INHERITANCE OF SPROUTING RESISTANCE IN COMMON BREAD WHEAT (*Triticum aestivum L.*)

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by

Margaret Auma Ogolla

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ABSTRACT

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The cultivar RL 4137, a spring wheat with a long dormancy period, was hybridized with two non-dormant lines. Sprouting resistance and hence dormancy was measured in terms of enzymatic (α-amylase) activity and also by visual assessment in order to simulate a practical plant breeding situation.

The two crosses were mildly field weathered, and α-amylase activity was measured on a single plant basis. Seeds of the parental plants (P_1 and P_2), their first and second hybrid generations (F_1 and F_2), and the first two backcross populations (BC_1F_1 and BC_2F_1) were used. The α-amylase activity was measured by a Model 191 Grain Amylase Analyzer (Perkin-Elmer Corp., Coleman Instruments Div., Dak Brook, IL 60521).

Generation means analyses indicated that gene action for α-amylase activity was primarily additive for both crosses, with a smaller insignificant dominance component.

Variance analyses indicated a moderate additive genetic variance for α-amylase activity. Dominance variance was only detected in one cross. Broad sense heritability and narrow sense heritability estimates were 0.87 and 0.39 respectively in one cross. None of the genetic variances were significant in the other cross, which was therefore thought to have narrow variability.

Head rows of the parents, F_3 progenies, BC_1F_2 and BC_2F_2 generations which simulated a practical plant breeding situation were severely field weathered. They were visually assessed for sprouting in the spikes as well as on the seed samples.

Chi-square for goodness of fit analysis was applied. In one cross, sprouting resistance was found to be controlled by two or three genes in the F_3 progenies, depending on the seed or spike visual assessments respectively. In the second cross, sprouting resistance was controlled by one or two recessive gene(s) in the F_3 progenies, depending on the seed or spike visual assessments.

For both crosses, two or three recessive genes were found to control sprouting resistance in the BC_2F_2 generations based on visual spike assessment. Visual, seed assessment in the BC_2F_2 generations indicated two genes for resistance in both crosses.

On the basis of this study, a general conclusion was made that both α-amylase activity and visual assessment of sprouting may be adequate as selection criteria for sprouting resistance.

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CHAPTER 1

INTRODUCTION

Pre-harvest sprouting is the unwanted germination in the ear of wheat, rye, barley, oats, triticale and even rice (Derera, 1980). It has also been reported in maize (Fong *et al.*, 1983) and in sorghum (Mathewson *et al.*, 1982).

Pre-harvest sprouting can be a serious problem when the harvest period is wet and humid (Harrington, 1949; McEwan, 1959; Gfeller and Svejda, 1960; Everson and Hart, 1961; Freed *et al.*, 1976; Derera, 1980; Ciha and Goldstein, 1983; and Reitan, 1983). In particular, it is of major concern in North West Europe and certain regions of South America and Africa. Other areas include the Pacific North West and New York State of the U.S.A.; the provinces of Ontario, Saskatchewan and Manitoba of Canada, and New Zealand and the Northern Wheat Belt of Australia (Derera, 1980; and Mitchell *et al.*, 1980).

Sprouting damage often occurs erratically and non-predictably. Stoy (1983) stressed that great economic losses in some years in various parts of the world may result from sprouting damage. Stoy strongly emphasised that research on this complicated problem should be carried out in the intervening 'favourable' period and should not fluctuate according to the weather.

Wheat varieties resistant to sprouting should withstand one or two weeks or longer of unfavourable weather after being ripe. Sprouting resistance however is a complex phenomenon which includes seed dormancy, plant morphology and environmental influences. Of these components, seed dormancy appears to be the most important.

Dormancy denotes any condition inherent in the seeds which prevents the germination of viable seeds for a definite period after harvest (Gfeller and Svejda, 1960). The usual criterion for germination is the emergence of the radicle. However, many processes take place in seeds prior to any visible growth (Ketring, 1973). Of these processes, the activity of the α-amylase enzyme can be the most damaging in wheat utilisation (Kruger, 1980; Meredith, 1983). Although increased α-amylase levels may not be a major concern in bread baking processes of Canada (La Croix *et al.*, 1976) and U.S.A. (O'Connel *et al.*, 1980), it is of significance for some export markets such as Japan and Great Britain.

It is not only desirable that most of the work on pre-harvest sprouting be centered on varietal trials, but there is also a need to know the genetics of sprouting. Studies on the inheritance of any of the components of sprouting may give insight into ways of exploitation in breeding for pre-harvest sprouting resistance.

The study reported herein attempted to determine the inheritance of pre-harvest sprouting resistance. The measurement of pre-harvest sprouting was determined in two phases:

1) α-amylase activity, which is a laborious and expensive laboratory procedure, but can be precisely determined to meet the industry's requirement.
2) Visual assessment, which is a quick and inexpensive field method. The main emphasis was placed on a plant breeder's ability to visually select the resistant plants under natural field conditions.

CHAPTER II

LITERATURE REVIEW

2.1 PHYSIOLOGY OF PRE-HARVEST SPROUTING

The physiology of sprouting damage revolves around the physiology of dormancy and germination, with particular reference to the activity of enzymes. This involves the interaction of hormones (gibberellins, abscisic acid and cytokinins). Another aspect includes the provision of energy and substrates, and resumption of protein synthesis for metabolism (Gordon, 1980).

The wheat caryopsis can be divided into three major parts; pericarp, endosperm and embryo. The pericarp or seed coat is maternal tissue derived from the ovary wall. The endosperm contains the aleurone layer and the starch reservoir. The embryo contains the scutellum, root and shoot meristems (Freed *et al.*, 1976).

When grain sprouts, several different things happen in a complex manner. Firstly, there is the initiation of embryo growth and endosperm breakdown. This is controlled by endogenous growth substances exerting either promoting or inhibiting effects on several key reactions (Stoy and Sundin, 1976). Of these, gibberellic acid is the main promoter of germination, while abscisic acid may block germination (Maguire, 1975). The first step in the embryo growth is the formation or release of bound gibberellins (Belderok, 1976 b), which activate the release of SH-containing glutathione and cysteine for use in protein synthesis (Belderok, 1968). Giberellins move from the embryo to the aleurone cells of the endosperm inducing the production of hydrolytic enzymes. These enzymes degrade the endosperm,

thereby providing sugars and amino acids for growth (Maguire, 1975).

2.1.1 Seed Coat Imposed Dormancy

In this type of dormancy, virtually no germination or only a slow germination occurs provided the seed coat layers are intact, even if the seeds are placed under optimum conditions of water, light, oxygen and temperature (Belderok, 1976). Because of varietal differences in dormancy between red and white grained wheats together with the germination - promoting effect of removal, or damage of the seed coat, it has been assumed that some causes of dormancy may reside in the seed coat.

Miyamoto *et al.*, (1961) reinvestigated the main hypotheses which were previously suggested as causes of dormancy in wheat. They found that dormancy was not caused by restricted water or oxygen uptake, a mechanically tough seed coat or immature embryos. Germination inhibitors located in the seed coat were found to have a significant effect on dormancy. At harvest and three weeks after harvest the red dormant cultivars had more inhibitors than the white non-dormant. The loss of dormancy during the weeks after maturation was correlated with the natural inactivation of the inhibitors. They felt that the low levels of inhibitors in the white wheats may be a possible reason why plant breeding research for dormant white wheats has been unsuccessful.

Studies by Wellington and Durham (1961) indicated that mechanically tough seed coat of wheat does not limit water uptake.

Durham and Wellington (1961) determined whether the germination behaviour of white and red wheat grains could be attributed

to either oxygen requirement of the embyros or the permeability of the covering layers. They found that the presence of the covering layers prevented any intact red grains from germinating in either 0.5% or 5% oxygen; very few germinated in air, although more germinated in 40% oxygen. In contrast, a few intact white grains germinated in 0.5% oxygen with marked increased germination in 5% oxygen, and in air, and only a further slight increase in 40% oxygen. In both varieties the covering layers of some grains were therefore sufficiently impermeable to prevent germination when the oxygen concentration in the external atmosphere was very low. With high external concentration there was a greater increase in germination of the white grains than of the red, possibly as a result of increased diffusion of oxygen through the covering layers.

The actual mechanism of impermeability to oxygen is not known. Belderok (1976) assumes that high molecular weight proteins are present in the testa layer of wheats during dormancy. These proteins easily swell after soaking the grains in water and thus make the testa impermeable to oxygen.

Gordon (1970) attributed premature germination to mechanical defects in the pericarp-testa. A relatively higher percentage of defective kernels was observed in certain varieties than in others. Because he found differential germination within an ear, he concluded that a mechanical failure caused germination. Rupture of the seed coat preceded any obvious growth of the embryo. Belderok (1976b) found that the testa of a sprouting resistant wheat maintained its integrity beyond grain maturity, whereas the structure became granular before maturity in wheats without sprouting resistance.

2.1.2 Embryo Dormancy

Embryo dormancy means that the germination inhibitors reside in the embryo, and that no germination takes place when the coat layers are damaged or removed, even if the seeds are placed under optimum conditions of water, light and temperature (Belderok, 1976). This dormancy has been described in freshly harvested *Hordeum spontaneum* (Urion and Chapon, 1955; cited by Belderok, 1968) and in *Avena fatua* (Naylor and Simpson, 1961). It has also been observed occasionally in other cereals (Schleip, 1938; Moormann, 1942; cited by Belderok, 1968). The proposed mechanism is based on cytokinin/inhibitor interaction (Belderok, 1976). The first step in the termination of dormancy is a decrease in the inhibitor level, followed by an increase in cytokinin content. If wheats with this cytokinin/inhibitor gene source were available, they would be useful in breeding programs for sprouting resistance. This may be of some importance for the breeders of white grained varieties, for the cytokinin/inhibitor mechanism is localized in the embryo and not in the seed coat.

2.1.3 Abscisic Acid (ABA)

ABA has been shown to induce natural dormancy in seeds and buds of many species. Stoy and Sundin (1976) demonstrated that ABA at a concentration of 5×10^{-5} M drastically reduced the germination of wheat seeds. Radley (1980) confirmed the inhibitory action of ABA and found gibberellic acid to have a reverse effect. Radley suggested that supraoptimal auxin levels may be even more important as germination inhibitors. The auxin was identified as indole acetic acid.

Undoubtedly, there are genes which control levels of endogeneous growth substances. Freed *et al.*, (1976) felt that an understanding of these gene systems may allow researchers to manipulate the chain of events involved in the germination sequence.

2.1.4 Gibberellic Acid (GA)

The first step in embryo growth of cereals is the formation of GA or the conversion of bound to free GA in the embryo. Endogeneous GA in the embryo scutellum stimulates synthesis of hydrolytic enzymes, most notably α-amylase in the aleurone layer (Varner, 1964). Thus any reduction in GA release or aleurone layer response to GA may be exploited in the reduction of deleterious effects of pre-harvest sprouting (Gale, 1976).

2.1.5 α-Amylase

This enzyme in mature wheat kernels is either the residue that formed during maturation (Kruger, 1972 ; Meredith, 1974) or is synthesised during germination (Kruger, 1972 b; Briggs, 1972; Meredith, 1974).

The natural function of α-amylase is to convert starch in the wheat endosperm to sugars and gummy substances for use by the growing plant (Sanders, 1961). Dronzek *et al.*, (1972) found that α-amylase began its attack in the aleurone layer and preferentially attacked large granules of starch. As germination proceeded, smaller granules were also eroded but at a slower rate than the large granules.

Thus starch that is resistant to α-amylase activity, at least to some degree, would in theory be attractive in breeding for

resistance. The other possible alternative would be to look for α-amylase inhibitors.

The physiology of pre-harvest sprouting seems to be very complex. Many factors are to be considered in seeking resistance to sprouting. Among the factors, physiological blocks to endosperm degradation may be exploited. As the activities of these processes are largely controlled by embryo processes, dormancy of the embryo may be desirable. However, it may also be useful to have a physiological block only at the aleurone cells of the endosperm.

2.2 FACTORS THAT INFLUENCE PRE-HARVEST SPROUTING

2.2.1 Plant Morphology

The degree of pre-harvest sprouting may be influenced by cultivar characters which affect moisture content of the ears. Such factors include glumes and awns, position of seed on spike and position of the spike.

2.2.1.1 Glumes and Awns

Glumes and awns have been implicated in sprouting resistance with particular reference to differential moisture uptake/loss and inhibitors. Pool and Patterson (1958) found that awned wheat lines had a significantly higher rate of water uptake than the awnless lines. They also found significant differences in rates of water absorption of waxy and non-waxy glumed cultivars. The non-waxy glumed cultivar was higher in moisture than the waxy glumed. Clarke (1982) found no differences in drying rate of two cultivars Manitou (awnless) and Napayo (awned) in either windrowed or standing treatments.

In a comparison of 26 awned cultivars with 25 awnless ones, King and Chadim (1983) found significantly slower wetting and slower germination in the awnless cultivars. Germination in the ear was significantly correlated with simultaneous, 30 h germination of isolated grain on filter paper ($r = 0.61$). Seed from awnless varieties germinated relatively poorly in the ear compared with seed on filter paper, while the awned varieties germinated better in the ear than on the filter paper. They concluded that the awns influence grain germination.

Mares (1983) found that the most sprout resistant cultivar had a lower rate of water uptake by the grain in the ear than that of the least resistant cultivar.

Low germination in whole heads has been attributed to inhibitors in the bracts. Smith (1948) found that removing the chaff from seeds of several stocks of wheat increased the percentage of germination, even in relatively fresh seeds. The ability of seeds of four varieties of common wheat to germinate in the chaff as reported earlier by Harrington (1932) was correlated with their tendency to germinate in the stook. Hutchinson *et al.*, (1948) felt that the glume stimulated some latent factor within the grain itself. Derera *et al.*, (1976) found highly significant differences in cultivar germination response when tests were carried out on media containing their own milled husk. In a subsequent study, Derera and Bhatt (1980) sucessfully transferred the germination inhibition of the cultivar Kleiber into the cultivar Gamut. They concluded that it appeared promising to use germination inhibition contributed by Kleiber.

Strand (1980), found the effects of chaff inhibitors to

be limited, and their role appears to be less well known.

2.2.1.2 Position of Seed on Spike

The relationship of rate of germination and position of the seed on the spike is relatively obscure (Hardesty and Elliot, 1956). Wellington (1956) observed that the first seeds of a white spring wheat variety to germinate were those selected from spikelets near the top of the parental spike. This phenomenon was not apparent in a red spring wheat. Observations similar to that of Wellington (1956) were made by Hardesty and Elliot (1956). They found that florets that reach anthesis earlier (1st and 2nd florets from spikelets in the centre of the spike) had the slowest rates of germination. Further support comes from the recent work of King and Chadim (1983), who observed that germination in the ear was always more advanced in the smaller grain in peripheral floret positions.

2.2.1.3 Position of Spike

In barley, ear nodding angles of greater than 120° from the upright reduce water damage (Brinkman and Luk, 1979). However, it is uncommon for wheat ears to nod at more than 90° from vertical (King and Chadim, 1983).

Strand (1980) found cultivar characteristics such as position (nodding vs. upright) and density of the ears to affect moisture uptake. However, the differences between cultivars were small and their effect on sprouting low. King and Chadim (1983) testing two varieties of wheat, found that ears held horizontally absorbed water more at the start of wetting than those held vertically.

Hong (1979) concluded that desirable criteria when selecting for resistance may include closed florets, early maturity, lax spike, tenacious glume, heavy waxy bloom on the spike, short plant height, and short or absent awns.

The plant factors that influence sprouting appear to be a balance of several factors. The relative importance of each factor changes with variety and the environment.

2.2.2 Environmental

The readiness to sprout is a result of the genetic constitution of the grain and the influence of the weather, both before and during the harvest season (Stoy, 1983).

A common observation is that grains germinate more readily when dry and hot, rather than when moist, cool conditions prevail during grain development (Belderok, 1966, 1968). Presumably, higher temperatures increase biochemical processes; further advanced reactions require a shorter after-ripening period in the germination sequence.

Nielsen *et al.*, (1984) found that high mean temperatures during the two weeks before physiological maturity increased susceptibility of wheat to sprouting after rain treatment.

Belderok (1968) showed that the developing grain is influenced in a critical period coinciding with the soft dough stage. The dormancy of a wheat variety was found to depend on the accumulated temperature (sum of daily mean temperatures above 12.5°C) during this critical period. Based on this system, a model for sprouting damage prediction warning has been developed for soft white wheats in The Netherlands. In other words, as soon as a certain critical accumulated temperature

level is exceeded, there is risk of sprouting if it rains during harvest.

Prediction schemes may have variable success as might be expected in such a complex process. Olsson and Mattson (1976) found Belderok's system unreliable for most cultivars in other environments. Possibly due to large genotype x environment interactions, as these have been reported (Kneen and Hads, 1945; Lacroix *et al.*, 1976; Olsson and Mattson, 1976; McCrate *et al.*, 1981; and Nielsen *et al.*, 1984) to have complex effects on sprouting. However, Hong (1979) found that genotype x environment effects were insignificant for sprouting, dormancy and α amylase activity.

Mitchell *et al.*, (1980) demonstrated that sprouting can occur before some predictive methods are applied to assess susceptibility.

In North Dakota, studies to facilitate the development of a sprouting model are underway by Bauer and Black (1983). They found sprouting to be a potential problem when two conditions are met. First, spike water concentration must have been reduced to harvest ripeness, about 120 to 140 g kg^{-1} water concentration. This reduction must occur at least once and be retained at this level, for a period differing with cultivars to overcome dormancy. Secondly, the spike must remain wet long enough for the grain to imbibe enough water to attain the threshold of about 450 to 490 g kg^{-1}. After these conditions are met the extent of sprouting is governed by the length of time the grain moisture level remains above the sprout threshold water concentration. They suggested that the key to issuing a warning of a threatening sprouting problem following a rain is a determination

of the ambient drying conditions.

Most of the factors tend to vary according to the variety location and year, thereby making sprouting a very complicated phenomenon.

2.3 EFFECTS OF PRE-HARVEST SPROUTING ON GRAIN UTILIZATION

Wheat is used mainly as food for man, to a lesser extent as feed for livestock and still much less for seed and industrial purposes. Of the wheat used throughout the world by far the greater part (endosperm) is milled into flour, from which a great variety of baked or cooked products can be made (Peterson, 1965). With the utilization in mind, it therefore follows that the effects of sprouting, i.e. damage to the endosperm and consequently the whole grain can be very detrimental to producers and processors.

For the producers, sprouting reduces yield and quality of the harvested grain. Yield loss due to sprouting occurs during threshing when the plumules and the radicles, together with the lighter, depleted grains, are winnowed out (Derera, 1980). A 10% yield loss is not uncommon (Belderok, 1968).

Reduction in quality which leads to lowering of grade or rejection of grain has been of major concern to many countries. The degree and proportion of sprouted grain in a consignment is one of the important degrading factors. According to the inspection divison of the Canadian Grain Commission (1983), kernels are classified as sprouted when there is clear evidence of growth at the germ area. In some cases the actual sprout is knocked off leaving de-germed kernels; such kernels are only considered sprouted if they are associated with

other kernels bearing visible sprouts. In Canada, the maximum allowable percentage of sprouted kernels in No. 1 CWRS wheat is 0.5% (Kruger and Tipples, 1983).

It is not easy to obtain figures of total damage. One estimate from the Canadian Grain Commission indicates that 0.07% of the total carlots of Hard Red Spring Wheat unloaded at terminal elevators during May to October, 1983 were downgraded due to excessive sprouting (per. Comm., Ward, 1983). Sprouting processes also decrease grain density and reduce weight per bushel which may further lower the grade (Derera, 1980).

The effects of sprouting are also noted by the processors in reduced kernel weight and decreased flour yield and quality. These effects arise from the fact that when grain sprouts, several things happen in a complex manner. Not only are the shoot and root growing, but there are vast changes occurring in the rest of the grain. In particular, enzymes are being formed that will enable the new plant to make use of the food reserves that are in the endosperm. The most important of these enzymes, as has been noted by many workers, is α-amylase, but there are many others. The natural function of α-amylase is to convert the starch to sugars and certain gummy substances.

Wheat with low α-amylase is required to produce bread of good quality (Gothard, 1976). Excess α-amylase activity produces a highly colored loaf with a sticky crumb due to the reduction of more starch to dextrins than necessary (Buchanan and Nicholas, 1980; Sanders, 1961). This reduces the water absorption and prevents production of a large loaf volume with regular crumb structure (Gothard, 1976).

The sticky crumb is a problem to the baker because it sticks to the blades of the slicing machine, so that the loaves tear instead of slicing. The activity of α-amylase is also the cause of problems in other industrial uses of flour and starch made from sprouted wheat (Meredith, 1983).

Sprouted wheat is also unsuitable for seed purposes, as damage to the embryo and subsequent attack by pathogenic fungi reduce the germination of the grain or vigour of the seedling (McEwan, 1959). For example, a report in New Zealand Wheat Review (1945-1946) on field germination tests on four wheat lines sorted according to the degree of sprouting, indicated that seedlings in the "unsprouted" class germinated as well as the control (about 90%). The "slightly sprouted" class germinated 15 to 20% lower. The "badly sprouted" class hardly germinated at all; the few seedlings were weak and stunted. The conclusion was that wheat lines with a large proportion of sprouted grain (more than 15%) should not be used for seed because great wastage would be involved.

Sprouted grain kept for feed is liable to go mouldy (Meredith, 1983). According to McEwan (1967), wheat damaged by sprouting can be used satisfactorily for livestock feed, but it is generally sold at a lower price than grain for milling. Such wheat could be fed to animals with caution as there could be danger of mycotoxins.

There is no doubt that sprouting can be a problem in the agricultural economy of various parts of the world. It therefore calls for measures to remedy the situation. To this end some suggestions have been presented in the literature. For example, Derera (1980) suggested the neutralization of the hydrolytic enzymes by

industrial means. Alternatively, a more economical use for the damaged grain could be sought. Briggle (1980) reported that early harvesting coupled with the use of grain dryers has reduced sprouting damage in the Michigan wheat crops.

The Flour Milling and Baking Research Association at Chorleywood, England, has attempted to eliminate α-amylase as a problem with the development of a microwave baking system (Chamberlain, 1973).

Hagberg noted in 1951 that it is possible to remove sprouted grains from a mixture by taking advantage of their lower density (Meredith 1983). Meredith (1983) reported that the Wheat Research Institute, Christchurch, New Zealand, tried the process of cutting off the germ end of the grain where amylase is most concentrated. The problem would rarely be serious enough to justify the added expense of using density and cutting processes.

Lastly, if the baker and his customers can accept a lower standard of product, then this would be the cheapest solution to the problem.

2.4 MEASUREMENT OF PRE-HARVEST SPROUTING RESISTANCE

Different methods for determining sprouting resistance have been described in the literature. The majority are based on dormancy. Dormancy is a complicated phenomenon and is usually investigated by germination tests. Such tests scored as percent germinated can be done using threshed grains which are either placed in petri dishes (Belderok, 1968), or on sand (Harrington, 1949) or the whole ear is used. The ears are placed in a room with high relative humidity (Schmidt, 1934; Svensson and Lagerstrom, 1966; cited in Johansson,

1976) or placed in a rainfall simulator (McMaster and Derera, 1976) or placed on damp sand (Harrington, 1949). Clarke (1983) concluded that screening of genotypes for sprouting resistance in the field should be done in simulated windrows. Simulated windrows tend to facilitate good differential between genotypes.

An alternative to germination tests is to determine enzyme levels, especially α-amylase activity (Johansson, 1976). In principle, the measurement of α-amylase activity should yield better results in relation to quality required by the industry. This is because results obtained are the sum-effects of all cultivar characters expressed as resistance to pre-harvest sprouting (Strand, 1980).

To measure the activity of an enzyme, a substrate is provided and a measure is made of its action on the substrate. The substrate in this case is starch. Starch consists of two fractions: amylose (a linear polymer of glucose units linked through α–(1-4) bonds) and amylopectin, which is similar to amylose except that the polymer is branched because of the presence of α-(1-6) bonds (Robyt and Whelan, 1968). α-Amylase is an endo-hydrolase enzyme, and it can attack internal segments of the starch chain thereby producing dextrins and very small amounts of maltose and glucose (Reddy *et al.*, 1984). The maltose and dextrins are further degraded by glucosidases, β-amylase and phosphorylase to glucose and glucose-6-phosphate (Briggs, 1972).

The ability of α-amylase to hydrolyse starch has been utilised in many tests. A test devised by Hagberg (1960; 1961) for measuring α-amylase activity has proved to be very practical. Commonly speaking it is known as Falling Number (FN). FN is defined as time in seconds required to stir and allow stirrer to fall a measured distance through

a hot aqueous flour gel undergoing liquefaction. Although the original FN method was intended to apply only to flour, Perten (1964) has shown how the test can be successfully applied to wheat.

The Amylograph (AACC, 1983) is a recording viscometer that may be used to determine the effect of α-amylase on viscosity of flour.

Tipples (1969) developed a microviscometric method for measuring α-amylase activity in small samples of wheat and flour. The reciprocal of time taken for the viscosity to decrease to one-half of its original value is used as the basis of α-amylase activity. Because it can be applied to single kernels, it may prove valuable in breeding work aimed at developing sprouting resistant cultivars.

As an alternative to the mentioned viscometric methods, a method based on the production of maltose formed by the breakdown of starch from flour could be measured by the maltose test (AACC, 1983).

Starch also gives a blue color with iodine, and the disappearance of this color can be measured. A method by Farrand (1964) measures the α-amylase activity by using a β-limit dextrin as a standard substrate. Residual dextrin after predetermined reaction time is determined by measuring the color developed after addition of iodine.

The method of Perten (1966) also measures α-amylase activity using β-limit dextrin as a substrate. The decrease with time of the intensity of color obtained with diluted iodine solution is used as

an index of starch degredation.

The gel-diffusion method of Briggs (1962) uses β-limit dextrin or starch as substrate. α-Amylase activity can be readily estimated by allowing a solution of the enzyme to diffuse from a filter paper disc into an agar gel containing substrate. The substrate is degraded in the zones into which the enzyme penetrates so that, when the gel-surface is flooded with a solution of iodine, circular clear zones are obtained against the substrate-iodine background. The diameter of the clear zone is proportional to the logarithm of enzyme concentration. A modification of Brigg's method has been made by Hayter and Allison (1972) which enables total diastatic activity to be measured. They found the method to be of potential use in plant breeding as an aid to selection for low or high diastatic power.

Gothard (1976) developed a non-destructive method. The method is based on gel diffusion. As halved grains are applied directly to the agar surface, the need for enzyme extraction is eliminated. After incubation the half grains are removed with forceps and the agar surface irrigated with dilute iodine solution. The diameter of the clear zone is measured. Because only half the grain is used for each analysis, the remaining half with embryo can subsequently be grown if required in a breeding program. Hejgaard and Gibbons (1979) presented a gel diffusion method which uses a dye labelled starch as a substrate for α-amylase assay.

On the same basis of color-substrate complex, other methods involve incorporating some dye molecules into starch. Such a commercially available substrate is phadabas tablet, which consists of starch cross linked to a cibacron blue dye. The digestion of the

modified starch will liberate dye into solution so that color can be measured by a colorimeter. The amount of blue color produced is proportional to the degree of hydrolysis. Such methods include those of Fuller (1972) Barnes and Blakeney (1974) and Mathewson *et al.*, (1982).

Finally, the effect of α-amylase activity can also be measured by a reduction in the amount of cloudiness of a starch solution. Recently, such a method was described by Campbell (1980). The method uses β-limit dextrin as a substrate for α-amylase activity. The basic principle by which the unit (Perkin-Elmer model 191 Grain Amylase Analyzer) measures amylase activity is that the right angle scattering of light by suspended starch particles decreases as amylase cleaves the starch into smaller particles. Kruger and Tipples (1981) modified Campbell's method in order to obtain maximum sensitivity.

The majority of the methods of assessing sprouting damage as described above have been shown to be correlated. Thus the visual assessments have been shown to be correlated with analytical methods and also analytical methods correlated amongst each other.

Bhatt *et al.*, (1976) determined visible sprouting (% sprouted) and α-amylase activity (gel diffusion method) on four groups of wheat. They found correlation ($r = 0.75, 0.74, 0.58$ and 0.82) between visible sprouting and α-amylase activity for groups 1, 2, 3 and 4 respectively. Nielsen *et al.*, (1984) also found a highly significant positive correlation ($r = 0.88$) between percent sprouting and α-amylase activity in wheat.

Tipples (1969) reported that the microviscometric method compared more favourably with the Farrand method than with the ICC colorimetric method. Gothard (1976) found a correlation (r = 0.984) between gel diffusion and Farrand method. Barnes and Blakeney (1974) found correlations (r = 0.863) between Amylograph and gel diffusion. O'Connell *et al.*, (1980) reported a correlation (r = 0.973) between Falling Number and Grain Amylase Analyzer (Perkin-Elmer model 191, GAA).

More correlations concerning the foregoing can be found in Barnes and Blakeney (1974), Mathewson and Pomeranz (1978), Mathewson *et al.*, (1982) and D'Appolonia *et al.*, (1982).

Generally, the method of choice seems to depend on the cost and convenience. It may also depend on the amount of delay that can be tolerated to obtain a result of the desired precision. Almost instantaneous results can be obtained by visual selection which is also inexpensive. In some varieties, even if visible sprouting does not occur, the α-amylase level may be considerably high. Thus the α-amylase activity cannot be reliably estimated by determining the percentage of sprouted kernels.

2.5 GENETIC ASPECTS OF PRE-HARVEST SPROUTING

Resistance to pre-harvest sprouting is a complex character (Derera *et al.*, 1976; Stoy and Sundin, 1976). Dormancy and red grain color were the earliest to be considered as components of resistance (Nilsson-Ehle, 1914). More recently, low α-amylase synthetic potential and gibberellic acid insensitivity have been included as components of resistance in white grained wheat (Derera *et al.*, 1976). Varietal

differences have been observed for both these components (Gale and Marshall, 1973; Derera *et al.*, 1976).

2.5.1 Association of Dormancy and Seed Coat Color

In wheat, white grained and red grained varieties are distinguished; in the latter the grains are not really red, but display a wide range of shades of brown (Belderok, 1968). The earliest report on heritable varietal differences in predisposition to sprouting, associated mainly with seed coat color was made by Nilsson-Ehle (1914). He reported that the white varieties which did not have "red factors" germinated very early; the "one factorial" red varieties germinated next and the "more factorial" red varieties germinated last. He concluded that it was not necessarily the red pigmentation in the seed coat that inhibited germination, but that the genes responsible for red color may be closely associated with the genes influencing the structure of the seed coat.

Studies by other workers seem to be in line with Nilsson-Ehle's findings. For example, Gfeller and Svejda (1960) postulated that the degree of seed dormancy was likely to be controlled by the multiple genes which govern seed coat color. Everson and Hart (1961) found that red varieties had post-harvest dormancies ranging from the inhibition of germination for 5 or more days to germination in less than 3 days. The white varieties germinated in less than 3 days after they reached maturity, except Brevor which had a weak inhibitory system. Further support comes from the work of Bingham and Whitmore (1966) who found significant differences in dormancy between red and white grained varieties.

Other workers who found close positive associations between red seed coat color and dormancy, include Everson and Hart (1961); Piech *et al.*, (1970); Freed (1972) cited in Freed *et al.*, (1976); McEwan (1976); and McCrate *et al.*, (1981).

Some workers have reported that the association between red grain color and dormancy is not complete and can be very low. For example Reitan, (1980) found that red seed color intensity was poorly correlated with seed dormancy ($r = 0.387$). He concluded that red seed color may not be necessarily a useful selection criterion for dormancy. Moreover, Gordon (1979) reported a red-grained line (Sonora 64A) with little or no sprouting resistance. McEwan (1980) found that high levels of resistance could be conferred by single red grain factors in the homozygous condition. He concluded that an explanation simply in terms of gene dosage levels does not account for the variation in sprouting resistance in red-grained wheats.

Based on the foregoing, there appears to be some interaction between the factors for red grain color and the genetical background controlling dormancy. Many breeders commonly seek sprouting resistance amongst the red grained varieties and their derivatives.

2.5.2 Inheritance of Dormancy and Seed Coat Color

In a preliminary test to determine the inheritance of dormancy, Deming and Robertson (1933) based dormancy on % germinated of F_1 of a cross between dormant and non-dormant parents. Dormancy was determined 10, 15 and 20 days after harvest. They found that dormancy appeared to be dominant over lack of dormancy.

Bhatt and Derera (1980) determined dormancy on randomly

selected F_4's of a cross between dormant and non-dormant parents. The tests were done 10, 20 and 30 days after harvest ripeness. Because there was such a gap between dormant and the rest of the segregants, the differences in dormancy were treated as a qualitative one. Classifications were made into dormant (50% or less), intermediate (51-75%) and non-dormant (76-100%). They felt that because very few dormant types were recovered there was tendency towards recessiveness of the factors controlling dormancy. In a subsequent paper, Bhatt *et al.*, (1983) conducted inheritance studies of dormancy 10 days after harvest ripeness in three wheat crosses. Genetic analysis of the segregating F_2 and BC_2 generations revealed in two crosses that dormancy was controlled by two recessive genes. They concluded that a situation where dormancy is controlled by dominant factors could hasten the breeding process. However, Hong (1979) found dormancy to be controlled primarily by additive gene action. Monogenic recessive or digenic dominant control explained the inheritance of sprouting resistance, depending upon the parents used.

Maternal inheritance has also been reported to condition seed dormancy (Gale, 1976; Freed and Everson, 1972; Hong, 1979; Reitan, 1980; Noll *et al.*, 1982).

Gordon (1980) determined narrow sense heritability of embryo dormancy in wheat at three levels of precision i.e. plot by plot regression estimates based on serial data collected during grain development, spot tests at harvest time on single heads monitored for moisture content and spot tests at harvest time on plot bulks. He found heritability estimates (41%, 22% and 0%) of embryo dormancy for the levels (1, 2 and 3) respectively. Reitan (1980) reported

broad and narrow sense heritability estimates for seed dormancy of 76% and 57% respectively.

Gfeller and Svejda (1960) and Baker (1981) reported that three genes controlling red color act in an additive way in that each additional gene results in at least some intensification of the red color. These reports have confirmed those of Nilsson-Ehle (1914).

Generally, the red color appears to be dominant to white and is controlled in an additive manner. The inheritance of dormancy seems to vary according to the donor parents and methods of analysis used. From the plant breeding point of view, it appears possible to select for sprouting resistant genotypes using dormancy and/or color.

2.5.3 Genetic Sources of GA Insensitivity and Low α-Amylase Synthetic Potential

Gale and Marshall (1973) discovered that germinating seeds of Tom Thumb and Minister Dwarf had low initial levels of α-amylase and were not responsive to added GA. Norin 10-Brevor 14 and Minister, which were typical of other tall varieties, gave large responses to GA, with minimum sensitivity at 10^{-6} or 10^{-7} M solutions and an optimum reaction at 10^{-4} or 10^{-3} M. Minister Dwarf and Tom Thumb both showed very little response at any of these concentrations. In these genotypes, the base endogeneous levels of α-amylase were also lower than the other varieties and the increase after the second 24 hr incubation period was small.

The gene controlling GA insensitivity in dwarf varieties such as Tom Thumb, Tom Pouce and Minister Dwarf has been assigned

the symbol Gai 3 (review, Gale and Law, 1976). The gene Gai 3 is reported to be extremely potent and dominant to gai 3. The location of Gai 3 was shown to be on chromosome 4A (Gale *et al.*, 1975). The location of Rht 3 (gene for dwarfism in Tom Thumb) was also found to be on chromosome 4A (Morris *et al.*, 1972). Considerable work by Gale and Marshall (1973), Gale and Gregory (1977) and Flintham and Gale (1980) has led to the postulation that GA insensitivity could be a pleiotropic effect of one or more of the dwarfing genes or the effect of separate genes linked to those in the dwarfing system.

Because the GA insensitivity in germinating seeds of Tom Thumb and its derivatives has been confirmed a number of times, many workers such as Gale and Marshall (1975), Gale (1976), Derera *et al.*, (1976), McMaster (1976), Bhatt *et al.*, (1977), Flintham and Gale (1983) and Mares *et al.*, (1983) have proposed that the insensitivity could be a mechanism for pre-harvest sprouting resistance. Indeed, work is underway at the Plant Breeding Institute at Cambridge, England, and the University of Sydney in Australia, utilising the GA insensitivity in search of sprouting resistance.

2.5.4 Inheritance of α-Amylase Activity

There is very little information concerning inheritance of α-amylase *per se*. Bingham and Whitmore (1966) found large differences between varieties in α-amylase activity thought in part to be due to the differences in susceptibility to germination in the ear. The germination in the ear always resulted in increased α-amylase activity. There were also large differences between varieties in the α-amylase activity of the grain which had no visible indication

of germination. They concluded that in breeding for sprouting resistance, progress would be most rapid if both low α-amylase activity and resistance to germination in the ear are considered.

Gale (1976) using means components of variation found that additive and dominance genetic effects had significant contribution to α-amylase activity in two out of three crosses of wheat. The additive genetic variability for α-amylase activity suggests that there is scope for selection of the desired level of α-amylase activity.

Bhatt *et al.*, (1976) found an F_2 segregation ratio of 359 low:256 high α-amylase activity which fitted to a 9:7 ratio (x^2 = 1.1275; p lies between 0.50 and 0.25). This suggested that the character may be governed by two major complementary genes.

Heritability estimates of α-amylase activity seem to be very low. Day *et al.*, (1955) working with three barley crosses found broad sense heritability estimates (34.2%, 30.6% and 32.5%) of diastatic activity for crosses 1, 2 and 3 respectively. Bhatt *et al.*, (1976) working with wheat found broad sense heritability estimate of 59% for α-amylase activity. Gordon (1980) also working with wheat found very low narrow sense heritability estimates of 12% and 13% for base level and sprouted level of α-amylase activity respectively. He concluded that because of the low heritability α-amylase may not always be a useful selection criterion. Bingham and Whitmore (1966) stated that although no full genetic analysis of the inheritance of α-amylase activity was available, as a breeding objective there was little difficulty in isolating acceptable lines. Persson (1976) was able to develop a rye variety (Otello) as a result of low α-amylase selection for sprouting resistance.

On the whole, the genetic aspects of pre-harvest sprouting are still not well understood. Many factors are involved in an interacting manner, thus making it difficult to do genetic analysis. Because correlations have been frequently made between high α-amylase activity and high pre-harvest sprouting (Bingham and Whitmore, 1966; Bhatt *et al.*, 1976; and Derera *et al.*, 1976) selection procedures in breeding for resistance have often involved selection of lines with low α-amylase activity (Hwang and Varriano-Marston, 1980).

CHAPTER III

MATERIALS AND METHODS

3.1 PARENTAL CHARACTERIZATION

RL 4137, UM 55-3 and BW 47 lines of common bread wheat (*Triticum aestivum* L.) were used as parental lines. RL 4137 has a long stable dormancy, resistance to sprouting and low α -amylase activity. Owing to its excellent sprouting resistance, RL 4137 was used in the development of the sprouting resistant cultivar 'Columbus' (Campbell and Czarnecki, 1981).

UM 55-3 and BW 47 are both characterised as sprouting susceptible and have a high α-amylase activity. Both RL 4137 and BW 47 were developed at the Agriculture Canada Research Station, Winnipeg, Manitoba. The line UM 55-3 was developed by the Plant Science Department, University of Manitoba. The origin and pedigrees of the parental lines are indicated in Table 1.

3.2 CROSSING PROCEDURE

The crosses made were as follows:

1. UM 55-3 x RL 4137
2. BW 47 x RL 4137

In the spring of 1982, F_1 and parental generations were space planted in the field. F_1 plants were selfed to produce F_2 and simultaneously backcrossed to the parents yielding BC_1 and BC_2. Similar crossing procedure was done in the growth chamber in the summer of 1982 to supplement seed from the field. Additional F_1 seeds from each cross which were required were generated in the greenhouse, in

TABLE 1 Origin and Pedigree of Parents

PARENT	ORIGIN	PEDIGREE[1]
RL 4137	Agric. Canada Winnipeg, Canada	Frontana /3/ McMurachy / Exchange //2* Redman /4/ Thatcher* 6/ Kenya Farmer
UM 55-3	Plant Sci. Dept. U. of Manitoba	Glenlea / High Protein Intercross Composite
BW 47	Agric. Canada Winnipeg, Canada	Neepawa* 6 / Pompe

1 Pedigree is written according to Purdy *et al.*, (1968).

the fall of 1982. At the same time F_2, BC_1 and BC_2 of both crosses were selfed in the greenhouse yielding single heads per plant of F_3, BC_1F_2 and BC_2F_2 respectively.

The inheritance of sprouting was undertaken in two parts. Part one involved the measurement of α-amylase activity on single plant basis. Part two involved visual assessments of sprouting.

3.3 INHERITANCE AS MEASURED BY α-AMYLASE ACTIVITY

3.3.1 Field Plots

Designated populations given in Tables 2 and 3 were field grown in the spring of 1983. The single plants were completely randomized within each cross in order to equalize environmental variance due to soil heterogeneity. Seeds were hand planted at a spacing of 61 x 61 cm apart in order to eliminate the effects of interplant competition on the estimation of genetic parameters. Because of the abnormally hot and dry periods during the growing season, a number of the plants did not reach maturity. This of course reduced the number of harvested plants in every generation.

In the middle of the growing season, the plants were tagged for identification purposes. On August 29, 1983 the tillers of individual plants were tied into bundles and then cut.

A common observation in Western Canada has been that windrowed wheat is more susceptible to sprouting than standing wheat. Clarke (1983) attributed the difference in sprouting susceptibility between standing and windrowed wheat to higher moisture contents and slower drying following rains in windrowed compared to standing. With this

TABLE 2 Generations and Numbers of Plants per Generation, Planted in the Field for Cross UM 55-3 x RL 4137

GENERATION	NUMBER OF PLANTS
P_1 (UM 55-3)	30
P_2 (RL 4137)	30
F_1	30
F_2	220
BC_1 (P_1 x F_1)	160
BC_2 (P_2 x F_1)	160

TABLE 3 Generations and Numbers of Plants per Generation, Planted in the Field for Cross BW 47 x RL 4137

GENERATION	NUMBER OF PLANTS
P_1 (BW 47)	30
P_2 (RL 4137)	30
F_1	30
F_2	220
BC_1 (P_1 x F_1)	160
BC_2 (P_2 x F_1)	160

in mind, on August 29, 1983, the single plant bundles were placed in a single layer on a thick bed of wheat border row in order to simulate a windrow (Fig. 1).

The susceptible parents were used to monitor sprouting levels before harvesting. Falling Number as described by Hagberg (1961) was determined on 7 gms of ground wheat of the two susceptible parents at harvest. The FN of the susceptible parents UM 55-3 and BW 47 were 390 and 428 seconds respectively. Thirteen days later, the FN dropped to 63 and 125 seconds for UM 55-3 and BW 47 respectively. Because of the tremendous drop in FN, the single plants were then removed from the field on September 13, 1983. The weather parameters during the first two weeks prior to windrowing and two weeks after are given in Appendix Table 1.

The heads were threshed using a single plant thresher. The seeds were further separated from chaff to ensure that only clean seed remained for laboratory determination of α-amylase activity.

3.4 INHERITANCE AS MEASURED BY VISUAL ASSESSMENT

3.4.1 Field Plots

Designated populations given in Tables 4 and 5 were field grown in the spring of 1983. Seed from a single head was planted in a 3 M long row using a four-row seeder. The rows were 30 cm apart. The main emphasis was placed on a plant breeder's ability to visually select the resistant plants under natural field conditions.

On August 22, 1983, the rows were lodged in sequence, i.e. one row onto the next to simulate a windrow as indicated in Fig. 2. The simulated windrows were periodically checked for visual sprouting on a weekly basis for approximately eight weeks. The weather parameters during the first two weeks prior to windrowing and eight weeks after

FIG. 1 Single Plant Bundles

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TABLE 4 Generations and Numbers of Head Rows per Generation Planted in the Field for Cross UM 55-3 x RL 4137

GENERATION	NUMBER OF ROWS
P_1 (UM 55-3)	20
P_2 (RL 4137)	20
F_3	208
BC_1 F_2 (S)[1]	104
BC_2 F_2 (S)[1]	104

1 (S) indicates selfed backcrosses

TABLE 5 Generations and Numbers of Head Rows per Generation Planted in the Field for Cross BW 47 x RL 4137

GENERATION	NUMBER OF ROWS
P_1 (BW 47)	20
P_2 (RL 4137)	20
F_3	208
BC_1 F_2 (S)[1]	104
BC_1 F_2 (S)[1]	104

1 (S) indicates selfed backcrosses

FIG. 2 Lodged Head Rows

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are given in Appendix Table 2. On October 14, 1983 substantial differential sprouting among the generations had occurred, hence they were cut and visual sprouting scores made on the spikes.

3.5 MEASUREMENTS OF SPROUTING

3.5.1 Determination of α-Amylase Activity

Grain of single plants given in Tables 2 and 3 were used in the determination of α-amylase activity. However, as indicated earlier, not all the plants indicated were used as some plants did not reach maturity and some did not yield adequate seed. α-Amylase activity was measured by a model 191 Grain Amylase Analyzer (Perkin-Elmer Corp., Coleman Instruments Div., Oak Brook, IL 60521). The procedures used were based on those described by Kruger and Tipples (1981) who modified the procedures of Campbell (1980). The instrument measures the light scattered from the disperse phase of a suspension at right angles to the axis of the exciting light. The intensity of this light is directly related to the concentration of the dispersed particles. As α -amylase cleaves the starch substrate into smaller soluble products, the light reflected diminishes and the rate of clearing can be measured. The more the enzyme activity, the greater the rate of clearing. A more horizontal line on the chart paper indicates a higher activity. Conversely, a more vertical line indicates a lower activity.

3.5.1.1. Preparation of Buffer

The sodium acetate buffer was prepared by dissolving sodium acetate (4.1 g) and calcium chloride (0.1110 g) in distilled deionized

water. The pH was brought to 5.5 with dilute acetic acid. An Accumet pH Meter Model 610 was used to set the pH of the buffer throughout the experiment. The final solution was brought to 1 litre with distilled deionized water.

3.5.1.2 Preparation of Substrate

One gram of β-limit dextrin of waxy maize starch was added into a 250 ml flask containing 200 mls of 0.05 M sodium acetate buffer (pH 5.5, 1mM $CaCl_2$). The solution was magnetically stirred until all the β-limit dextrin was dispersed in the solution. With flask covered to prevent dust contamination the solution was heated in a boiling (100°C) water bath for 10 min to give a uniform and stable suspension, then allowed to cool while stirring for 1 hr. The solution was then centrifuged for 10 min in a bench top centrifuge at 3,000 r.p.m. (setting No. 7) to remove undissolved particles. The pooled supernatants were kept in the refrigerator (4°C).

3.5.1.3 Preparation of Stock α Amylase Solution

The α-amylase used was from *Aspergillus oryzae*. Zero point one gram of the enzyme was slowly added to magnetically stirring 50 mls of 0.05 M sodium acetate buffer (pH 5.5, 1 mM $CaCl_2$). When dissolved, the solution was brought to 100 mls with drops of the buffer. The solution was then centrifuged in Beckman model J2-21 centrifuge, at 10,000 r.p.m. for 10 min. The clarified stock solution was kept in the refrigerator (4°C).

3.5.1.4 Preparation of Samples

The samples were prepared in two phases - (1) grinding and (2) enzyme extraction.

A grain sample of 5 g was taken from each single plant (Tables 2 and 3) for grinding. The sample was ground in a Udy cyclone mill using a 0.5 mm round hole mesh sieve. The grist was thoroughly mixed.

A grist sample of 1 g was then placed into a rubber capped test tube. To this grist, 5.0 ml of 0.05 M sodium acetate buffer (pH 5.5, 1 mM $CaCl_2$) was added and the tubes capped. An initial gentle hand shaking of the mixture was required to facilitate easy mixing. The tubes were then held tightly in position on a Multipurpose Rotator model 150V. The rotator was set to rotate at 10 r.p.m. during which the enzyme was extracted for one hour. The extract solution was allowed to settle on the bench for 30 mins. The partly cloudy extraction solution was then filtered through a Whatman Glass Microfibre filter paper of 5.5 cm diameter (GF/C). The clear extract was kept at 4°C.

3.5.1.5 Estimation of α-Amylase Activity

A standard curve was prepared using a commercial α-amylase preparation of *Aspergillus oryzae*. One ml of stock amylase solution was diluted with 0.05 M sodium acetate buffer (pH 5.5, 1 mM $CaCl_2$) to 50.0 ml. Six levels of α-amylase activity used to produce standard were prepared by diluting 10, 20, 50, 100, 150 and 200 ml of the above solution (0.001 gm/ml) to 10.0 ml of buffer. Activity in all test samples were reported in terms of this standard α-amylase. The day to day standard curves are meant to take care of minor day to day variations in substrate turbidity. One assumption here is that the activity of the commercial

enzyme preparation is relatively stable and thus will allow comparison of test activities over a long period of time.

The procedures for the standard enzyme dilutions and test samples were the same. Three millilitre aliquots of the substrate solution were measured into cuvettes, the cuvettes capped and placed in the incubation block of the instrument. The cuvettes were allowed to equilibrate at 37°C for a minimum of 20 min. 200 ul of enzyme solution was added to an equilibrated cuvette, the cuvette capped and inverted gently 5 times to mix enzyme and substrate. The cuvette was immediately placed in the measurement well of the instrument and cover closed. The recorder was turned on and needle set to 95 on the nephelos scale. The chart paper was set to move (1 in/min) and reaction allowed to proceed for approximately 3 mins. The recorder units taken after 3 mins were used to determine the unknown enzyme activity.

It is worth noting that the enzyme activity of the samples was expressed in ug/ml relative to the activity of *Aspergillus oryzae*. The *Aspergillus oryzae* was used for the standard curves throughout the experiment. Possibly, if a different enzyme is used, the relative activity values may be different. However, the main purpose in the study was to distinguish the different genotypes. Therefore as long as the same enzyme is used throughout, the genetic analysis should not be offset.

3.5.2 Visual Assessment of Sprouting

Visual assessment was done in two phases. First it was done on the spikes of the head rows in the field. Secondly, it was later done on threshed grain of the head rows.

On October 14, 1983, lodged head rows of populations in Table 3 and Fig. 2 were cut and tied into bundles of comparable sizes. First, the parental materials were observed visually for visible sprouting (roots and shoots) on the spikes. Secondly, the subsequent generations were observed. It seemed possible in some generations to broadly classify the material into three classes based on visible sprouting. The three classes were as follows:

1) Extensively sprouted (a majority of the heads had visible sprouts)
2) Moderately sprouted (intermediate number of visible sprouts)
3) Lightly sprouted (approximately less than 5% of the heads had visible sprouts)

It was assumed that materials in the extensively sprouted class would not provide for any differential sprouting on the threshed grain. For this reason, all materials (except UM 55-3) that fell into the extensively sprouted class were omitted in the visual assessment of threshed grain. All of the two BC_1F_2's, and some F_3's were omitted.

The remaining materials were threshed and seeds of individual rows bulked. A sample of 100 seeds was then taken from each bulk for visual assessment of sprouting. Grain was considered sprouted where the seed coat above the embryo had completely ruptured exposing a visible shoot. The number of seeds sprouted was expressed as a

percentage of the total seeds observed.

3.6 STATISTICAL METHODS

The mean, standard deviation, standard error of the mean and variance of α-amylase activity were calculated for each generation according to Steel and Torrie (1980). Variances of the means for each generation were tested for homogeneity using a Bartlett's test (Steel and Torrie, 1980).

The means of the six generations were used to estimate the magnitudes of gene action governing the trait investigated. A simple additive-dominance model was tested. The methodology used was a joint scaling test proposed by Cavalli (1952) and outlined by Mather and Jinks (1982). This involved either a weighted or unweighted least squares analysis. A weighted least squares analysis assumes that the variances of the mean are not homogeneous. Therefore the generation means are weighted accordingly, with the appropriate weights being the reciprocal of squared standard errors of each generation mean. Unweighted least squares analysis assumes that the variances of the mean are homogeneous, i.e. the means are known with equal precision. A Chi-square analysis of observed and expected generation means was used to test the fit of the genetic model to the data.

An analysis using the variances of the generations was also performed on both crosses. The variation observed in the parental and F_1 generations, was used to estimate the non-heritable variation while the overall variance in the F_2 and the backcross generations, was partitioned into additive, dominance and environmental components as outlined by Mather and Jinks (1982). The model was estimated using

unweighted least squares approach.

Heritability estimates were also calculated from the estimated values of genetic components of variation in the variance analysis.

The visually assessed materials of the head rows were genetically tested in two phases. In phase one, Chi-square testing for the goodness of fit of observed to the expected ratios if one, two or three genes are involved in sprouting, were done on the spike visual assessment of the head rows. Similar Chi-square tests were done in phase two using the observed % sprouted on the threshed seeds of the head row spikes.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 INHERITANCE AS MEASURED BY α-AMYLASE ACTIVITY

4.1.1 General Comparisons

The data for the two crosses are presented in Tables 6 and 7. The generation means were compared in pairs by the t-test at α = 0.05 level of significance.

In the cross UM 55-3 x RL 4137 (Table 6), the mean α-amylase activity of the sprout resistant parent (RL 4137) was significantly lower than that of P_1. The mean F_1 value was slightly lower than the mid-parent value of 1.85, which suggested a partial dominance for low α-amylase activity in this cross. The mean of F_1 was significantly different from those of the two parents. The mean of P_1 was not significantly lower than that of its backcross (BC_1F_1).

The mean of P_2 was however significantly below that of its backcross (BC_2F_1). The F_2 mean was significantly above that of F_1.

For the cross BW 47 x RL 4137 (Table 7), the mean α-amylase activity of the sprout resistant parent was significantly lower than that of the susceptible parent. The F_1 mean approximated the mid-parent value of 1.23, which suggested additive gene action in this cross. The mean of F_1 was significantly different from those of the two parents. The mean of P_1 was not significantly above that of its backcross (BC_1F_1). However, the mean of P_2 was significantly lower than that of its backcross (BC_2F_1). The means of F_1 and F_2 were not significantly different from each other.

In both crosses RL 4137 had the lowest mean α-amylase activity

TABLE 6 α-Amylase Activity of UM 55-3, RL 4137 and Subsequent Generations

GENERATION	No. of PLANTS	MEAN[1] (µg/ml)	VARIANCE
UM 55-3 (P_1)	16	3.17 ± 0.19	0.57
RL 4137 (P_2)	17	0.54 ± 0.09	0.15
F_1	16	1.38 ± 0.16	0.42
F_2	170	2.82 ± 0.14	3.15
BC_1F_1	124	3.33 ± 0.15	2.68
BC_2F_1	102	1.96 ± 0.15	2.36

1 X^2 homogeneity for the variance of the mean = 10.18

P (0.05) = 11.1

TABLE 7 α-Amylase Activity of BW 47, RL 4137 and Subsequent Generations

GENERATION	No. of PLANTS	MEAN[1] (µg/ml)	VARIANCE
BW 47 (P_1)	19	1.92 ± 0.23	0.99
RL 4137 (P_2)	20	0.54 ± 0.10	0.18
F_1	24	1.21 ± 0.19	0.84
F_2	168	1.12 ± 0.01	1.13
BC_1F_1	85	1.41 ± 0.12	1.26
BC_2F_1	110	0.91 ± 0.08	0.67

1 X^2 homogeneity for the variance of the mean = 88.37

P (0.05) = 11.1

compared with the other generations means. The susceptible parents appeared to have the highest α-amylase activity compared with those of the other generations.

The consistently low α-amylase activity of RL 4137 is expected. Possibly, due to the strong dormancy in RL 4137, the two weeks of field weathering (Appendix Table 1) did not activate its enzyme system to the same extent as in the other generations. Alternatively, RL 4137 could possess less base level α-amylase than the other generations. Campbell and Czarnecki (1981) described RL 4137 as a red seeded cultivar with outstanding resistance to sprouting and weathering.

An examination of the variances for each of the generations of the cross UM 55-3 x RL 4137 (Table 6) indicated a large genetic component to the variability of α-amylase activity, as the variances for the segregating populations were larger than the mean of the parental and F_1 variances. The F_2 variance was the largest, whereas the variance of RL 4137 was the smallest compared to variances of other generations.

The variances of each of the generations of the cross BW 47 x RL 4137 (Table 7) indicated a relatively minor genetic component to the variability of α-amylase activity as the variances for the segregating populations were not much larger than the mean of the parental and F_1 variances.

Again, in this cross (Table 7), RL 4137 had the lowest variance compared with variances of other generations, while BC_1F_1 had the highest variance.

In both crosses, (Table 6 and Table 7), the variances of

RL 4137 and those of its backcrosses tended to be relatively low. Whereas the variances of the susceptible parents and those of their backcrosses tended to be relatively large. The reason for such relatively large variabilities is not readily understood. Possibly, if a sample contains a single grain that is highly sprouted, the α-amylase activity of such a sample may well be inflated. Kruger and Tipples (1980) reported that one additionally highly sprouted kernel in 90 grams would noticeably change Falling Number or Amylograph.

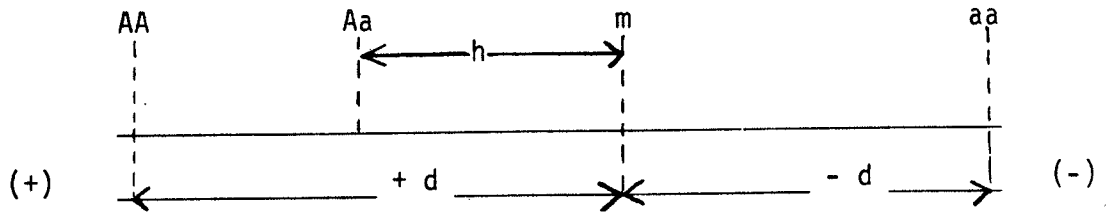
4.2 GENETIC ANALYSIS ON α-AMYLASE ACTIVITY

4.2.1 Means Analyses

Genetic components of variation that are frequently partitioned are additive and dominance effects. The other genetic variations include epistatic effects. The additive component describes genes either at the same locus or at different loci which act independently by increasing or decreasing the phenotypic expression. The dominance part describes the intra-allelic interaction between genes at the same locus such that one allele masks the effect of the other to varying degrees. While the epistatic part involves the interaction between genes at different loci such that the expression at one gene locus is influenced by another gene locus (Allard, 1960).

In this study, additive and dominance gene effects were estimated for α-amylase activity. The means of the six standard generations were used in a procedure known as the joint scaling test proposed by Cavalli (1952) and outlined by Mather and Jinks (1982). The method assumes that the parental lines are true breeding. The genetic relationship between the two parents and their F_1 may be

illustrated in the following manner:



The joint scaling test based on the above scale estimates m, [d] and [h]. Where m, [d] and [h] represent mid-parent value, additive gene effects and dominance gene effects respectively.

Taking the mid-parent as the origin used in measuring the effect of various combinations of genes at a single locus, d represents the additive gene effect either in the positive or negative direction from the origin, and h indicates the non-additive influence. On the same scheme, a single locus situation, for example, may be used to obtain genotypic and phenotypic expectations of parents and their subsequent generations as follows:

Generation	Genotypic Expectation	Phenotypic Expectation
P_1	AA	m + d
P_2	aa	m - d
F_1 AA x aa	Aa	m + h
F_2 Aa x Aa	¼ AA + ½ Aa + ¼ aa	¼ (m+d) + ½ (m+h) + ¼ (m-d) ¼ m + ¼ d + ½ m + ½ h + ¼ m - ¼ d = m + ½ h
BC_1 Aa x AA	½ AA + ½ Aa	m + ½ d + ½ h
BC_2 Aa x aa	½ Aa + ½ aa	m - ½ d + ½ h

In the case of a phenotype of a homozygous individual with respect to a character governed by a number of genes, the phenotype will have an average measurement of

$$m + S(+d) + S(-d)$$

where S (+d) indicates the summed effects of the loci where the genes contribute a positive effect on the character in question and vice versa for S (-d). Meanwhile m is a constant "base" level dependent on the effects of genes not under consideration and effects of environment.

A summary of the phenotypic expectations is as follows:

GENERATION	PHENOTYPIC EXPECTATION		
	m	[d]	[h]
P_1	1	1	0
P_2	1	-1	0
F_1	1	0	1
F_2	1	0	0.5
BC_1	1	0.5	0.5
BC_2	1	-0.5	0.5

The joint scaling test estimates the parameters m [d] and [h]. Six equations are prepared for the estimation and these are obtained by equating the observed family means to their expectations in terms of these three parameters. The coefficients used for the components of means are as summarized for the phenotypic expectations.

To solve the six equations, a weighted least squares analysis program Version 83.1 by Dr. L. Sernyk (of Dept. of Plant Science, U. of Manitoba) was used. The weighted analysis assumes that the means are not known with equal precision. Therefore the generation means and their expectations are weighted accordingly, with the appropriate weights being the reciprocal of squared standard errors of each generation mean.

The expected generation means are derived from the estimates of the three parameters. For example, mean of P_1 in cross UM 55-3 x

RL 4137 is expected to be 1 m + [d]. The estimates are tested for difference from zero. The X^2 goodness of fit of the model to the data is tested. If the estimates are not significantly different from zero then their contribution to the character is regarded as non-significant. On the other hand, if the estimates are significantly different from zero, then it implies that they have a significant contribution to the character in question.

A non-significant X^2 implies that the model fits the data. A significant X^2 implies that the model is not adequate for the data. In a case where an estimate is not significantly different from zero but X^2 is non-significant, analysis can be re-done excluding such a parameter, thereby providing a better estimate of the significant components and increasing the degrees of freedom.

The estimates of the genetic parameters for α-amylase activity for both crosses using generation means shown in Tables 6 and 7 are presented in Tables 8, 9, 10 and 11.

In the cross UM 55-3 x RL 4137 (Tables 8 and 9), the estimate of additive gene effects were large and significant compared to the non-significant dominance effects. The sign of h was negative indicating some dominance towards low α-amylase activity.

Similarly, additive effects were large and significant in the cross BW 47 x RL 4137. Again, the dominance effects were relatively small and insignificant. This may not necessarily indicate that the individual values of h (dominance) for the various loci must be zero. Warner (1952) stated that the individual values of h for the various loci may each have a real magnitude but be of different signs such that their algebraic sum is zero. The magnitude of [h] is a measure of

TABLE 8 Estimates of Genetic Parameters for α-Amylase Activity for Cross UM 55-3 x RL 4137 3 Parameter Model

PARAMETER	
M	2.24 ± 0.48*
[d]	1.33 ± 0.47*
[h]	- 0.09 ± 0.89
χ^{2}[1]	1.67 (3df)
P (0.05)	7.81

1 Test of the fit of the model to the data.

* Significant at the 0.05 probability level.

TABLE 9 Estimates of Genetic Parameters for α-Amylase Activity for the Cross UM 55-3 x RL 4137 2 Parameter Model

PARAMETER	
m	2.20 ± 0.26*
[d]	1.33 ± 0.41*
χ^{2}[1]	1.68 (4df)
P (0.05)	9.49

1 Test of the fit of the model to the data.

* Significant at the 0.05 probability level.

TABLE 10 Estimates of Genetic Parameters for α-Amylase Activity for Cross BW 47 x RL 4137 3 Parameter Model

PARAMETER	
m	1.16 ± 0.10*
[d]	0.60 ± 0.10*
[h]	0.02 ± 0.19
X^{2}[1]	1.39 (3df)
P (0.05)	7.81

1 Test of the fit of the model to the data.

* Significant at the 0.05 probability level.

TABLE 11 Estimates of Genetic Parameters for α-Amylase Activity for Cross BW 47 x RL 4137 2 Parameter Model

PARAMETER	
m	1.16 ± 0.05*
[d]	0.60 ± 0.08*
X^{2}[1]	1.40 (4 df)
P (0.05)	9.49

1 Test of the fit of the model to the data.

* Significant at the 0.05 probability level.

phenotypic dominance while genic dominance refers to the magnitude of the various individual values of h. Thus the presence of phenotypic dominance ([h] ≠ 0) is an indication of the presence of genic dominance, at least in some pairs, while an absence of phenotypic dominance (no dominance, [h] = 0) does not preclude the presence of genic dominance.

Worthy of note is that the sign of the parameter d depends upon the parents being considered as P_1 or P_2. For example, in cross UM 55-3 x RL 4137, if UM 55-3 is considered as P_1 and RL 4137 as P_2, the estimate of parameter d is positive. However, if RL 4137 is considered as P_1 and UM 55-3 as P_2, the estimate is negative. The sign of h is unaffected (see phenotypic expectation equations).

The major contribution by additive gene effects in both crosses was indicated by the relative magnitude of parameter d to m. In addition, the estimate of additive effects was significant in both crosses.

Gale (1976) also using means of components of variation found that additive and dominance gene effects had a significant contribution to α -amylase activity in two out of three crosses. The additive genetic variability for α-amylase suggests that there is scope for selection of the desired level of α -amylase activity.

4.2.2 Variance Analyses

An analysis using the variances of the six generations was also performed on data of both crosses based on procedures outlined by Mather and Jinks (1982). They have demonstrated from a symbolical analysis of variance that the genetic variance of an F_2 population is equal to ½ D + ¼ H, where D represents that portion of the variance attributable to the additive gene effects and H represents the portion due to deviations

from additivity (dominance). Similarly, they showed that the sum of genetic variances of BC_1 and BC_2 is equal to ½ D + ½ H where BC_1 and BC_2 represent the backcrosses to the two parents. Because the variation in the non-segregating generations (P_1, P_2 and F_1) is exclusively environmental, they provide estimates of E_w (environmental variance). F_2 is the first segregating generation in which the total variance has two components i.e. genetic and environmental. Under the assumption that E_w is comparable in all three segregating generations (F_2, BC_1 and BC_2) the following equations can be obtained:

$$V_{F_2} = (\tfrac{1}{2})\ D + (\tfrac{1}{4})\ H + E_w$$

$$V_{BC_1} + V_{BC_2} = (\tfrac{1}{2})\ D + (\tfrac{1}{2})\ H + 2E_w$$

A summary of the phenotypic variance expectations from Mather and Jinks (1982) is given below: these were used as coefficients for the components of variance analyses.

GENERATION	PHENOTYPIC EXPECTATION			
	D	H	F	E
P_1	0	0	0	1
P_2	0	0	0	1
F_1	0	0	0	1
F_2	0.50	0.25	0	1
BC_1	0.25	0.25	-0.50	1
BC_2	0.25	0.25	0.50	1

The analyses of the variances of the experimental generations as outlined by Mather and Jinks (1982) provided estimates of D, H, and E as well as a fourth component (F) which arose from partitioning of the variation in the backcross generations. The estimates of the genetic parameters for α -amylase activity for both crosses are shown in Tables

12, 13, 14 and 15.

The results of the analysis of variances of the experimental generations for the cross UM 55-3 x RL 4137, are presented in Tables 12 and 13. There was clearly a large genetic component of the observed variance comprised of both an additive and a dominance component. The dominance component was large compared to the additive component. The standard error associated with the dominance was large relative to that for the additive component. A significant environmental component was also detected, as expected.

For the cross BW 47 x RL 4137, the results of the analysis of variances of the experimental generations are presented in Tables 14 and 15. The estimate of the additive component was larger compared to that of the dominance component. The precision of the estimates was poor, as evidenced by the large standard errors associated with the estimates, and in particular with the dominance component. The estimates were not significant, this of course reflects the smaller difference between the parental lines for the trait. Consequently the segregants that would result from a cross of these parents would be less variable as previously observed in Table 7.

4.2.3 Heritability Estimates

Heritability specifies the proportion of the total variability that is due to genetic causes, or the ratio of the genetic variance to the total variance. It can be described by the formula:

$$H = \frac{V_g}{V_g + V_e}$$

where V_g = genetic variation

and V_e = environmental variation

TABLE 12 Estimates of the Components of Variance for α-Amylase Activity for the Cross UM 55-3 x RL 4137 4 Parameter Model

COMPONENT	
D	2.72 ± 1.09*
H	5.62 ± 1.64*
F	- 0.42 ± 0.41
E	0.38 ± 0.13*
X^2[1]	0.09 (2df)
P (0.05)	5.99

1 Test of the fit of the model to the data.

* Significant at the 0.05 probability level.

TABLE 13 Estimates of the Components of Variance for α-Amylase Activity for the Cross UM 55-3 x RL 4137 3 Parameter Model

COMPONENT	
D	2.51 ± 1.08*
H	6.04 ± 1.61*
E	0.38 ± 0.13*
X^2[1]	0.15 (3df)
P (0.05)	7.81

1 Test of the fit of the model to the data.

* Significant at the 0.05 probability level.

TABLE 14 Estimates of the Components of Variance for α-Amylase Activity for the Cross BW 47 x RL 4137 4 Parameter Model

COMPONENT	
D	0.67 ± 2.11
H	0.52 ± 3.14
F	- 0.59 ± 0.61
E	0.67 ± 2.70*
X^{2}[1]	0.37 (2df)
P (0.05)	5.99

1 Test of the fit of the model to the data.

* Significant at the 0.05 probability level.

TABLE 15 Estimates of the Components of Variance for α-Amylase Activity for the Cross BW 47 x RL 4137 3 Parameter Model

COMPONENT	
D	0.66 ± 2.08
H	0.52 ± 3.11
E	0.67 ± 0.25*
X^{2}[1]	0.54 (3df)
P (0.05)	7.81

1 Test of the fit of the model to the data.

* Significant at the 0.05 probability level.

Two kinds of heritabilities are well noted, i.e. the broad sense and the narrow sense. The broad sense heritability encompasses all types of gene action including dominance, additive and epistasis, while the narrow sense heritability is a ratio of the additive genetic variation over total phenotypic variation (Allard, 1960). The narrow sense heritability is of particular interest to the plant breeder because it represents that portion of the genetic variance that will respond to selection or is fixable.

Techniques for estimating the degree of heritability are varied and include the use of variance components from an analysis of generation variances. This technique was used in this study.

The heritabilities of α-amylase activity, were calculated using the estimates of the components of variance determined earlier. The following formulae from Allard (1960) were used.

$$\text{Heritability broad sense} = \frac{\frac{1}{2}(D) + \frac{1}{4}(H)}{\frac{1}{2}(D) + \frac{1}{4}(H) + E_W}$$

$$\text{Heritability narrow sense} = \frac{\frac{1}{2}(D)}{\frac{1}{2}(D) + \frac{1}{4}(H) + E_W}$$

where D = variation due to additive gene action

H = variation due to dominance gene action

E_W = variation due to environmental effects

Heritability in the broad sense for α-amylase activity was .87. The narrow sense was .39 which indicated that approximately half of the genetic variance was fixable.

It is worth noting that due to the lack of significant genetic components of variation in the cross BW 47 x RL 4137, heritability

estimates were not calculated for this cross.

The heritability estimates for α -amylase activity *per se* are very scarce in the literature. Bhatt *et al.*, (1976) found broad sense heritability estimate of 0.59 for α -amylase activity in wheat. Gordon (1980) also working with wheat found very low narrow sense heritability estimates of 0.12 and 0.13 for base level and sprouted level of α-amylase activity respectively. In barley, Day *et al.*, (1955) found broad sense heritability estimates of 0.34, 0.30 and 0.32 for diastatic activity of three crosses. Thus the range in the material and methods used for the estimations seem to reflect the amounts of heritabilities.

A general observation using the means analyses indicated that the gene action for α-amylase activity in both crosses is primarily additive. The dominant component was small and insignificant in both crosses. Meanwhile, the variance analyses indicated significant additive, dominance and environmental components in one cross. However, in the other cross only the environmental component was significant.

4.3 INHERITANCE AS MEASURED BY VISUAL ASSESSMENT

4.3.1 General Comparisons

The results on visual assessment of sprouting are given in Table 16.

In the cross UM 55-3 x RL 4137, all the lines of the susceptible parent fell into the extensively sprouted class. Meanwhile, the resistant lines (RL 4137) fell into the lightly sprouted class. The F_3 and BC_2F_2 families segregated into the three classes with more families being in the extensively sprouted class. The BC_1F_2 all fell into the extensively sprouted class.

TABLE 16 No. of Families per Generation and Their Mean % Sprouted (in Parentheses) of Both Crosses

CROSS	GENERATION	CLASSES			N
		EXTENSIVELY SPROUTED	MODERATELY SPROUTED	LIGHTLY SPROUTED	
UM 55-3	UM 55-3	5 (71.0)			5
x	RL 4137			5 (6.4)	5
RL 4137	F_3	84	18 (29.5)	1 (13.0)	103
	BC_1F_2	10			10
	BC_2F_2	51 (25.1)	32 (15.2)	17 (7.2)	100
BW 47	BW 47		5 (13.0)		5
x	RL 4137			5 (6.4)	5
RL 4137	F_3	67	32 (20.4)	5 (9.8)	104
	BC_1F_2	10			10
	BC_2F_2	58 (16.1)	28 (12.9)	17 (8.7)	103

The susceptible parents had a higher percentage sprouted than that of the resistant parent. In every generation, the trend was such that the extensively sprouted class had the highest percentage sprouted followed by the moderately sprouted and finally the lightly sprouted class. This observation may appear to indicate a general agreement of visual assessment of the spikes and visual assessment of the threshed grain.

It is worth noting that the families in cross UM 55-3 x RL 4137 as groups were generally more advanced in their sprouting stages than those of the other cross.

4.4 GENETIC ANALYSES ON VISUAL ASSESSMENT

The material assessed as lightly sprouted on the spikes were classified as resistant and those in the moderately and extensively sprouted classes were classified as susceptible. For the seed analysis, the plants showing 6% sprouted or less were classified as resistant and those > 6% were classified as susceptible. Only a few resistant plants were recovered. This observation tended to imply that resistance appears to be governed by factors that appear to behave recessively.

To illustrate the expected genotypic ratios a one gene difference will be assumed where AA and aa represent susceptible and resistant parents respectively. The resulting F_1 between the two parents would be expected to be Aa genotype. The F_2's from selfed F_1's would be expected to segregate genotypically into the following ratio ¼AA: ½Aa: ¼aa. The backcross to the susceptible parent would segregate into 1AA: 1Aa ratio. The segregation of the backcross to the resistant parent would be 1Aa: 1aa. Since it may be very difficult to distinguish phenotypically between Aa and AA genotypes, they may be combined into one category.

Segregation ratios in F_3 and BC_2F_2 families were tested for both spike and seed visual assessments. All ratios were tested for goodness of fit to the 3 susceptible: 1 resistant and 1:1 ratios expected if susceptibility is controlled by one gene, the 15:1 and 3:1 ratios expected if two genes are involved and 63:1 and 7:1 ratios expected if three genes are involved in F_3 and BC_2F_2 families respectively.

The results of X^2 tests based on spike assessments are presented in Tables 17 and 18. In cross UM 55-3 x RL 4137, the observed susceptible and resistant F_3's fitted to the ratio of 63:1 expected if three genes govern sprouting (Table 17). The BC_2F_2 data for this cross showed a good fit to a 7:1 ratio which confirmed the F_3 segregation ratio of 63:1 (Table 18).

For cross BW 47 x RL 4137 the observed susceptible and resistant F_3's fitted to the ratio of 15:1 expected for a two gene difference (Table 17). The BC_2F_2 data for this cross fitted to ratios of 3:1 and 7:1 expected for a two and three gene difference respectively (Table 18).

The results of X^2 tests based on the seed from the previous spikes are presented in Tables 19 and 20. In cross UM 55-3 x RL 4137, the observed susceptible and resistant F_3's fitted to a 15:1 ratio expected for a two gene difference (Table 19). The BC_2F_2 data for this cross fitted to a 3:1 ratio which confirmed the F_3 segregation ratio of 15:1 (Table 20).

For cross BW 47 x RL 4137 the F_3 data fitted to a 3:1 ratio (Table 19) while the BC_2F_2 data fitted to a 3:1 ratio (Table 20).

In the light of the available information it is likely that there are more genes governing sprouting in the cross UM 55-3 x RL 4137 than in the other cross.

TABLE 17 Segregation of Susceptible and Resistant Families in the F_3 Generations of Both Crosses

Cross	S[1]	R[1]	Total	X^2 goodness of fit to expected ratios: 3:1	15:1	63:1
UM 55-3 x RL 4137	102	1	103	31.40	4.04	0.013* (P = 0.05)
BW 47 x RL 4137	99	5	104	21.55	0.16* (P = 0.05)	5.17

* Fitted to ratio at P = 0.05 level of significance.

1 Spike data

TABLE 18 Segregation of Susceptible and Resistant Families in the BC_2F_2 Generations of Both Crosses

Cross	S[1]	R[1]	Total	X^2 goodness of fit to expected ratios: 1:1	3:1	7:1 or 56:8
UM 55-3 x RL 4137	83	17	100	42.25	3.00* (P = 0.05)	1.46* (P = 0.05)
BW 47 x RL 4137	86	17	103	44.89	3.52* (P = 0.05)	1.17* (P = 0.05)

* Fitted to ratio at P = 0.05 level of significance.

1 Spike data

TABLE 19 Segregation of Susceptible and Resistant Families in the F_3 Generations of Both Crosses

Cross	Sus.[1]	Res.[1]	Total	X^2 goodness of fit to expected ratios: 3:1	15:1
UM 55-3 x RL 4137	19	0	19	5.07	0.42* (P = 0.05)
BW 47 x RL 4137	31	6	37	1.09* (P = 0.05)	4.69

* Fitted to ratio at P = 0.05 level of significance.

1 Seed data.

TABLE 20 Segregation of Susceptible and Resistant Families in the BC_2F_2 Generations of Both Crosses

Cross	Sus.[1]	Res.[1]	Total	X^2 goodness of fit to expected ratios: 1:1	3:1
UM 55-3 x RL 4137	75	25	100	24.01	0.01* (P = 0.05)
BW 47 x RL 4137	74	29	103	18.80	0.39* (P = 0.05)

* Fitted to ratio at P = 0.05 level of significance.

1 Seed data.

CHAPTER V

GENERAL DISCUSSION AND CONCLUSIONS

In breeding for sprouting resistance, the breeder may be interested in knowing the amount of genetic variation available in relation to the environmental variation. In particular, the breeder looks for the additive genetic variance because it can respond to selection or is fixable. Once a knowledge of the gene action governing sprouting resistance is known the breeder can then formulate appropriate breeding methodology for rapid progress.

For both crosses, the means analyses revealed that gene action for α-amylase activity was primarily additive, with a much smaller, insignificant dominance component. Since the dominance were insignificant and measured with a low precision as reflected in their standard errors, it can be assumed that indeed dominance had an insignificant role in the crosses studied. Gale (1976) found significant additive and dominance gene action for α-amylase activity in two out of three crosses.

A moderate additive component of genetic variance in α-amylase activity was also noted for the cross UM 55-3 x RL 4137 using the variance analyses. This was reflected in the moderately low narrow sense heritability estimate for α-amylase activity.

The cross BW 47 x RL 4137 failed to show any significant genetic component of variation. This of course reflects the low variability for the trait between the two parents.

The additive component of gene action conditioning the phenotypic expression of sprouting resistance as measured by α-amylase

activity, implies that improvement can be made for resistance by using a breeding method that would accumulate favourable genes. The backcross method has been frequently used in breeding for sprouting resistance (McEwan 1967, and Campbell and Czarnecki, 1981).

For the visual assessment of sprouting the main emphasis was placed on a plant breeder's ability to visually select the resistant plants under natural field conditions. The genetic analysis, though probably oversimplified implied that sprouting resistance seems to be governed by recessive factors. Bhatt and Derera (1980) were also of the same opinion. While Bhatt *et al.*, (1983) found that sprouting resistance as measured by germination percentage was controlled by recessive factors. This implies that the breeder may be able to fix the desirable genotypes in the later generations. The later generations may seem appropriate because they contain more homozygous material and hence no masking effects by the dominant factors.

There seemed to be more genes governing sprouting in the cross UM 55-3 x RL 4137 than in the other cross. This was also evidenced in the previous analysis by the relatively larger variability in the former cross than in the latter. The gene difference in the cross UM 55-3 x RL 4137 could be two or three. While the other cross could have either one or two based on F_3's or two or three based on the backcross families. The exact number of genes is however not clear. One of the reasons for this discrepancy could be the population sizes, particularly in the F_3's which were rather low.

Both studies of sprouting resistance revealed that it is possible to breed for sprouting resistance. The additive gene action for α-amylase activity implies that favourable genes for resistance

may be accumulated in the desired cultivar. The moderately low narrow sense heritability also indicates that the trait may be expected to respond to selection. The recessive nature of the visual sprouting means that the trait may be fixed in the later homozygous material. The consistently low α-amylase activity and sprouting resistance of RL 4137 after weathering for two and eight weeks (Appendix Tables 1 and 2) respectively makes RL 4137 a valuable source of sprouting resistance for breeding and genetic studies.

In conclusion, therefore, a test for low α-amylase activity together with visual assessment of pre-harvest sprouting may be adequate as selection criteria for pre-harvest sprouting resistance.

LIST OF REFERENCES

Allard, R.W. 1960. Principles of Plant Breeding. John Wiley and Sons. Toronto, p. 75-108.

American Association of Cereal Chemists Approved Methods, 1983. Vol. 1. AACC Method 22-10.

American Association of Cereal Chemists Approved Methods 1983. Vol. 1. AACC Method 22-15.

Baker, R.J., 1981. Inheritance of Seed Coat Color in Eight Spring Wheat Cultivars. Car. J. Plant Sci. 61: 719-721.

Barnes, W.C. and Blakeney, A.B. 1974. Determination of Cereal Alpha Amylase Using a Commercially Available Dye-Labelled Susbtrate. Die Starke 26: 193-197.

Bauer, A. and Black, A.L. 1983. Sprouting in Intact Mature Spikes of Hard Red Spring Wheat. Agron. Jour. 75: 1016-1022.

Belderok, B. 1966. Report on Trials in 1963 on Dormancy and Sprouting (in Cereals). Fld. Crop. Absts. 19: 104-105.

Belderok, B. 1968. Seed Dormancy Problems in Cereals. Fld. Crop. Absts., Vol. 21, No. 3; 202-211.

Belderok, B. 1976. Physiological-biochemical Aspects of Dormancy in Wheat. Cereal Res. Comm. Vol. 4, No. 2: 133-137.

Belderok, B. 1976 b. Changes in Seed Coat of Wheat Kernels During Dormancy and After Ripening. Cereal Res. Comm. Vol. 4, No. 2: 165-171.

Bingham, J. and Whitmore, E.T. 1966. Varietal Differences in Wheat in Resistance to Germination in the Ear and α Amylase Content of the Grain. J. Agric. Sci. 66: 197-201.

Bhatt, G.M. and Derera, N.F. 1980. Potential Use of Kenya 321-Type Dormancy in a Wheat Breeding Programme Aimed at Evolving Varieties Tolerant to Pre-Harvest Sprouting. Cereal Res. Comm., Vol. 8, No. 1: 291-295.

Bhatt, G.M., Derera, N.F. and McMaster, G.J. 1977. Utilization of Tom Thumb Source of Wheat Breeding Programme. Euphytica 26: 565-572.

Bhatt, G.M., Derera, N.F. and McMaster, G.J. 1976. Breding White-Grained Spring Wheat for Low Alpha-Amylase Synthesis and Insensitivity to Gibberellic Acid in Grain. Cereal Res. Comm. Vol. 4, No. 2: 245-249.

Bhatt, G.M., Ellison, F.W. and Mares, D.J. 1983. Inheritance Studies in Dormancy in Three Wheat Crosses. In: Inter. Symp. on Pre-Harvest Sprouting in Cereals. (ed: Kruger, J.E. and LaBerge, D.E.), 274-278. Westview.

Briggle, L.W. 1980. Pre-Harvest Sprout Damage in Wheat in the U.S. Cereal Res. Comm., Vol. 8, No. 1, 245-250.

Briggs, D.E. 1962. Gel-diffusion Method for the Assay of α-Amylase. J. Inst. Brew. 68: 27-32.

Briggs, D.E. 1972. Hormones and Carbohydrate Metabolism in Germinating Cereal Grains. In: Biosynthesis and its Control in Plants. (ed: Milborrow, B.V.) Proc. Phyto. Chem. SOC. Symp. Academic Press, London. pp. 219-277.

Brinkman, M.A. and Luk, T.M. 1979. Relationship of Spike Nodding Angle and Kernel Brightness Under Simulated Rainfall in Barley. Can. J. Pl. Sci. 59: 481-485.

Buchanan, A.M. and Nicholas, E.M. 1980. Sprouting, Alpha Amylase and Bread Making Quality. Cereal Res. Comm., Vol. 8, No. 1, 23-28.

Canadian Grain Commission, Official Grain Grading Guide 1983. P. 21.

Campbell, J.A. 1980. A New Method for Detection of Sprout-Damaged Wheat Using a Nephelometric Determination of α-Amylase Activity. Cereal Res. Comm., Vol. 8, No. 1: 107-113.

Campbell, A.B. and Czarnecki, E.G. 1981. Columbus Hard Red Spring Wheat. Can. J. Pl. Sci., 61: 147-148.

Cavalli, L.L. 1952. An Analysis of Linkage in Quantitative Inheritance. In: Quantitative Inheritance. (Eds. Reeve, E.C.R. and Waddington, C.H.) H.M.S..O., London, pp. 135-144.

Chamberlain, N. 1973. Microwave Energy in the Baking of Bread. Food Trade Review. 43: 8-12.

Ciha, A.J. and Goldstein, W.A. 1983. Effects of Fertility and Rain Simulation During Grain Fill on Protein Content, Starch Quality and Alpha-Amylase Activity in Winter Wheat. In: Third Inter. Symp. on Pre-Harvest Sprouting in Cereals. (ed: Kruger, J.E. and LaBerge, D.E.). 72-78. Westview.

Clarke, J.M. 1980. Measurement of Relative Water Uptake Rates of Wheat Seeds Using Agar Media. Can. J. Pl. Sci. 60: 1035-1038.

Clarke, J.M. 1982. Effect of Awns on Drying Rate of Windrowed and Standing Wheat. Can. J. Pl. Sci. 62: 1-4.

Clarke, J.M. 1983. Wheat Weathering Damage in Windrower/Combine vs. Direct-Combine Harvesting Systems. In: Third Inter. Symp. on Pre-Harvest Sprouting in Cereals. (ed: Kruger, J.E. and LaBerge, D.E.). 287-293. Westview.

Day, A.D., Down, E.E. and Frey, K.J. 1955. Association Between Diastatic Power and Certain Visible Characteristics and Heritability of Diastatic Power in Barley. Agron. J. 47: 163-165.

D'Appolonia, B.L., MacArthur, L.A., Pisesookbunterng, W. and Ciacco, C.F., 1982. Comparison of the Grain Amylase Analyzer with the Amylograph and Falling Number Methods. Cereal Chem. 59: 254-257.

Deming. G.W. and Robertson, D.W. 1933. Dormancy in Small-Grain Seeds. Colorado Exp. Stn. Tech. Bull. No. 5, 1-12.

Derera, N.F. 1980. The Audit of Sprouting. Cereal Res. Comm., Vol. 8, No. 1: 15-22.

Derera, N.F., McMaster, G.J. and Balaam, L.N. 1976. Pre-Harvest Sprouting Resitance and Associated Components in 12 Wheat Cultivars. Cereal Res. Comm., Vol. 4, No. 2: 173-179.

Derera, N.F., Bhatt, G.M. and McMaster, G.J. 1977. On the Problem of Pre-Harvest Sprouting of Wheat. Euphytica 26: 299-308.

Derera, N.F. and Bhatt, G.M. 1980. Germination Inhibition of the Bracts in Relation to Pre-Harvest Sprouting Tolerance in Wheat. Cereal Res. Comm. Vol. 8, No. 1: 199-201.

Dronzek, B.L., Hwang, P. and Bushuk, W. 1972. Scanning Electron Microscopy of Starch from Sprouted Wheat. Cereal Chem. 49, 232-239.

Durham, V.M. and Wellington, P.S. 1961. Studies on the Germination of Cereals 4. The Oxygen Requirement for Germination of Wheat Grains During Maturation. Ann. Bot. N.S. 25: 197-205.

Everson, E.G. and Hart, R.B. 1961. Varietal Variation for Dormancy in Mature Wheat. Q. Bull. Mich. St. Univ. Agric. Exp. Stn. 43 No. 4: 820-829.

Farrand, E.A. 1964. Flour Properties in Relation to the Modern Bread Processes in the United kingdom, with Special Reference to Alpha Amylase and Starch Damage. Cereal Chem. 41: 98-111.

Fong, F., Koehler, D.E. and Smith, J.D. 1983. Fluridone Induction of Vivipany During Maize Seed Development. In: Third Inter. Symp. on Pre-Harvest Sprouting in Cereals. (ed: Kruger, J.E. and LaBerge, D.E.). 188-196. Westview.

Flintham, J.E. and Gale, M.D. 1980. The Use of Gai/Rht 3 as a Genetic Base for Low α-Amylase Wheats. Cereal Res. Comm., Vol. 8, No. 1: 283-290.

Flintham, J.E. and Gale, M.D. 1982. The Tom Thumb Dwarfing Gene, Rht 3 in Wheat; 1. Reduced Pre-Harvest Damage to Bread Making Quality. Theor. Appl. Gent. 62: 121-126.

Flintham, J.E. and Gale, M.D. 1983. The Tom Thumb Dwarfing Gene Rht 3 in Wheat; 2. Effects on Height Yield and Grain Quality. Theor. Appl. Genet. 66: 249-256.

Freed, R.D. 1972. Seedcoat Color and Dormancy in Wheat, *Triticum aestivum L.* - Ph.D. Thesis, Dept. of Crop and Soil Sciences, Michigan State University, E. Lansing, MI (Cited by Freed *et al.*, 1976).

Freed, R.D. and Everson, E.H. 1972. Seed Colour and Dormancy in Wheat. *Triticum Aestivum* X., Agron. Absts. Annual Meeting, p. 7.

Freed, R.D., Everson, E.H., Ringlund, R. and Gullord, M. 1976. Seed Coat Colour in Wheat and the Relationship to Seed Dormancy at Maturity. Cereal Res. Comm., Vol. 4, No. 2: 147-149.

Fuller, P. 1972. Rapid Sorting Test for α-Amylase Activity in Home Grown Wheat. Milling 154: 29-30.

Gale, M.D. 1976. High α-Amylase Breeding and Genetical Aspects of the Problem. Cereal Res. Comm., Vol. 4, No. 2: 231-243.

Gale, M.D. and Marshall, G.A. 1973. Insensitivity to Gibberellin in Dwarf Wheats. Ann. Bot. 37: 729-735.

Gale, M.D. and Marshall, G.A. 1975. The Nature and Genetic Control of Gibberellin Insensitivity in Dwarf Wheat Grain. Heredity 35: 55-65.

Gale, M.D., Law, C.N., Marhshall, G.A. and Norland, A.J. 1975. The Genetic Control of Gibberellic Acid Insensitivity and Coleoptile Length in a 'Dwarf' Wheat. Heredity 34: 393-399.

Gale, M.D. and Law, C.N. 1976. The Identification and Exploitation of Norin 10 Semi Dwarfing Genes. Part III. Plant Breeding Institute, Cambridge, Annual Report, 21-35.

Gale, M.D. and Gregory, R.S. 1977. A Rapid Method for Early Generation Selection of Dwarf Genotypes in Wheat. Euphytica 26: 733-738.

Gfeller, F. and Svejda, F. 1960. Inheritance of Post Harvest Seed Dormancy and Kernel Colour in Spring Wheat Lines. Can. J. Pl. Sci. 40: 1-6.

Gordon, A.G. 1970. Premature Germination in Cereal Grains. Can. J. Pl. Sci. 50: 191-194.

Gordon, I.L. 1979. Selection Against Sprouting Damage in Wheat 11 Harvest Ripeness, Grain Maturity and Germinability. Aust. J. Agric. Res. 30: 1-17.

Gordon, I.L. 1980. Germinability, Dormancy and Grain Development. Cereal Res. Comm., Vol. 8. No. 1: 115-129.

Gothard, PG. 1976. A Simple Gel-Diffusion Assay of α-Amylase in Ungerminated Wheat Grains. J. Sci. Fd. Agric. 27: 691-694.

Hagberg, S. 1960. A Rapid Method for Determining Alpha-Amylase Activity. Cereal Chem. 37: 218- 222.

Hagberg, S. 1961. Note on a Simplified Rapid Method for Determining Alpha-Amylase Activity. Cereal Chem. 38: 202-203.

Hardesty, B. and Elliot, F.C. 1956. Differential Post-Ripening Effects Among Seeds from the Same Parental Wheat Spike. Agron. Jour. 48: 406-409.

Hayter, A.M. and Allison, M.J. 1972. A Gel Diffusion Assay for Diastatic Activity, and its Use in Plant Breeding. J. Inst. Brew. 78: 310-313.

Harrington, G.T. 1923. Forcing the Germination of Freshly Harvested Wheat and Other Cereals. Jour. Agric. Res., 23: 79-100.

Harrington, J.B. 1932. The Comparative Resistance of Wheat Varieties to Sprouting in the Stook and Windrow. Sci. Agric. 12: 635-645.

Harrington, J.B. 1949. Testing Cereal Varieties for Seed Dormancy. Sci. Agric. 29: 538-550.

Hejgaard, J. and Gibbons, G. 1979. Screening for α Amylase in Cereals. Improved Gel-Diffusion Assay a Dye-Labelled Starch Substrate. Carlsberg. Res. Commun. 44: 21-25.

Hong, B.H. 1979. Genetic and Environmental Aspects of Pre-Harvest Sprouting and Related Traits in *Triticum Aestivum*, L.E.M. Thell. Ph.D. Thesis, Washington State University.

Huang, G. and Varriano-Marston, E. 1980. Alpha Amylase Activity and Pre-Harvest Sprouting Damage in Kansas Hard White Wheats. J. Agric. Food Chem. 28: 509-512.

Hutchinson, J.B., Green, E.N. and Brett, C.C. 1948. resistance of Wheat to Sprouting in the Ear: Preliminary Investigations. Emp. Journ. Exp. Agric. 16: 23-32.

Johansson, N. 1976. Enzymatic Activity and Germination Capacity as a Selection Criteria for Sprouting Resistance. Cereal Res. Comm., Vol. 4, No. 2: 255-261.

Ketring, D.L. 1973. Germination Inhibitors. Seed Sci. & Technol., 1: 305-325.

King, R.W. and Chadim, H. 1983. Ear Wetting and Pre-Harvest Sprouting of Wheat, In: Third Inter. Symp. on Pre-Harvest Sprouting in Cereals, (ed: Kruger, J.E. and LaBerge, D.E.) 36-42. Westview.

Kneen, E., and Hads, H.L. 1945. Effects of Variety and Environment on Amylases of Germinated Wheat and Barley. Cereal Chem. 22: 407-413.

Kruger, J.E. 1972. Changes in the Amylases of Hard Red Spring Wheat During Growth and Maturation. Cereal Chem. 49: 379-391.

Kruger, J.E. 1972 b. Changes in the Amylases of Hard Red Spring Wheat During Germination. Cereal Chem. 49: 391-399.

Kruger, J.E. 1980. Progress in the Chemistry of Some Quality-Affecting Enzymes Resulting from Pre-Harvest Sprouting Damage. Cereal Res. Comm. Vol. 4, No. 2: 39-48.

Kruger, J.E. and Tipples, K.H. 1980. Relationships Between Falling Number, Amylograph Viscosity and α-Amylase Activity in Canadian Wheat. Cereal Res. Comm. Vol. 8, No. 1: 97-105.

Kruger, J.E. and Tipples, K.H. 1981. Modified Procedure for Use of the Perkin-Elmer Model 191 Grain Amylase Analyzer in Determining Low Levels of α Amylase in Wheats and Flours. Cereal Chem. 58: 271-274.

Kruger, J.E. amd Marchylo, B.A. 1982. High-Performance Aqueous Gel Permeation Chromatographic Analysis of Beta-Limit Dextrin Hydrolysis by Malted Hard Red Spring Wheat, Malted Durum Wheat, and Fungal (*Aspergillus Oryzae*) Alpha-Amylases. Cereal Chem. 59: 488-492.

Kruger, J.E. and Tipples, K.H. 1983. Some Experiences with Monitoring Alpha-Amylase Levels in Canadian Wheat. In: Third Inter. Symp. on Pre-Harvest Sprouting in Cereals. (ed: Kruger, J.E. and LaBerge, D.E.) 125-131. Westview.

LaCroix, L.J., Waikakul, P. and Young, G.M. 1976. Seasonal and Varietal Variation in Dormancy of Some Spring Wheats. Cereal Res. Comm. Vol. 4, No. 2: 139-146.

Maguire, J.F. 1975. Seed Dormancy. In: Advances in Research and Technology of Seeds. Part 1. (ed: Bradnock, W.T.). Centre for Agricultural Publishing and Documentation, Wageningen: 45-53.

Mares, D.J. 1983. Preservation of Dormancy in Freshly Harvested Wheat Grain. Aust. J. Agric. Res. 34: 33-38.

Mares, D.J., Ellison, F.W., and Derera, N.F. 1983. Gibberellic Acid Insensitivity Genes and Pre-Harvest Sprouting Damage Resistance. In: Third Inter. Symp. on Pre-Harvest Sprouting in Cereals. (ed: Kruger, J.E. and LaBerge, D.E.). 22-28. Westview.

Mathewson, P.R. and Pomeranz, Y. 1978. A Research Note on the Relationship Between Alpha Amylase and Falling Number in Wheat. J. Food. Sci. 43: 652-653.

Mathewson, P.R., Gahrenholz, C.H. and Pomeranz, 1982. Applicability of the Colorimetric Alpha-Amylase Assay to Evaluate Sprouted Sorghum. Cereal Chem. 59: 156-157.

Mather, K. and Jinks, J.L. 1982. Biometrical Genetics. University Press, Cambridge. pp. 37-170.

McCrate, A.J. 1981. Pre-Harvest Sprouting and Seed Dormancy in Hard White Winter Wheat *Triticum Aestivum* L. Plant Br. Abst. 52, No. 3, 170.

McCrate, A.J., Nielsen, M.T., Paulsen, G.M. and Heyne, E.G. 1981. Gibberellic Acid Effects on Germination and α Amylase Activity in Winter Wheats. Euphytical 30: 875-880.

McEwan, J.M. 1959. Sprouting in New Zealand Wheat Varieties. New Zealand Wheat Review, No. 7: 61-64.

McEwan, J.M. 1967. Breeding Wheat Varieties Resistant to Sprouting. New Zealand Wheat Review, No. 7: 87-89.

McEwan, J.M. 1976. Relative Sprouting Resistance of Closely-Related Wheats Differing in Grain Colour. Cereal Res. Comm., Vol. 4, No. 2: 151-155.

McEwan, J.M. 1980. The Sprouting Reaction of Stocks with Single Genes for Red Grain Colour Derived from Hilgendorg 61 Wheat. Cereal Res. Comm. Vol. 8, No. 1: 261-264.

McMaster, G.J. 1976. Response to GA_3 in α Amylase Synthesis of Four Wheat Cultivars. Cereal Res. Comm., Vol. 4, No. 2: 227-230.

McMaster, G.J. and Derera, N.F. 1976. Methodology and Sample Preparation When Screening for Sprouting Damage in Cereals. Cereal Res. Comm., Vol. 4, No. 2: 251-254.

Meredith, P. 1974. Development of Wheat Grains. New Zealand Wheat Review No. 12: 38-43.

Meredith, P. 1983. Sprout Damage. New Zealand Wheat Review, No. 15: 78-83.

Mitchell, B., Armstrong, C., Black, M. and Chapman, J. 1980. Physiological Aspects of Sprouting and Spoilage in Developing

Triticum Aestivum L. (Wheat) Grain. In: Seed Production (ed: Hebbeltheaite, P.D.) 339-356. London Butterworths.

Miyamoto, T., Tobert, N.E. and Everson, E.H. 1961. Germination Inhibitors Related to Dormancy in Wheat Seeds. Plant Physiol. 36: 739-746.

Moormann, B. 1942. [Studies on Dormancy in Oats and Barley.] Kuhn-Arch. 56: 41-79. (Cited by Belderok 1968.)

Morris, R., Schmidt, J.W. and Johnson, V.A. 1972. Chromosomal Location of a dwarfing Gene in "Tom Thumb" Wheat Derivative by Monosomic Analysis. Crop Sci. 12: 247-249.

Naylor, J.M. and Simpson, G.M. 1961. Dormancy Studies in Seed of *Avena Fatua*. 2. A Gibberellin Sensitive Inhibitory Mechanism in the Embryo. Can. J. Bot. 39: 281-295.

New Zealand Wheat Review No. 24 (1945-1946). Sprouted Wheat for Seed. 10-11.

Nielsen, M.T., McCrate, A.J., Heyne, E.G. and Paulsen, G.M. 1984. Effect of Weather Variables During Sprouting of Hard White Winter Wheat. Crop. Sci. 24: 779-782.

Nilsson-Ehle, H. 1914. Zur Kenntnis der mit der Keimungsphysiologie des weizens in Zusammenhang Stehenden innerer Faktoren. S. Pflanzenzuecht 2: 153-185.

Noll, J.S. and Czarnecki, E.M. 1980. Methods for Extending the Testing Period for Harvest Time Dormancy in Wheat. Cereal Res. Comm., Vol. 8, No. 1: 233-238.

Noll, J.S., Dyck, P.L. and Czarnecki, E. 1982. Expression of RL 4137 Type of Dormancy in F_1 Seeds of Reciprocal Crosses in Common Wheat. Can. J. Pl. Sci. 62: 345-349.

Olsson, G., and Mattson, B. 1976. Seed Dormancy in Wheat Under Different Weather Conditions. Cereal Res. Comm. Vol. 4 No. 2: 181-185.

O'Connell, B.T., Rubenthaler, G.L. and Murbach, N.L. 1980. Evaluation of a Nephelometric Method for Determining Cereal Alpha-Amylase. Cereal Chem. 57: 411-415.

Persson, E. 1976. Otello - A Result of Amylase Selection for Sprouting Resistance. Cereal Res. Comm. Vol. 4, No. 2: 101-106.

Perten, H. 1964. Application of the Falling Number Method of Evaluating Alpha-Amylase Activity. Cereal Chem. 41: 127-140.

Perten, H. 1966. A Colorimetric Method for the Determination of Alpha-Amylase Activity (ICC Method). Cereal Chem. 43: 336-342.

Peterson, R.F. 1965. Wheat Botany, Cultivation and Utilization. Interscience Publishers Inc., N.Y. p. 307.

Piech, J., Ruszkowski, M. and Jaworka, K. 1970. Inheritance of the Seed Dormancy Stage Duration in Winter Wheat (*Triticum Aestivum* L.). Genetica Polonica, 11: 227-239.

Pool, M. and Petterson, F.L. 1958. Moisture Relations in Soft Red Winter Wheats. 11. Awned Versus Awnless and Waxy Versus Non-Waxy Glumes. Agron. J. 50: 158-160.

Purdy, L.H., Loegering, W.Q., Konzak, C.F., Peterson, C.J. and Allan, R.E. 1968. A Proposed Standard Method for Illustrating Pedigrees of Small Grain Varieties. Crop. Sci. 8: 405-406.

Radley, M. 1980. Role of Growth Substances in the Regulation of Germination in Developing and Ripening Wheat Grains. Cereal Res. Comm., Vol. 4, No. 2: 131-138.

Reddy, L.V., Ching, T.M. and Metzger, R.J. 1984. Alpha-Amylase Activity in Wheat Kernels Matured and Germinated under Different Temperature conditions. Cereal Chem. 61: 228-231.

Reitan, L. 1980. Genetical Aspects of seed Dormancy in Wheat Related to Seed Coat Colour in an 8 x 8 Diallel Cross. Cereal Res. Comm., Vol. 8, No. 1: 275-282.

Reitan, L. 1983. Early Generation Mass Selection for Seed Dormancy in Barley. In: Third Inter. Symp. on Pre-Harvest Sprouting in Cereals. (ed. Kruger, J.E. and LaBerge, D.E.) 244-250. Westview.

Robyt, J.F. and Whelan, W.J. 1968. In: "Starch and its Derivatives" Radley J.A., AEd. 4th ed. Chapman and Hall, Ltd., London, p. 430-433.

Sanders, I.D. 1961. Wheat Sprout Damage and the Hagberg Test. New Zealand Wheat Review. No. 8: 76-77.

Schleip, H. 1938. [Studies on sprouting Resistance in Wheat.] Landw. Jbr. 86: 795-822 (cited by Belderok 1968).

Schmidt, E. 1934. Experimentelle Untersuchungen uber Auswunchsneigung und Keimreife als Sorteneigenschaften des Getreides. Angew. Botan. 16: 10-50 (cited by Johansson 1976).

Smith, L. 1948. The Effect of Chaff of Cereals on Germination of Seeds and on the Growth of Mold. Agron. Jour. 40: 32-44.

Steel, R.G.D. and Torrie, J.H. 1980. Principles and Procedures of Statistics, a Biometrical Approach. (2nd ed.) McGraw-Hill Book Company, Toronto, p. 633.

Stoy, V. 1983. Progress and Prospect in Sprouting Research. In: Third Inter. Symp. on Pre-Harvest Sprouting in Cereals. (Ed: Kruger,

J.E. and LaBerge, D.E.). 3-7. Westview.

Stoy, V. and Sundin, R. 1976. Effects of Growth Regulating Substances in Cereal Seed Germination. Cereal Res. Comm., Vol. 4, No. 2: 157-163.

Strand, E. 1980. A Seed Dormancy Index for Selection of Cultivars of Cereals Resistant to Pre-Harvest Sprouting. Cereal Res. Comm. Vol. 8, No. 1: 219-223.

Svensson, G. and Lagerstrom, G. 1966. Bestimmung der Auswuchsresistenz bei Weizen, Gerste und Hafer. Agric. Hort. Gen. 24: 11-47 (cited by Johansson 1976).

Tipples, K.H. 1969. A Viscometric Method for Measuring Alpha-Amylase Activity in Small Samples of Wheat and Flour. Cereal Chem. 46: 589-597.

Urion, E. and Chapon, L. 1955. Contribution to the Study of the Dormancy of Barley. Proc. Eur. Brew. Conv. 172-202 (cited by Belderok 1968).

Varner, J.E. 1964. Gibberellic Acid Controlled Synthesis of α Amylase in Barley Endosperm. Plant Physiol. 39: 413-417.

Warner, J.N. 1952. A Method for Estimating Heritability. Agron. J. 44: 427-430.

Wellington, P.S. 1956. Studies on Germination of Cereals. 1. The Germination of Wheat Grains in the Ear During Development, Ripening, and After Ripening. Ann. Bot. 20: 105-120.

Wellington, P.S. and Durham, V.M. 1961. Studies on the Germination of Cereals. 3. The Effect of the Covering Layers on the Uptake of Water by the Embryo of the Wheat Grain. Ann. Bot. N.S. 25: 187-196.

APPENDIX

APPENDIX TABLE 1 Diurnal Temp. Range, Mean Temperatures and Precipitation Two Weeks Before and After Windrowing Single Plants

	WEEKLY PERIOD	DIURNAL TEMP. RANGE (°C)		MEAN TEMPS (°C)		PRECIPITATION (mm)	
		1983	LT AVE.	1983	LT AVE.	1983	LT AVE.
1.	Aug. 16						
		13.9	13.7	19.4	18.0	18	15
	Aug. 22						
2.	Aug. 23						
		13.3	13.1	23.0	17.1	15	15
	Aug. 29						
3.	Aug. 30						
		14.1	12.8	22.7	16.0	-	15
	Sept. 5						
4.	Sept. 6						
		11.8	12.6	12.6	13.8	11	10
	Sept. 12						

LT AVE. = Long Term Average

APPENDIX TABLE 2 Diurnal Temp. Range, Mean Temperatures and Precipitation Two Weeks Before and Eight Weeks After Windrowing of Head Rows

	WEEKLY PERIOD	DIURNAL TEMP. RANGE (°C)		MEAN TEMPS. (°C)		PRECIPITATION (mm)	
		1983	LT AVE.	1983	LT AVE.	1983	LT AVE.
1.	Aug. 9 – 15	15.4	13.6	22.3	18.7	-	15
2.	Aug. 16 – 22	13.9	13.7	19.4	18.0	17	15
3.	Aug. 23 – 29	13.3	13.1	13.0	17.1	15	15
4.	Aug. 30 – Sept. 5	14.1	12.8	22.7	16.0	-	15
5.	Sept. 6 – 12	11.8	12.6	12.6	13.8	11	10
6.	Sept. 13 – 19	8.5	12.0	10.3	12.4	-	10
7.	Sept. 20 – 26	13.8	11.9	10.0	10.4	-	10
8.	Sept. 27 – Oct. 3	8.2	12.5	9.8	9.3	40	10
9.	Oct. 4 – 10	10.5	11.7	7.7	8.0	-	10
10.	Oct. 11 – 16	11.1	11.5	2.4	6.6	-	5

LT AVE = Long Term Average

APPENDIX TABLE 3 α-Amylase Activity (μg/ml) of UM 55-3, RL 4137 and F_1 Generations

GENERATIONS																
UM 55-3								RL 4137								F_1
1	3.79							1	0.04							1	1.81
2	4.56							2	0.04							2	1.17
3	2.70							3	0.28							3	2.25
4	2.22							4	0.44							4	1.16
5	3.19							5	0.15							5	1.48
6	2.41							6	0.67							6	0.61
7	2.70							7	0.58							7	1.15
8	3.38							8	0.39							8	2.39
9	2.73							9	0.29							9	1.11
10	3.12							10	0.39							10	2.29
11	4.21							11	0.45							11	2.44
12	2.21							12	0.53							12	0.81
13	4.15							13	1.04							13	0.68
14	2.85							14	1.19							14	0.58
15	2.55							15	0.70							15	1.15
16	4.00							16	0.61							16	1.05
								17	1.41								

APPENDIX TABLE 4 α-Amylase Activity (µg/ml) of F_2 BC_1F_1 and BC_2F_1 Generations of the Cross UM 55-3 x RL 4137

GENERATIONS

F_2

1	0.80	57	1.55
2	2.54	58	2.00
3	0.82	59	4.51
4	6.00	60	2.55
5	2.96	61	2.55
6	2.14	62	3.26
7	1.66	63	1.00
8	2.58	64	1.87
9	4.63	65	2.62
10	3.20	66	2.62
11	1.17	67	0.81
12	4.68	68	1.53
13	1.36	69	3.68
14	7.57	70	1.26
15	3.83	71	1.26
16	3.87	72	0.58
17	2.11	73	4.75
18	2.19	74	0.96
19	2.08	75	3.38
20	5.30	76	0.33
21	1.30	77	0.93
22	5.66	78	2.43
23	2.02	79	2.57
24	3.24	80	1.89
25	0.93	81	3.74
26	1.05	82	4.08
27	0.35	83	2.43
28	5.94	84	4.13
29	0.92	85	2.33
30	0.80	86	4.87
31	2.73	87	3.35
32	2.86	88	2.92
33	2.28	89	0.43
34	7.42	90	1.36
35	6.33	91	2.43
36	1.25	92	1.59
37	0.80	93	1.58
38	1.83	94	1.01
39	1.32	95	2.92
40	1.64	96	1.63
41	2.47	97	1.49
42	1.77	98	4.74
43	2.56	99	1.10
44	2.76	100	5.07
45	2.41	101	5.59
46	1.65	102	2.35
47	0.83	103	5.98
48	2.56	104	1.72
49	1.97	105	4.16
50	4.46	106	2.54
51	1.90	107	1.10
52	1.70	108	2.73
53	1.70	109	0.48
54	3.06	110	3.97
55	1.65	111	0.43
56	3.66	112	0.91
113	6.52		
114	1.99		
115	0.86		
116	6.00		
117	3.89		
118	1.01		
119	6.57		
120	4.61		
121	0.70		
122	6.46		
123	3.07		
124	5.28		
125	4.46		
126	0.80		
127	5.89		
128	3.74		
129	4.77		
130	3.94		
131	2.32		
132	2.78		
133	0.35		
134	6.40		
135	1.60		
136	3.24		
137	4.97		
138	3.08		
139	1.70		
140	1.19		
141	2.21		
142	3.64		
143	1.14		
144	1.49		
145	6.25		
146	0.89		
147	3.39		
148	0.48		
149	1.39		
150	1.39		
151	1.39		
152	2.60		
153	5.07		
154	5.38		
155	2.34		
156	6.10		
157	3.06		
158	3.01		
159	2.33		
160	1.72		
161	1.87		
162	5.17		
163	3.78		
164	8.22		
165	2.59		
166	2.96		
167	2.08		
168	2.39		
169	3.21		
170	7.56		

BC_1F_1

1	6.36	57	3.11
2	0.97	58	3.35
3	4.68	59	4.28
4	3.09	60	4.13
5	5.73	61	5.74
6	0.81	62	3.50
7	5.20	63	1.45
8	4.56	64	0.53
9	1.02	65	2.72
10	1.79	66	2.18
11	4.95	67	5.79
12	2.46	68	2.96
13	0.52	69	5.11
14	6.12	70	3.21
15	3.36	71	4.07
16	2.43	72	6.41
17	3.31	73	1.96
18	2.92	74	1.25
19	2.41	75	1.82
20	1.70	76	3.25
21	4.79	77	4.26
22	4.72	78	5.35
23	2.67	79	2.78
24	6.27	80	2.76
25	1.44	81	3.48
26	5.04	82	6.00
27	1.57	83	0.86
28	4.92	84	1.32
29	2.15	85	1.15
30	0.83	86	5.02
31	1.51	87	4.20
32	5.87	88	6.00
33	5.47	89	2.86
34	3.66	90	1.42
35	5.87	91	4.82
36	2.17	92	4.09
37	2.47	93	2.92
38	6.03	94	2.14
39	2.02	95	3.12
40	2.52	96	4.15
41	3.38	97	0.89
42	5.43	98	3.85
43	1.19	99	3.49
44	4.55	100	3.29
45	2.09	101	3.64
46	0.88	102	5.18
47	2.78	103	2.32
48	1.11	104	4.00
49	3.53	105	5.78
50	1.19	106	1.75
51	1.19	107	4.21
52	0.88	108	3.44
53	2.93	109	4.56
54	2.25	110	0.99
55	3.50	111	2.67
56	4.85	112	1.96
		113	4.81
		114	3.32
		115	6.26
		116	4.19
		117	2.29
		118	5.54
		119	2.08
		120	6.10
		121	3.63
		122	4.71
		123	2.96
		124	2.10

BC_2F_1

1	0.15	57	1.89
2	1.17	58	0.82
3	0.83	59	1.16
4	1.29	60	1.36
5	0.52	61	2.67
6	1.06	62	2.04
7	1.56	63	2.18
8	1.05	64	2.25
9	3.35	65	3.38
10	0.65	66	2.87
11	1.18	67	2.68
12	2.20	68	2.06
13	5.28	69	1.68
14	1.62	70	4.31
15	2.29	71	0.62
16	1.02	72	5.36
17	0.65	73	2.20
18	0.39	74	1.87
19	0.90	75	0.30
20	0.38	76	1.39
21	3.98	77	2.63
22	0.32	78	1.49
23	0.92	79	0.19
24	8.22	80	3.64
25	2.79	81	1.58
26	4.40	82	0.65
27	6.07	83	2.66
28	0.80	84	4.15
29	3.82	85	2.09
30	0.42	86	1.16
31	0.39	87	2.50
32	1.35	88	0.78
33	2.71	89	0.99
34	0.95	90	1.65
35	1.43	91	0.58
36	2.46	92	0.99
37	1.70	93	2.06
38	3.41	94	1.09
39	1.50	95	1.26
40	4.01	96	0.43
41	3.81	97	1.98
42	1.60	98	1.62
43	0.75	99	2.24
44	0.37	100	5.07
45	4.11	101	2.75
46	1.20	102	0.12
47	2.26		
48	2.11		
49	1.03		
50	1.84		
51	7.32		
52	1.41		
53	0.31		
54	1.79		
55	0.26		
56	0.97		

APPENDIX TABLE 5 **α-Amylase Activity (μg/ml) of BW 47, RL 4137 and F_1 Generations**

GENERATIONS					
BW 47		RL 4137		F_1	
1	3.46	1	0.13	1	2.62
2	0.91	2	1.06	2	0.09
3	0.91	3	0.98	3	0.55
4	3.49	4	0.10	4	1.66
5	1.59	5	0.26	5	0.18
6	0.98	6	0.67	6	0.15
7	3.10	7	0.84	7	0.36
8	1.16	8	0.41	8	2.40
9	1.70	9	1.70	9	1.16
10	1.37	10	0.21	10	2.24
11	3.01	11	0.71	11	0.46
12	1.44	12	0.09	12	2.86
13	1.79	13	0.65	13	1.49
14	1.12	14	0.95	14	0.15
15	1.02	15	0.46	15	0.66
16	2.85	16	0.09	16	1.67
17	1.43	17	0.70	17	0.06
18	1.43	18	0.40	18	2.64
19	3.76	19	0.12	19	0.78
		20	0.18	20	1.20
				21	0.40
				22	1.79
				23	1.81
				24	1.60

APPENDIX TABLE 6 α-Amylase Activity (μg/ml) of F_2, BC_1F_1 and BC_2F_1 Generations of the Cross BW 47 x RL 4137

GENERATIONS																			
F_2						BC_1F_1								BC_2F_1					
1	1.22	57	1.82	113	0.17	1	1.59	57	0.91	1	0.24	57	0.08						
2	0.08	58	1.08	114	1.71	2	1.21	58	4.82	2	0.73	58	0.23						
3	0.80	59	0.34	115	3.35	3	0.84	59	2.41	3	0.08	59	0.31						
4	0.26	60	2.35	116	4.22	4	1.70	60	0.46	4	2.24	60	0.36						
5	1.39	61	0.18	117	0.12	5	2.42	61	1.49	5	0.68	61	0.57						
6	0.77	62	1.24	118	0.29	6	0.21	62	1.03	6	2.13	62	2.11						
7	0.05	63	1.08	119	0.63	7	1.43	63	0.04	7	0.14	63	1.80						
8	0.98	64	1.17	120	0.18	8	0.82	64	0.51	8	0.68	64	0.67						
9	0.57	65	1.58	121	6.35	9	1.43	65	2.21	9	1.48	65	1.80						
10	0.98	66	0.96	122	0.24	10	2.29	66	0.51	10	0.59	66	0.05						
11	1.80	67	2.09	123	0.89	11	2.37	67	3.69	11	0.32	67	1.08						
12	0.10	68	2.04	124	1.88	12	1.88	68	0.85	12	0.12	68	0.15						
13	0.18	69	0.44	125	0.34	13	0.22	69	0.73	13	0.04	69	0.57						
14	1.59	70	1.63	126	0.18	14	0.86	70	1.38	14	2.75	70	0.93						
15	0.67	71	1.63	127	1.06	15	0.76	71	0.41	15	1.59	71	2.83						
16	1.39	72	1.10	128	1.06	16	1.88	72	2.89	16	0.10	72	0.77						
17	2.52	73	0.28	129	1.38	17	2.62	73	1.92	17	2.50	73	0.62						
18	0.15	74	0.08	130	1.88	18	0.22	74	1.86	18	0.36	74	1.75						
19	0.36	75	0.08	131	0.40	19	0.65	75	2.56	19	0.65	75	2.24						
20	0.05	76	0.59	132	3.02	20	0.72	76	4.15	20	0.54	76	1.05						
21	0.77	77	0.28	133	0.29	21	1.49	77	0.59	21	0.23	77	0.51						
22	0.26	78	0.59	134	0.57	22	2.57	78	3.53	22	1.09	78	0.84						
23	1.32	79	0.03	135	0.07	23	1.76	79	1.05	23	2.48	79	1.59						
24	2.35	80	0.12	136	0.54	24	1.49	80	1.16	24	0.01	80	1.11						
25	2.13	81	1.56	137	1.31	25	0.84	81	0.76	25	0.69	81	0.47						
26	0.29	82	0.32	138	5.65	26	0.54	82	3.72	26	1.37	82	1.11						
27	0.08	83	0.98	139	2.29	27	4.49	83	0.51	27	0.76	83	1.11						
28	0.35	84	3.97	140	0.11	28	4.06	84	2.14	28	0.17	84	0.62						
29	2.02	85	2.31	141	1.05	29	1.49	85	0.01	29	0.66	85	0.09						
30	1.70	86	1.04	142	0.12	30	0.24			30	0.34	86	0.11						
31	1.59	87	1.37	143	0.84	31	1.82			31	0.49	87	0.95						
32	0.14	88	0.18	144	2.76	32	0.44			32	0.90	88	1.44						
33	0.08	89	1.80	145	0.95	33	0.18			33	1.15	89	0.12						
34	1.38	90	0.15	146	0.93	34	0.49			34	0.85	90	2.10						
35	1.11	91	0.06	147	0.88	35	1.36			35	0.12	91	0.40						
36	0.84	92	0.37	148	1.17	36	0.88			36	1.25	92	1.36						
37	0.12	93	1.05	149	0.20	37	2.56			37	0.58	93	0.33						
38	2.24	94	1.29	150	0.01	38	1.56			38	0.40	94	1.31						
39	0.46	95	6.68	151	0.14	39	0.28			39	1.70	95	0.25						
40	0.04	96	3.45	152	0.73	40	0.34			40	0.29	96	0.06						
41	0.21	97	0.17	153	1.60	41	1.50			41	0.07	97	0.17						
42	0.17	98	1.20	154	0.64	42	2.93			42	0.03	98	0.30						
43	0.10	99	1.54	155	0.18	43	0.39			43	1.39	99	0.12						
44	2.14	100	0.56	156	1.60	44	0.97			44	0.09	100	0.49						
45	2.99	101	1.73	157	0.51	45	0.49			45	0.39	101	0.12						
46	0.39	102	1.25	158	2.14	46	0.49			46	2.67	102	1.84						
47	3.89	103	0.17	159	0.45	47	0.81			47	0.02	103	2.90						
48	0.55	104	0.22	160	1.71	48	0.76			48	0.06	104	1.11						
49	1.13	105	1.93	161	4.48	49	0.49			49	0.28	105	1.65						
50	1.08	106	1.93	162	0.13	50	1.00			50	1.53	106	1.11						
51	0.87	107	0.90	163	0.40	51	1.11			51	2.20	107	3.39						
52	1.98	108	0.27	164	0.29	52	0.89			52	0.91	108	2.52						
53	0.44	109	1.49	165	0.78	53	0.18			53	0.02	109	2.58						
54	0.76	110	0.12	166	0.29	54	0.49			54	1.00	110	0.03						
55	1.29	111	3.15	167	0.94	55	3.60			55	1.48								
56	0.12	112	0.62	168	2.14	56	0.34			56	0.13								