution was added. The treatment solutions contained 10 mM $CaCl_2$, 30 uM chloramphenicol, and the appropriate treatment. The 3 main treatments were the control, 10 uM GA_3, and 50 uM ABA. The flasks were then incubated for 24 hours in the dark at room temperature on a shaking water bath oscillating at 120 cycles/minute. At the end of the incubation step, the media and quarter-seeds were placed in a tube. The flask was then rinsed 3X with 3 mL

of TES buffer with each rinse being added to the media and quarter-seeds. The samples were macerated using a polytron at a speed setting of 3.4 for 1.5 minutes. The extract samples were analyzed for -amylase activity.

3.10 Statistical Analysis

All statistical analysis was performed using SAS v.5.16. All data was analyzed by using analysis of variance. The LSD procedure using a significance level of 0.05 was utilized for the comparisons of treatment means. Transformations were performed on the following data prior to statistical analysis:

i) α-amylase activity - log transformation

ii) percet germination at time P - arcsine transformation.

Throughout this thesis, LSD values for the these two parameters will be from the analysis of the transformed data. The actual data that will be presented along with the LSD values will be the untransformed data.

4. RESULTS AND DISCUSSION

4.1 Protein Composition of the Wheat Lines

4.1.1 α-amylase activity in the mature seeds

The α-amylase activity of the mature, whole, dry seed was used as an indicator of sprouting damage at maturity. The level of α-amylase activity will have a positive correlation with the level of sprouting damage primarily in the form of reduced flour quality.

Bingham and Whitmore (1966) and Derera et al. (1977) reported significant cultivar differences in α-amylase activity within samples of mature, whole, dry seeds. A similiar situation was seen at the W1 site. At this site, the white wheats tended to have a higher level of α-amylase activity as compared to the red wheats (Table 1). Within the first group of five cultivars, both Columbus and Neepawa, the two red wheats, had a lower level of α-amylase activity as compared to the three white wheats, Fielder, Norquay, and RL4555. Columbus and Neepawa were both significantly different from Fielder and Norquay but not RL4555. There was no significant difference between Fielder and Norquay.

TABLE 1. α-amylase activity of whole, mature, dry seed samples from the wheat lines at the W1 site.

Cultivar	α-Amylase Activity[1] (IDC Units/Seed)
Columbus	40.2 de
Neepawa	38.8 e
Fielder	1839.9 ab
Norquay	2580.2 ab
RL4555	166.9 cd
Era	1133.9 bc
Era, white	3844.1 a
Glenlea	44.4 d
Glenlea, white	132.2 d

[1]Treatments with the same grouping letter are not significantly different ($\alpha = 0.05$).

One of the problems that may occur when comparing the first group of five cultivars is that two hard red spring wheats are being compared with two soft white spring wheats and one other white spring wheat. The genetic backgrounds of these wheats are definitely different from each other. Differences other than seed coat colour may exert some influence on the pre-harvest spouting characterisitics of the various cultivars. Thus, it is difficult to derive legitimate conclusions based on seed coat colour differences alone. Some of these problems may be overcome by comparing the red and white isolines of either Era or Glenlea, In both pairs of isolines, the white isoline had the higher level of α-amylase activity (Table 1). In the case of Era, there was a significant difference between the red and white isoline. However, in the case of Glenlea, there was no significant difference. This data, along with the data for the first group of five cultivars, suggests that seed coat colour does have some role in conferring pre-harvest sprouting resistance with respect to the production of α-amylase prior to harvest.

At the W2 site, there was no indication of a relationship between seed coat colour and the level of α-amylase activity (Table 2). There were no significant

TABLE 2. α-amylase activity of whole, mature, dry seed samples from the wheat lines at the W2 site.

Cultivar	α-Amylase Activity[1] (IDC Units/Seed)
RL4137	33.7 a
Neepawa	171.4 a
Garnet	163.1 a
Cypress	169.8 a
Pitic	86.9 a
Sonora 64	21.3 a
Sonora 64A	83.8 a
Fielder	94.0 a

1Treatments with the same grouping letter are not significantly different ($\alpha = 0.05$).

differences among this group of 8 cultivars. This could either indicate that seed coat colour had no influencing effect on the level of sprouting damage or that the environmental conditions did not induce appreciable sprouting damage. From what was evident in the data from the W1 site (Table 1), the latter of the two options would have to be assumed, however, further testing, especially under environmental conditions favourable for sprouting such as additional field weathering after physiological maturity, would verify whether or not seed coat colour influences the level of α-amylase within this group of cultivars. Another difficulty that also arises from this group of cultivars is the diverse genetic background of these cultivars similar to that encountered in the W1 site cultivars. It would be more desireable to select a group of cultivars with differing levels of red seed coat colour that have at least similar breeding pedigrees.

The cultivars from the L1 site were not evaluated for this parameter primarily due to the lack of an adequate seed supply. Preliminary studies indicated that the use of small sample sizes (25 seeds) was undesireable due to the variability within the seed sample (Table 3). A sample size of at least 100 seeds was satisfactory.

TABLE 3. Comparisons of Coefficients of Variations between sample sizes of 25 and 100 seeds for the α-amylase activity data for the whole, mature, dry seed samples from the W1 site.

Parameter	Coefficients of Variation	
	25 seeds	100 seeds
α-Amylase Activity	243.05	59.15
α-Amylase Activity (Transformed Data)	91.42	20.38

4.1.2 α-amylase inhibitor content

The levels of the α-amylase inhibitor within the mature, whole, dry, seeds for the cultivars from the W1 and W2 sites and for some of the cultivars from the L1 site are presented in Tables 4, 5,and 6 respectively. There were few significant differences between the wheat lines at all 3 sites. At the W1 site (Table 4), Columbus did have a significantly higher level of α-amylase inhibitor. Besides this observation, there were no significant differences among the other 8 cultivars. In addition, there was no apparent relationship between either seed coat colour or, more importantly, α-amylase activity and the α-amylase inhibitor content. At the W2 site, a similar situation exists. Both Neepawa and RL4137, a cultivar similar to Columbus for sprouting resistance, had a high level of α-amylase inhibitor, however, there were few significant differences (Table 5). There did appear to be a weak relationship between the number of genes for red seed coat colour and α-amylase inhibitor content, however, this relationship was not significant. At the L1 site, there were no significant differences between any of the cultivars (Table 6).

In addition to determining the inhibitor contents of

TABLE 4. α-amylase inhibitor content of the whole, mature, dry seed samples from the wheat lines at the W1 site.

Cultivar	α-Amylase Inhibitor Content[1] (ug/Seed)
Columbus	449.4 a
Neepawa	137.4 bcd
Fielder	104.5 d
Norquay	227.0 bc
RL4555	170.0 bcd
Era	139.2 bcd
Era, white	192.2 bcd
Glenlea	174.4 bcd
Glenlea, white	236.0 b

1Treatments with the same grouping letter are not significantly different ($\alpha = 0.05$).

TABLE 5. α-amylase inhibitor content of the whole, mature, dry seed samples from the wheat lines at the W2 site.

Cultivar	α-Amylase Inhibitor Content[1] (ug/Seed)
Cypress	130.4 ab
Fielder	81.4 b
Garnet	144.0 ab
Neepawa	225.7 a
Pitic	88.8 b
RL4137	145.0 ab
Sonora 64	103.2 b
Sonora 64A	155.6 ab

1Treatments with the same grouping letter are not significantly different ($\alpha = 0.05$).

TABLE 6. α-amylase inhibitor content of the whole, mature, dry seed samples from the wheat lines at the L1 site.

Cultivar	α-Amylase Inhibitor Content[1] (ug/Seed)
Columbus	154.2 a
Neepawa	170.4 a
Fielder	178.2 a
Glenlea	126.9 a
Glenlea, white	113.0 a
RL4137	103.4 a
RL4137, white	123.6 a

1Treatments with the same grouping letter are not significantly different ($\alpha = 0.05$).

the whole seeds, the distribution of this inhibitor between the embryo and the endosperm was determined. In Table 7, it is evident that the embryo has a significantly higher concentration of the α-amylase inhibitor as compared to the endosperm. These results are the opposite to what Hill et al. (1987) has reported for Conquest barley. This relationship remained constant under both the Winnipeg and Lampman growing conditions. Despite this constant relationship between the two sites, there was no evidence of a consistent relationship in distribution of the α-amylase inhibitor between the embryo and endosperm between the relatively dormant (L1 site cultivars) and non-dormant (W1 & W2 site cultivars). As indicated by the ratio of embryo:endosperm inhibitor content (Table 7), there was no preferential accumulation of the inhibitor in either the embryo or endosperm for the two different sites. This lack of trend could either suggest that the relative distribution of the inhibitor between the embryo and endosperm is not important with respect to dormancy and germinability or that the severe growing conditions at the L1 site may have distorted the actual trend for this particular set of data and possibly for the data obtained from the other measured parameters.

TABLE 7. α-amylase inhibitor content of embryo-rich and endosperm-rich samples from selected wheat lines from the W1, W2, and L1 sites.

Cultivar	α-Amylase Inhibitor Content[1] (ug/gm) Embryo	Endosperm	Ratio Emb.:Endo.
Columbus, W1	1336.8 a	537.1 cdef	2.5:1
Neepawa, W1	992.1 abc	373.9 edf	2.7:1
Fielder, W1	730.7 bcd	173.5 f	4.2:1
RL4137, W2	722.4 bcd	275.6 ef	2.6:1
Columbus, L1	659.1 cde	380.1 ef	1.7:1
Neepawa, L1	1176.1 ab	259.0 ef	4.5:1
Fielder, L1	445.2 def	269.3 ef	1.7:1
RL4137, L1	717.8 bcd	195.5 ef	3.7:1

1Treatments with the same grouping letter are not significantly different (α = 0.05).

4.2 Wheat line Characterization Using Germination Studies

4.2.1 Determination of germinability

The wheat lines were not exposed to conditions favourable for precocious germination. Only the Norquay and Fielder samples from the W1 site had any visible indications of sprouting. There was only a slight trace of sprouting (<0.1 percent) in both samples. The justification of using the germinability of the seed samples especially the seed samples with 0 days of afterripening is that the potential for precocious germination is being measured. Hagemann and Ciha (1984) stated that in general, germination tests are suited for predicting sprouting susceptibility of the wheat grains. It should be pointed out that only the ability of the embryo to germinate is being measured and that any characteristics associated with the head or chaff, whether favourable or undesireable, would not be taken into consideration.

From the W1 site with 0 days of afterripening, the red wheats tended to have a lower germinability as compared to the white wheats (Table 8), however, RL4555 was an exception to this general trend. Columbus had a

TABLE 8. Germinability of the wheat lines at the W1 site over an afterripening period of 90 days.

Cultivar	Germination Index[1]		
	0 day	45 day	90 day
Columbus	30.3 d	60.0 d	112.6 d
Neepawa	53.3 c	89.8 c	107.9 d
Fielder	56.4 c	93.3 c	135.3 bc
Norquay	86.5 a	131.1 a	153.2 a
RL4555	23.2 d	36.7 e	79.0 e
Era	53.7 c	90.5 c	110.2 d
Era, white	70.9 b	89.1 c	123.5 cd
Glenlea	69.7 b	99.1 bc	130.2 bc
Glenlea, white	86.4 a	110.0 b	144.4 ab

1Treatments with the same grouping letter are not significantly different ($\alpha = 0.05$).

significantly different from Norquay. RL4555 had the lowest germinability of the cultivars at the W1 site. At the L1 site, there was a similar trend (Table 9). Both Columbus and Neepawa had significantly lower germinabilities than Fielder and Norquay. Furthermore, Columbus had a significantly lower germinability than Neepawa. Overall, the cultivars at the L1 site had a lower level of germinability than the cultivars at the W1 site.

A more detailed comparison between seed coat colour and germinability can be made using the data from the red and white isolines of Era, Glenlea, and RL4137. From the W1 site, both white isolines of Era and Glenlea had a significantly higher germinability at harvest and throughout most of the afterripening period as compared to the red isolines (Table 8). At the L1 site, the white lines of both Glenlea and RL4137 had a higher germinability, however, the differences were not significant at harvest but were significant for the Glenlea isoline pair after the 90 day afterripening treatment (Table 9).

At the W2 site, there was little relationship between seed coat colour and germinability (Table 10). There was a large variability just within the four cultivars

TABLE 9. Germinability of the wheat lines at the L1 site over an afterripening period of 90 days.

Cultivar	Germination Index[1]	
	0 day	90 day
Columbus	7.24 cd	84.98 cde
Neepawa	34.25 b	89.89 cd
Fielder	52.56 a	115.26 b
Norquay	67.53 a	131.35 a
Glenelea	17.79 bc	94.42 c
Glenlea, white	22.29 bc	125.99 ab
RL4137	0.00 d	70.75 e
RL4137, white	7.63 cd	78.59 de

1Treatments with the same grouping letter are not significantly different ($\alpha = 0.05$).

TABLE 10. Germinability of the wheat lines at the W2 site over an afterripening period of 90 days.

Cultivar	Germination Index[1]		
	0 days	45 days	90 days
Cypress	21.2 d	76.3 c	101.3 de
Fielder	45.6 c	69.6 c	104.9 de
Garnet	61.4 b	102.0 b	131.1 bc
Neepawa	47.0 c	77.9 c	108.3 cd
Pitic	22.1 d	53.6 d	84.4 e
RL4137	14.0 d	47.6 d	86.8 de
Sonora 64	77.4 a	119.9 a	146.9 ab
Sonora 64A	68.9 b	109.5 ab	159.6 a

1Treatments with the same grouping letter are not significantly different ($\alpha = 0.05$).

that had 3 genes for red seed coat colour. Of these cultivars, RL4137 and Cypress had a low germinability, Neepawa had an intermediate germinability, and Garnet had a high germinability. There was also a large difference in germinabilities between Pitic and Sonora 64 & 64A all of which have 1 gene for red seed coat colour. Pitic had a low germinability while Sonora 64 & 64A had a high germinability. Fielder, a white wheat cultivar, had a germinability similar to that of Neepawa. Thus, based on the data from the W2 site, there is no clear-cut relationship between seed coat colour and germinability.

The germinability data especially the data from the red and white isoline pairs suggests that seed coat colour may have an influencing factor on germinability. There may, however, be other factors involved that are not connected to seed coat colour. DePauw and McCaig (1983) suggested there were both seed coat colour related and non-related mechanisms involved in pre-harvest sprouting resistance. RL4137 was specifically mentioned as a cultivar that possessed pre-harvest sprouting resistance that was not associated with seed coat colour. In Tables 8, 9, and 10, there is evidence for the presence of both of these mechanisms. The data from the isoline pairs from the W1 and L1 sites suggests that seed coat colour may

indeed have an influence on germinability. On the other hand, cultivars with an RL4137 backround, namely the red and white isolines of RL4137 and Columbus, or other cultivars such as RL4555 and Pitic all display a relatively high level of dormancy (or a low germinability). Other factors that could also be responsible for this low germinability will be discussed later. These cultivars, especially RL4555 and the RL4137 cultivars are prime candidates for possessing pre-harvest sprouting mechanisms that are not associated with seed coat colour. At the W2 site, the lack of any possible relationships between seed coat colour and germinability are probably due to the different genetic backgrounds that are present within this group of international wheat lines. Non-seed coat colour factors may also be overiding any influences that seed coat colour may have on the germinability of the wheat lines. Once again, it is evident that it would be more desireable to work with a group of cultivars differing in seed coat colour and the number of seed coat colour genes, but at the same time also possessing some common genetic backgrounds.

A study of the two components of the germination index, namely the final percent germination (P) and the time at which half the final percent germination occurred

(1/t), for all three sites was made. For the cultivars from the W1 site, at maturity, dormancy as inferred from the 100 - P value was primarily important only for Columbus and RL4555 (Table 11). The component that accounted for most of the differences in germinability was the rate of germination as indicated by the 1/t values. At the L1 and W2 sites (Tables 12 and 13), both dormancy and the rate of germination accounted for the differences in germinability within each group of cultivars. The cultivars at the W1 site after being exposed to some weathering may have lost some of their dormancy so that dormancy was no longer a prime consideration in germinability for most of the cultivars.

Two questions that may arise from the P data as an estimate of the dormancy present are, firstly, "Is poor seed vigour a factor especially in the seed samples with a low final percent germination?" and, secondly, "Is 3 days adequate enough to observe germination of all germinable seeds?". If a particular seed lot has a low percent germination due to low seed vigour, one would expect to see a low percent germination relative to the other cultivars throughout the afterripening period. If an initial low percent germination is truly due to dormancy, the percent germination should increase throughout

TABLE 11. Components of germinability for the wheat lines at the W1 site.

	P[1]			1/t[1]		
Cultivar	0 day	45 day	90 day	0 day	45 day	90 day
Columbus	51.3 d	86.7 d	97.3 a	0.59 d	0.69 d	1.16 de
Neepawa	85.3 bc	94.7 ab	92.7 a	0.62 cd	0.95 c	1.17 d
Fielder	82.7 bc	90.7 bcd	94.0 a	0.68 c	1.03 bc	1.44 abc
Norquay	93.3 a	93.3 abc	95.3 a	0.93 a	1.40 a	1.61 a
RL4555	39.3 e	64.7 e	80.7 b	0.58 d	0.57 d	0.98 e
Era	79.3 c	92.0 abc	94.7 a	0.68 c	0.99 c	1.16 de
Era, w	88.7 ab	88.0 cd	92.7 a	0.80 b	1.01 bc	1.33 cd
Glenlea	88.0 ab	92.0 abc	93.3 a	0.79 b	1.08 bc	1.40 bc
Glenlea, w	94.0 a	96.0 a	92.7 a	0.92 a	1.15 b	1.56 ab

1Treatments with the same grouping letter are not significantly different ($\alpha = 0.05$).

TABLE 12. Components of germinabiltiy for the wheat lines at the L1 site.

	P^1		$1/t^1$	
Cultivar	0 day	90 day	0 day	90 day
Columbus	9.0 c	97.7 a	0.54 ab	0.86 c
Neepawa	58.0 ab	97.7 a	0.63 ab	0.92 c
Fielder	67.0 a	97.7 a	0.78 a	1.18 b
Norquay	75.7 a	95.7 b	0.89 a	1.37 a
Glenlea	31.0 b	100.0 a	0.56 ab	0.94 c
Glenlea, w	33.3 b	100.0 a	0.67 ab	1.26 ab
RL4137	0.0 c	86.3 b	0.00 c	0.82 c
RL4137, w	9.0 c	95.7 ab	0.28 bc	0.82 c

1Treatments with the same grouping letter are not significantly different ($\alpha = 0.05$).

TABLE 13. Components of germinability for the wheat lines at the W2 site.

	P^1			$1/t^1$		
Cultivar	0 day	45 day	90 day	0 day	45 day	90 day
Cypress	34.7 d	92.0 bc	92.0 a	0.61 d	0.83 cd	1.10 de
Fielder	62.0 c	80.0 de	80.7 b	0.74 c	0.87 cd	1.30 c
Garnet	74.0 b	92.7 abc	87.3 ab	0.83 ab	1.10 b	1.50 b
Neepawa	61.3 c	85.3 cd	88.0 ab	0.76 bc	0.91 c	1.23 cd
Pitic	36.0 d	68.7 e	84.0 ab	0.61 d	0.78 de	1.00 e
RL4137	27.3 d	68.7 e	85.3 ab	0.51 e	0.70 e	1.01 e
Sonora 64	86.7 a	97.3 a	90.0 ab	0.89 a	1.23 a	1.63 ab
Sonora 64A	80.7 ab	94.7 ab	90.7 a	0.85 a	1.15 ab	1.76 a

1Treatments with the same grouping letter are not significantly different ($\alpha = 0.05$).

afterripening and should approach 85-100 percent once most of the dormancy is lost. With these two considerations in mind, only RL4555 from the W1 site (Table 11) could be considered to have either a low seed vigour or an exceptionally long dormancy period. Considering that RL4555 is a poorly adapted cultivar and, at the W1 site, was exposed to a relatively larger amount of weathering due to lodging problems, a low seed vigour would probably be the correct assumption. If this low seed vigour was accounted for, RL4555 would still be one of the least germinable cultivars with a ranking approximately similar to Columbus.

If 3 days of germination is sufficient to observe the germination of all non-dormant seeds, by increasing the germination time to 7 days, one would expect to see a higher final percent germination. The P values for a 7 day germination test for the cultivars at the L1 site (Table 14) do not indicate a significant increase in final percent germination over the 3 day germination test values (Table 12). Thus, the use of a 3 day germination test is adequate for determining the germinability of this particular set of seed samples.

TABLE 14. Final percent germination for the wheat lines at the L1 site after 7 days of germination.

Cultivar	P[1]
Columbus	3.3
Neepawa	43.3
Fielder	80.0
Norquay	80.0
Glenlea	40.0
Glenlea, white	20.0
RL4137	0.0
RL4137, white	3.3

1Treatments with the same grouping letter are not significantly different ($\alpha = 0.05$).

As the period of afterripening increased, there was a corresponding loss of dormancy and increase in germinability at all 3 sites (Tables 8, 9, 10, 11, 12, and 13). This trend was earlier reported by LaCroix et al. (1976) in a study involving similar cultivars and lines under comparable Manitoba environmental conditions. All cultivars increased in germinability as the afterripening period increased. For the wheat lines with the lower germinabilities at day 0, the increase in germinability with afterripening was due to both a loss of dormancy and an increase in the rate of germination. For the wheat lines with a higher germinability at day 0, the increase in germinability with afterripening was primarily due to an increase in the rate of germination with the loss of dormancy being only a relatively minor factor.

During afterripening, there were few changes in the rankings among the cultivars at all 3 sites during the 90 day period of afterripenning. Thus, for studies involving only testing for differences in pre-harvest sprouting potential, it would be adequate just to test the germinability of the seed samples at harvest for determining these differences. The use of afterripening periods would only be required for studies specifically

involved with studying the processes that occur during afterripening including the loss of dormancy and in increase in germination rates.

4.2.2 Determination of α-amylase activity

The α-amylase activity of the seed samples were determined after three days of germination. This enzymatic assay can quantify the actual sprouting damage that occurs (Hageman and Ciha, 1984). In these studies, an estimate of the potential for each wheat line to produce α-amylase in conjunction with precocious germination was obtained. Larsson (1987) has found that using a germination time of 3 days within a breeding program was satisfactory for selecting wheat lines with improved pre-harvest sprouting resistance. This time period was also convenient for these main characterization studies as well. A germination period of 3 days was sufficient so that germination was complete for most wheat lines by the end of the germination trial. Furthermore, the use of a 3 day germination period partially overcame fungus contamination that was prevalent in trials utilizing a longer germination time period. While the fungus contamination did not interfere in the germinability measurements, this contamination

severely affected the α-amylase activity measurements. The presence of any significant amount of fungus contamination produced an enormously high α-amylase activity meausurement. Due to this contamination problem, relatively small sample sizes were used in these studies. This use of small sample sizes could lead to difficulties with experiment variability.

Based on the α-amylase activity data for sites W1 (Table 15), W2 (Table 16), and L1 (Table 17), there was no clear cut relationship between seed coat colour and α-amylase activity after 3 days of germination. There were no significant differences between neither the red and white isoline pairs of Era and Glenlea at the W1 site nor the red and white isoline pairs of Glenlea and RL4137 at the L1 site. The data for all the cultivars at all 3 sites indicated that for both red and white cultivars, there was arange of cultivars exhibiting either low or high levels of α-amylase activity after 3 days of germination. Within the red wheats, Garnet and Sonora 64 & 64A were examples of red cultivars with high α-amylase activity while RL4137 and Pitic were examples of red cultivars with low α-amylase activities. Within the white wheats, RL4555 and Fielder were examples of white cultivars with low α-amylase activities while Norquay was

TABLE 15. α-amylase activity of 3 day germinated seed samples from the wheat lines at the W1 site over an afterripening period of 90 days.

Cultivar	α-Amylase Activity[1] (IDC Units/Seed) Day 0	Day 45	Day 90
Columbus	5855 e	26098 bcd	1865 cd
Neepawa	11997 cd	44939 ab	12041 ab
Fielder	8620 d	17197 d	1244 d
Norquay	16718 abc	35146 abc	14666 ab
RL4555	4918 e	6511 e	8194 abcd
Era	16464 bc	28507 bcd	17865 ab
Era, w	17790 ab	16928 cd	7495 bcd
Glenlea	18617 ab	50992 ab	11215 abc
Glenlea, w	23643 a	63810 a	23695 a

1Treatments with the same grouping letter are not significantly different (α = 0.05).

TABLE 16. α-amylase activity of 3 day germinated seed samples from the wheat lines at the W2 site over an afterripening period of 90 days.

Cultivar	α-Amylase Activity[1] (IDC Units/Seed) Day 0	Day 45	Day 90
Cypress	9318 b	31515 a	23052 a
Fielder	9835 b	9548 bc	7389 a
Garnet	36042 a	34446 a	23635 a
Neepawa	22731 a	16128 bc	8283 a
Pitic	5127 c	8270 c	7978 a
RL4137	5821 bc	8678 c	10858 a
Sonora 64	21448 a	21466 ab	14886 a
Sonora 64A	20868 a	17528 abc	7363 a

1Treatments with the same grouping letter are not significantly different (α = 0.05).

TABLE 17. α-amylase activity of 3 day germinated seed samples from the wheat lines at the L1 site over an afterripening period of 90 days.

Cultivar	α-Amylase Activity[1] (IDC Units/Seed) Day 0	Day 90
Columbus	791 abc	34313 de
Neepawa	3491 ab	42071 bc
Fielder	1254 a	32347 de
Norquay	6158 a	43019 bc
Glenlea	1060 ab	57327 a
Glenlea, w	1434 ab	44921 b
RL4137	59 bc	30072 e
RL4137, w	663 c	37947 cd

1Treatments with the same grouping letter are not significantly different ($\alpha = 0.05$).

an example of a white cultivar with a high α-amylase activity.

McCrate et al. (1981) and Gordon (1979) observed that enzyme activity was not always correlated with visible sprouting. Similar observations were made in this study. In the case of Neepawa and Fielder, even though Neepawa had either a similar or lower germinability than Fielder (Tables 8, 9, and 10), Fielder had a lower level of α-amylase activity after 3 days of germination (Tables 15, 16, and 17). In most cases, Fielder, one of the most germinable of the cultivars at the W1 and L1 sites, had a lower level of α-amylase acitivty than Columbus, one of the least germinable cultivars at the W1 and L1 sites (Tables 8, 9, 15, and 17). Cypress and Garnet were also examples of cultivars that had an intermediate germinability but had a considerably higher level of α-amylase activity. These differences between the red and white wheats, especially in the case of Neepawa and Fielder, could be primarily just to the selection criteria in breeding programs. In the case of the hard red spring wheats such as Neepawa, these wheats tend to have a dormancy period after maturity and pre-harvest sprouting is relatively not as large of a concern as in the white wheats. If

germination does not occur, α-amylase production is essentially nill. In the case of the soft white spring wheats such as Fielder, these wheats tend to have problems with precocious germination especially under normal irrigated conditions. Once germination occurs, α-amylase production begins, and sprouting damage begins to occur. Thus, in selecting for these white wheats, lines with minimal sprouting and a low level of sprouting damage due to α-amylase are selected. Overall, α-amylase activity is more likely to be rigidly selected for in the white wheats than the red wheats, possibly indirectly accounting for the differences in α-amylase production between Neepawa and Fielder.

During the afteripening period, there was a trend towards an increase in the ability to produce α-amylase activity. At the W1 and W2 sites (Tables 15 and 16), there was an increase in the level of α-amylase activites for the cultivars with an afterripening period of 45 days. The only exceptions to this trend was the white line of Era at the W1 site and Neepawa, Garnet, Sonora 64A, and Fielder at the W2 site. In the case of Era (white), Garnet, Sonora 64A, and Fielder, the α-amylase activities remained at a similar level. In the case of Neepawa, there was a notable decrease in the level of

α-amylase activities after 3 days of germination. By 90 days of afterripening, there was an overall decline in α-amylase activity to levels either similar or lower than the α-amylase activity levels present at Day 0. The only exceptions to this trend were RL4555 at the W1 site and RL4137 at the W2 site. At the L1 site, there was a 12 - 500 fold increase in the levels of α-amylase activity after 90 days of afterripening (Table 17).

4.3 Responsiveness of the Aleurone Layer

4.3.1 α-amylase activity

Quarter-seeds from the cultivars at the W1 site with 0 and 90 days afterripening were studied for their response to 10 μM GA3 and 50 μM ABA. The classic responses as described by Varner and Chrispeels (1967) were obtained such that GA_3 considerably stimulated the increase in α-amylase activity while ABA decreased the level of α-amylase activity (Table 18). This trend was present in both the 0 day and 90 days afterripening samples. At 0 days afterripening, there were no significant differences among Columbus, Neepawa, Fielder, Norquay, and RL4555 for both the control treatments and ABA treatments. There were significant differences for

TABLE 18. α-amylase activity of quarter-seeds in response to GA_3 and ABA from the wheat lines at the W1 site over an afterripening period of 90 days.

	α-Amylase Activity[1] (IDC Units/Quarter-Seed)					
	0 day			90 day		
Cultivar	H_2O	GA_3	ABA	H_2O	GA_3	ABA
Columbus	12.9 b	6484 a	4.5 b	21.7 b	17275 ab	5.8 b
Neepawa	167.8 a	6288 a	17.0 ab	173.0 a	21890 a	29.2 ab
Fielder	273.3 a	4365 abc	20.1 a	120.1 a	6849 bc	77.8 a
Norquay	81.1 ab	1246 c	39.5 a	227.4 a	3488 cd	65.0 a
RL4555	83.5 ab	323 d	9.3 a	254.3 a	1333 d	51.6 a
Era	62.9 ab	2354 abc	7.3 ab	101.1 a	9149 abc	17.2 ab
Era, w	45.0 ab	1721 bc	19.0 ab	99.0 a	5994 c	34.1 ab
Glenlea	198.8 a	6240 a	10.5 a	147.0 a	13942 ab	75.9 a
Glenlea, w	146.1 ab	5061 ab	6.6 ab	158.2 a	18224 ab	25.8 ab

1Treatments with the same grouping letter are not significantly different (α = 0.05).

the GA_3 treatments. Both Columbus and Neepawa, the two hard red spring wheats, had a higher level of α-amylase activity than all 3 of the white wheats. The difference was not significant in the case of Fielder and Norquay but was significantly different in the case of RL4555. RL4555 was the least responsive to GA_3 of the 5 cultivars. There were no significant differences between the red and white isolines of either Era or Glenlea for all three treatments.

The samples with 90 days afterripening had similar trends to that of the 0 days afterripening samples. There were no significant differences between the cultivars for the control treatment, however, there were significant differences among the cultivars in response to ABA. α-amylase activity was inhibited the most in Columbus followed by Neepawa. Columbus had the lowest level of α-amylase activity for both the control and ABA treatments at both 0 and 90 days afterripening. The GA_3 trends exhibited in the 0 days afterripening samples were more pronounced in the 90 days afterripening samples. Both Columbus and Neepawa were significantly different from all 3 white cultivars. RL4555 was also the the least responsive of the 5 cultivars to GA_3. There were no significant differences in α-amylase activity between

the GA_3 treatments. Both Columbus and Neepawa, the two hard red spring wheats, had a higher level of α-amylase activity than all 3 of the white wheats. The difference was not significant in the case of Fielder and Norquay but was significantly different in the case of RL4555. RL4555 was the least responsive to GA_3 of the 5 cultivars. There were no significant differences between the red and white isolines of either Era or Glenlea for all three treatments.

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the red and white isolines of Glenlea and Era for all three treatments.

After 90 days afterripening, the quarter-seeds in general synthesized a higher level of α-amylase activity. This trend was evident in all treatments with the exception of the Glenlea control treatment which had a small decrease in the level of α-amylase activity. The increases ranged from small for most of the control and ABA treatments to considerable for the GA_3 treatments of Columbus and Neepawa. In addition to α-amylase activity, the aleurone layers were capable of synthesizing a larger amount of TES soluble protein in general (Table 19). This increase in TES soluble protein with afterripening could be either due to increased synthesis of these proteins by the aleurone layer, increased hydrolysis of the endosperm proteins into smaller TES soluble protein fragments, or, more probable, the combination of these two factors.

In these quarter-seed studies, the red wheat cultivars appears to be more responsive to a level of 10 uM GA_3 as compared to the white wheat cultivars. This trend was further enhanced with an afterripening period of 90 days. This trend was also exhibited in preliminary half-seed studies involving Columbus and Fielder (Table

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TABLE 19. TES soluble protein content of the embryoless quarter-seed samples stimulated by GA_3 and ABA from the wheat lines at the W1 site over an afterripening period of 90 days.

	Protein Content (mg/Quarter-Seed)					
	H2O		GA_3		ABA	
Cultivar	Day 0	Day 90	Day 0	Day 90	Day 0	Day 90
Columbus	1.48	2.47	2.15	3.55	1.78	2.77
Neepawa	1.86	3.15	2.25	4.19	1.48	3.25
Fielder	1.37	2.70	1.44	3.92	0.77	2.76
Norquay	1.10	3.16	1.94	3.92	1.30	4.00
RL4555	1.75	4.08	1.97	3.91	1.42	3.97
Era	1.10	2.63	1.43	4.01	0.98	2.63
Era, w	1.44	3.08	1.43	3.43	1.31	2.79
Glenlea	1.27	4.01	1.85	4.21	1.72	3.94
Glenlea, w	1.43	4.83	1.72	3.89	1.78	3.21

20). In this particular study, Columbus and Fielder were treated with either 10 μM GA_3, 50 μM ABA, or a combination of the two. Columbus was stimulated to a much greater degree by GA_3 either in the presence orabsence of ABA than Fielder. In the case of Fielder, GA_3 produced only a slight increase in α-amylase activity in this particular study, and in some other unpublished preliminary studies, GA_3 at times actually promoted a decrease in α-amylase activity. However, white seed coat colour is not the factor involved in this low GA response. There were no significant differences in GA_3 stimulation betweeen the red and white isolines of either Era or Glenlea. The differences between Columbus and Neepawa and the other 3 white wheats may be attributed to the genetic background of the cultivars rather than specifically to seed coat colour. Fielder and the other GA_3 non-responsive wheats may either possess or lack one or more genes that confer GA insensitivity or GA sensitivity respectfully. In the case of RL4555, poor seed vigour, as implicated in the germinability studies, may be involved as well. Sen and Osborne (1977) have shown that low vigour seed lots in rye could have an 80% reduction in protein synthesis during germination as compared to high vigour seed lots. Therefore, other

TABLE 20. Response of Fielder and Columbus half-seeds to GA_3 and ABA.

Cultivar	α-Amylase Activity (Total IDC Units)			
	H_2O	ABA	GA_3	ABA+GA_3
Fielder	1696	4	2888	1222
Columbus	168	0	1790	344

genes besides those for seed coat colour are involved in GA responsiveness.

In this study, the results obtained from the quarter-seed work were not fully satisfactory. The main problem was, again, the diversity of genetic background among the selection of cultivars. In future research, cultivars should be carefully selected equally for importance with respect to sprouting characteristics and, especially, for a common genetic background. The three pairs of red and white isolines would be excellent material for studying the effects of seed coat colour. These quarter-seed experiments would be quite useful for studying tissue sensitivity to either ABA or GA. In the case of studying ABA sensitivity, considering that the control normally has a low level of α-amylase activity, it would be probably more effective to measure the ability of ABA to inhibit the GA-stimulated responses as in Table 20 rather than just measuring the inhibitory response of ABA applied alone.

4.4 General Discussion

The differences in pre-harvest sprouting resistance between the cultivars can be explained by involving two

groups of plant substances. The first group includes the plant growth substances, GA and ABA. The second group includes potential germination inhibitors. Two such inhibitors that will be discussed includes the α-amylase inhibitor and the catechin-tannins. The involvement of the plant growth substances will be discussed first.

The differences in α-amylase content between the cultivars (Table 1) can possibly be explained based on the plant growth substances, GA_3 and ABA. Gale et al. (1987) have observed that germinative α-amylase activity accumulates originally and more rapidly in the embryo region suggesting that the embryo is involved in the process of early germinative α-amylase synthesis. The most likely explanation is that the embryo releases a stimulus, namely GA, which diffuses towards the scutellum and aleurone layers and subsequently, promotes α-amylase synthesis. GA_1 has been suggested to be this stimulus. However, only the precursor of GA_1, namely GA_{20}, has been detected in developing wheat grains (Gaskin et al., 1980). Walker-Simmons (1987) has further implicated the hormone, ABA, in pre-harvest sprouting resistance. ABA levels during early filling, and more importantly, ABA sensitivity at maturity has been shown to influence the germinability of isolated embryos. Considering that ABA

also inhibits the synthesis of α-amylase (Varner and Chrispeels, 1967), ABA could quite conceivably inhibit the production of α-amylase by either the aleurone layer or the scutellum layer during grain filling. Furthermore, ABA has also been shown to inhibit the promotive effects of GA on α-amylase sythesis (Baulcombe et al., 1984; Chandler et al., 1984). Thus, the differences observed among the various wheat lines for α-amylase content could be either due to the release of GA by the embryo during grain filling or due to the inhibitory action of ABA either directly through the levels of ABA or indirectly through the sensitivity of the seed tissues. In reality, both of these mechanisms are probably present at the same time counteracting each other's effect. Whether or not the production of germinative α-amylase prior to maturity occurs will depend on which of the two plant growth substances predominates during grain filling.

In the case of Fielder and Norquay, both of which tend to germinate more readily as compared to Columbus and Neepawa, the promotive effects of GA on α-amylase synthesis probably predominate due to the release of GA during the initial phases of germination. Thus, the end result is a higher level of α-amylase activity as shown

in Table 1. In the case of Neepawa and especially Columbus, both of which tend to have a more pronounced dormancy, the inhibitory effects of ABA probably predominates primarily due to the sensitivity of the aleurone layer to ABA. Thus, the end result is a lower level of α-amylase activity as shown in Table 1. In the case of RL4555, it's low level of α-amylase activity could be due to additional factors, namely, low seed vigour and/or GA insensitivity in the aleurone and scutellum layers. The potential for low seed vigour is indicated by the W1 data for the components of germinability (Table 11) in which RL4555 has a low percent germination and a low rate of germination throughout the afterripening period. The possibility of insensitivity of RL4555 to GA is indicated by the quarter-seed data (Table 18) in which RL4555 is the least responsive to GA_3 of the 9 cultivars.

The differences in dormancy between the cultivars as indicated in Tables 11, 12 and 13 could be due to the involvement of either the levels of ABA present within the embryo or, more likely, the sensitivity of the embryo to ABA (Walker-Simmons, 1987). Furthermore, the loss of dormancy during the afterripening period can be also explained based on this potential involvement of ABA.

ABA levels within the embryo and/or embryo sensitivity to ABA may decrease during afterripening, thus, decreasing the level of dormancy. Slominski et al. (1979) has shown a correlation with extractable ABA with germination at times after maturity. Furthermore, during afterripening, if the embryo tissue also becomes less sensitive to ABA, the embryos will more likely respond to germination stimulators such as GA. Thus, dormancy is eventually lost.

A similar argument as used for the loss of dormancy can be also used for the apparent capacity of the aleurone layers to synthesize increased levels of α-amylase with additional afterripening (Table 18). Either ABA levels or more likely, the aleurone layers' sensitivity to ABA, decreases during afterripening. Thus, the inhibitory effects of ABA on the production of α-amylase by aleurone layers could possibly be either lessened or removed so that increased α-amylase production occurs. The quarter-seeds, after an afterripening period of 90 days, appeared to be less responsive to applied ABA (Table 18). This is suggested by the increased levels of α-amylase activity within the ABA treatments with an afterripening period of 90 days. α-amylase activity levels for the majority of the

cultivars are 2-7X higher than the ABA treated quarter-seeds with 0 days afterripenning. The only cultivar that did not follow this general trend was Columbus. Columbus quarter-seeds still appeared to be sensitive to applied ABA. However, due to the low levels of α-amylase present, this discussion is more speculative rather than concrete. It is difficult with this particular data to distinguish whether this increase in α-amylase activity is due either to decreased ABA sensitivity or to just the general increase of α-amylase activity due to afterripening. More direct assays of ABA sensitivity will have to be utilized to make more solid conclusions regarding afterripening and ABA sensitivity.

In contrast to the quarter-seed results, the data for α-amylase activities in whole, 3 day germinated seeds (Tables 15, 16, and 17) does not present as clear as picture. With an increase in afterripening, there was a decrease rather than an increase in total α-amylase synthesized in some cultivars. This decrease in α-amylase activity could be due to reduced levels of stimulators such as GA as a consequence of poor seed vigour. Sen and Osborne (1977) have reported that a decrease in protein synthesis during imbibition and germination is a characteristic of reduced seed vigour in rye.

The second group of plant substances that may account for differences in pre-harvest sprouting resistance between the cultivars are inhibitory compounds such as the catechin-tannins and the α-amylase inhibitor. The potential involvement of catechin-tannins will be discussed first.

Studies involving catechin-tannins, the precursors for the red seed coat pigments have shown that catechin-tannins are inhibitory on germination (Miyamoto and Everson, 1958). Furthermore, during afterripening, along with a increase in germinability, there was also a corresponding decrease in catechin-tannin levels (Miyamoto et al., 1961). Thus, seed coat inhibitors such as catechin-tannins could be involved in the overall process of germinability. Quite possibly, cultivar differences, especially differences for the rate of germination between red and white cultivars (Tables 11, 12, and 13), could be explained partially by cultivar differences in catechin-tannin levels. The red cultivars which had a lower rate of germination would be expected to have higher levels of catechin-tannins as it is a precursor for the red seed coat pigment. Thus, red wheats would be expected to have a higher level of catechin-tannin inhibitory activity as possibly indicated

by the rates of germination data for the 3 sites (Tables 11, 12, and 13).

The α-amylase inhibitor data from this study indicated no significant correlation between the level of α-amylase inhibitor in the mature, dry seed and either percent germination or the rate of germination. This can be seen by comparing the α-amylase inhibitor content for the cultivars at all 3 sites (Tables 4, 5, and 6) with the data for both percent germination and the rate of germination (Tables 11, 12, and 13). Furthermore, there was no correlation between the α-amylase inhibitor content of embryo-rich fractions (Table 7) with either percent germination or the rate of germination. Munck et al. (1985) has also shown this lack of correlation between germinability and α-amylase inhibitor levels in the mature, dry seed. These studies suggest that the α-amylase inhibitor has no association with sprouting and sprouting resistance.

Another factor to consider is the possible effects of ABA during filling on the accumulation of the α-amylase inhibitor. Based on the previous discussions about the possible role of ABA during filling, especially in Columbus and Neepawa, and on the fact that ABA promotes

the synthesis of the α-amylase inhibitor (Mundy, 1984), one would expect Columbus and Neepawa to have a higher level of α-amylase inhibitor. Columbus, indeed, does have a significantly higher level of α-amylase inhibitor as compared to the other cultivars (Table 4). Thus, differences in α-amylase inhibitor contents between cultivars may just be a reflection of either differences in ABA levels during filling and/or differences in ABA sensitivity in the seed tissues. Another point to consider with the α-amylase inhibitor is the difference in levels of inhibitor content between the embryo and the endosperm (Table 7). Since ABA is involved with the promotion of α-amylase inhibitor synthesis (Mundy, 1984), the differences between inhibitor levels of the embryo and endosperm could be due to either higher levels of ABA within the embryo or higher level of ABA sensitivity for the embryo tissue, or a combination of these two.

5. CONCLUSION

To summarize, one of the major stumbling blocks in this study was the use of a diverse international range of cultivars that consequently had a diverse genetic background. This diverse genetic background made it difficult to make any concrete conclusions especially those concerning the involvement of seed coat colour. The use of cultivars with similar genetic background such as the red and white isolines used in these studies is definitely preferable.

The one parameter that appeared to have the strongest association with seed coat colour was germinability, or more specifically, the rate of germination. Data from the germination studies, especially from the red and white isolines, indicated that the white wheats tended to have higher germination rates than the red wheats. One strong possiblity that should be considered would be the role of catechin-tannins, the precursor for the red seed coat colour pigments, in this relationship. These precursors could act as inhibitors on the α-amylase synthesis system. One other parameter that was associated with seed coat colour, but to a lesser degree, was the level of α-

amylase activity present within the mature, dry seed. Inhibitors, such as the catechin-tannins may also be involved with this association as well, however, the possibility for the involvement of growth substances, especially in the later stages of maturation, is more probable. The other measured parameters did not have a strong association with seed coat colour and are more likely to be involved with resistance mechanisms not associated with seed coat colour.

In contrast to the case of seed coat colour, the diverse group of cultivars, particularly those at the W2 site, did not confuse the answer for the effects of afterripening on the parameters used to measure pre-harvest sprouting resistance. In the case of the germination experiments, the three main effects of afterripening were to lessen the effects of dormancy, to increase the rate of germination, and to increase the capacity of the aleurone layer to produce α-amylase activity. Most of these effects have been speculated upon either through the involvement of growth substances, especially ABA and ABA sensitivity, or through the involvement of inhibitors including the catechin-tannins. Of these possibilities, reduction in tissue sensitivity to ABA has been strongly suggested to explain the loss of

dormancy and the increased α-amylase activity. In the case of the increased rates of germination, seed coat inhibitors, particularly the precursors to the red seed coat pigment, have been speculated to be the main factor involved. The results from the quarter-seed experiments essentially verified the germination results. There was a general increase in α-amylase activity with afterripening. In addition, aleurone tissue sensitivity to GA3 was increased with afterripening and tissue sensitivity to ABA was speculated to decrease during afterripening.

With respect to the α-amylase inhibitor, there was no apparent association between the inhibitor and any of the parameters used to indicate pre-harvest sprouting resistance. The α-amylase inhibitor may become important in the processing industry, however, in the field, it does not appear to have any association with either pre-harvest sprouting damage in terms of the presence of α-amylase activity or germinability and the components of dormancy.

Finally, while our currently licensed Canadian hard red and soft white spring wheat cultivars apparently follow the old general rule of resitant red wheats and

susceptible white wheats, it is just as evident that there are also both susceptible red wheats and resistant white wheats. Unravelling the exact role that seed coat colour may have in pre-harvest sprouting resistance would be desireable, however, studying resistance mechanisms in specific cultivars with unique characteristics would be just as beneficial and possibly, more desireable. A prime candidate would be RL4137. This breeding line has already been incorporated into Canadian breeding programs primarily for its excellent sprouting resistance. An overall study into exactly what sprouting mechanisms RL4137 uses may be more beneficial to plant breeders and crop physiologists. By identifying these mechanisms, more direct selection methods could be developed to specifically identify breeding lines with the desired traits. A study into the lack of GA responsiveness and low α-amylase levels in RL4555 would also be interesting. If these responses are indeed genetically based and not just due to poor seed vigour, these traits could be used in breeding programs to generate cultivars that develop minimal sprouting damage with respect to α-amylase damage even under adverse environmental conditions. A third potential cultivar that may prove useful would be Takahe, another highly resistant cultivar, however, there are problems working with this cultivar. It requires a

slight vernalization treatment, and subsequently, seed set during any given year seems to be either a hit or miss situation. In conclusion, while it may be desireable to determine the role of seed coat colour in pre-harvest sprouting resistance, studying the non-colour resistance mechanisms in other cultivars may be more important, and most of all, more beneficial, to the development of sprouting resistant wheat cultivars.

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