GLOBOID COMPOSITION DURING SEED SET AND SEEDLING GROWTH

CHANGES IN THE ELEMENT COMPOSITION OF GLOBOIDS IN WHEAT GRAINS (*TRITICUM AESTIVUM* L. CV. A.C. REED AND CELTIC) DURING SEED SET AND EARLY SEEDLING GROWTH

By

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TITLE: Changes in the Element Composition of Globoids in Wheat Grains (*Triticum aestivum* L. cv. A.C. Reed and Celtic) During Seed Set and Early Seedling Growth.

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ABSTRACT

In wheat grains, mineral nutrients are stored in spherical particles called globoids. Globoids are located within the protein bodies of cells from the aleurone layer, scutellum and embryonic axis of the wheat grain. Composed of phytate, globoids contain an essential source of myo-inositol, P, K, Mg, Ca, Mn, Fe and Zn which are used by the growing seedling during early seedling growth. Changes to the element composition within the globoids from the aleurone, scutellum and embryo axis, during seed set and early seedling growth were examined in two cultivars of wheat through the use of energy dispersive X-ray analysis.

During seed set and early seedling growth the composition of the globoids within all tissues changed. In each of the tissues in both cultivars the P levels decreased during seed set and remained relatively constant during early seedling growth. K levels increased during seed set and decreased during early seedling growth. Mg and Ca levels generally decreased during seed set and increased during early seedling growth. Mn and Zn were detected only within globoids within the embryonic axis and no changes were noted for these two elements.

Energy dispersive X-ray analysis showed that A.C. Reed and Celtic grains both followed the same general trends during seed set and seedling growth indicating that the differing protein content of the two cultivars had little effect on the timing of mineral

nutrient accumulation and utilization within the globoids. Atomic absorption analysis of whole grain tissue for P, K, Mg and Ca revealed that Celtic grains had higher concentrations of P and K while being grown in identical conditions to that of A.C. Reed grains. These results indicate that mineral nutrient levels within the grain seem to be influenced by the cultivar, and possibly the protein content of the particular cultivar.

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CHAPTER 1 : INTRODUCTION

1.1 - OBJECTIVES AND OUTLINE OF THIS STUDY

Extensive studies have been focused on the morphology and composition of the dry wheat kernel (Hoseney, 1986; Pomeranz, 1971). A majority of this work has concentrated on the protein and carbohydrates within the grain. These studies were pursued mainly due to the influence that protein and carbohydrate levels have on wheat quality and uses of wheat flour. In contrast, very few studies have looked at mineral nutrient levels or the changes to mineral nutrient levels during grain development, germination and seedling growth. These mineral nutrient stores are also of importance and deserve attention, due to their influence on the grain's nutritional quality, and their role during germination and seedling growth as a source of phosphorus, myo-inositol, and essential cations.

Mineral nutrients are stored in particles called globoids within the protein bodies of the aleurone layer, scutellum, and embryo axis of the wheat grain (Batten and Lott, 1986; Lott and Spitzer, 1980). During grain development these phytate-rich globoids are formed and stored within the maturing grain and then utilized by the seedling during stages of early seedling growth. The actual changes in concentration of the mineral nutrients within the globoids throughout grain development, germination and seedling growth has never been

followed in wheat, or any other cereal crop.

The main goal of this research was to study the changes that occur to the mineral nutrient stores throughout seed set and early seedling development within two cultivars of wheat. The two cultivars examined were A.C. Reed, a low protein wheat, and Celtic, a high protein wheat . These two cultivars were selected so that the effect that protein level may have on mineral nutrient levels could be determined. A.C. Reed and Celtic grains were also of interest to this study because they are currently being grown commercially in southern Ontario. A correlation between high protein, and high potassium levels, and low protein and low potassium levels, has been observed in dry seed samples from different genera (Blevins, 1985). In this study the relationship between protein and potassium was measured during seed set, germination and early seedling growth within two cultivars of wheat. Mineral nutrient levels were examined from three areas of the grain, the aleurone, the scutellum, and the embryo axis. These three areas were chosen due to the fact that they are known to contain globoids (Batten and Lott, 1986; Lott and Spitzer, 1980), and are distinct areas of the grain which could be dissected with relative ease and precision. Further dissection of the embryo axis into its component parts was not feasible for this study. With a scanning transmission electron microscope, energy dispersive x-ray analysis was used to determine element levels within the globoids from each stage of growth and from the three grain regions chosen for study. Globoids from each portion of the grain were analysed for phosphorus, potassium, magnesium, calcium, manganese, iron, zinc, sulphur and chlorine. In addition to x-ray analysis, whole grain concentrations of P, K, Mg, and Ca, were determined for stages during both grain development and seedling growth for both cultivars.

Whole grain protein content was also determined at intervals during grain development and seedling growth for both cultivars to allow me to determine if protein and mineral nutrient accumulations are correlated. Although reallocation of materials was expected within the grain during early seedling growth, whole seedling mineral nutrient concentrations were expected to remain relatively constant since no nutrient sources were added to the growing seedlings.

The introduction that follows will include;

- an overview of x-ray analysis.
- a summary of the structure and composition of globoids.
- a brief description of grain morphology.
- a look at some of the major storage reserves of the mature grain.
- a summary of some of the biological roles of P, K, Ca, Mg, Mn, Fe and Zn within plant tissues.

1.2 - ENERGY DISPERSIVE X-RAY ANALYSIS

Globoids from the aleurone layer, embryo axis, and scutellum were analysed by energy dispersive x-ray (EDX) analysis. In order to obtain an accurate account of the changes that occurred within the globoids it was essential to have a method of preparation that would not substantially alter mineral nutrient levels within the globoids. Since phytate is partially soluble, conventional aqueous fixation, dehydration, and embedding techniques were not suitable. Instead a rapid freezing of sample material in liquid nitrogen, followed by freeze

drying of the tissue in a vacuum chamber was used as a method of preparation. Although this resulted in a great deal of tissue damage, there was little chance of extracting mineral nutrients from the globoids.

When the high energy electron beam from the scanning transmission electron microscope comes into contact with a specimen, in this case a globoid, there are a number of possible interactions. For energy dispersive x-ray analysis, an interaction that causes the release of energy in the form of an x-ray is needed. The electron beam causes an electron to be ejected from its orbital. With the expulsion of an electron the atom is placed into an excited state. This excited, or ionized state is not an energetically favourable condition, and is alleviated by an electron falling from a higher energy level to replace the lost electron. As the higher energy electron falls there is energy loss, which is equal to the difference in the energy levels of the orbitals. This energy may be emitted as an x-ray photon. The amount of energy the x-ray has is dependent on the particular inter-orbital transition involved. With the use of an x-ray detector and analysis system in conjunction with the scanning transmission electron microscope, the x-ray emissions from a small portion of the specimen can be collected, and the energy of the x-rays analysed. As x-rays hit the detector they are converted to voltage pulses of different amplitudes depending on the energy of the x-ray, and spectra can be generated (Chandler, 1977; Morgan, 1985). As well as the production of characteristic x-rays, bremsstrahlung x-rays are produced by the slowing of electrons as they pass within the coulombic fields of the nuclei of the atoms (Bozzola and Russell, 1992). The closer the electrons come to the nuclei, the greater the slowing effect. These background, or bremsstrahlung x-rays are subtracted from the total count to obtain a true peak value of

incoming x-rays, which is associated with the concentration of the elements being analysed (Barbi, 1979). As the electrons fall to return the atom to a stable state the possible interorbital transitions which occur may produce multiple peaks for a certain element, each of these peaks is a fraction of the total counts produced for the element. Primary peaks produced are called K_α, secondary are peaks called K_β and so on. The size of the secondary peaks are related to the size of the K_α peak. Often the peak of an element may be overlapped by the secondary peak of another element. An example of peak overlap within this study would be the Ca K_α peak which was overlapped by the K_β of K. Because the size of the K K_α peak is known, the size of K K_β peak can be determined. Knowing the size of the K K_β peak, the actual size of the Ca K_α peak can be determined by subtraction. Other overlapping peaks in this study included the Fe K_α peak which is overlapped by the Mn K_β peak and the Zn K_α peak which is overlapped by the Cu K_β peak.

1.3 - GLOBOID PARTICLES

Globoids are spherical, electron-dense particles located within the protein bodies of cells from the aleurone layer, scutellum and embryo axis of the mature grain (Batten and Lott, 1986; Lott and Spitzer, 1980). The main component of the globoid is phytate, a myo-inositol hexakisphosphoric acid salt. Six negatively charged phosphate groups of each phytate molecule enable it to bind to cations of such elements as Mg, K, Zn, Mn, and Fe, and other trace elements, making phytate a rich source of mineral nutrients and myo-inositol (Lott and Vollmer, 1973; Ockenden and Lott, 1989). There are 12 potentially hydrolysable protons on

the phytate molecule (Cosgrove, 1966), eight of which are highly acidic, and four of which are weakly acidic (Johnson and Tate, 1969). Studies looking at the composition of the globoids from the mature wheat grain commonly find P, Na, K, Ca, Mg, Cu, Fe and Zn to be present within the particle (Bassiri and Nahapetian, 1977; Karlen and Whitney, 1980; Lott and Spitzer, 1980; Mazzoline *et al*, 1985). Work by Nolan and Duffin, (1987), has shown that ions of Mg, Mn, Fe, and Zn, have a higher affinity for phytate than that of calcium. Concentrations of mineral nutrients have been shown to vary throughout different portions of the grain, and within the different portions there has also shown to be a selection as to which mineral nutrients are bound (Lott and Spitzer, 1980). The concentration of cations bound to phytate is dependent on the pH, the concentration of cations, and the phytic acid available (Nolan and Duffin, 1987). Cations bound to phytate in globoids vary from species to species, but there is evidence that the cations are actively selected by the developing seed or grain, and changes to soil, and environmental conditions tend to have little effect on the composition of the globoids (Lott, 1975). Although environmental conditions have little effect on the composition of the globoids there is evidence that mineral nutrient concentration can alter the size of globoids. Wheat grown in high phosphorus concentrations tend to have larger globoids than those grown in limited phosphorus concentrations; however, those grown in lower phosphorus concentrations do tend to have higher numbers of smaller globoids (Batten and Lott, 1986). Work by Lott *et al* 1985 and Lott *et al* 1994 has shown that the ratio of (Mg and Ca):K can alter globoid size and number. Lower (Mg and Ca): K ratios produce protein bodies with numerous smaller globoids whereas higher (Mg and Ca):K ratios tend to produce protein bodies with single large globoids. Plants with high K concentrations

often have no globoids located within the protein bodies even though phytate is present.

The actual synthesis of phytate is not fully understood. It is agreed, however, that phytate is synthesized within the tissue in which it is stored by the stepwise addition of phosphate groups to myo-inositol (Greenwood 1989). Work by Bechtel (1985) on wheat showed that phytate is synthesized within the endoplasmic reticulum, packaged within the golgi apparatus, and transported to the vacuoles (protein bodies) as small membrane bound particles. Inorganic phosphorus levels within the developing wheat grain and gibberellins secreted from the embryo may be involved in the regulation and synthesis of phytate.

During early seedling growth phytate stores are broken down by increasing levels of phytase enzymes. Like phytate biosynthesis, this breakdown of phytate is not fully understood, but the end result is the release of cations, inorganic phosphorus, and myo-inositol (and phosphoric esters of myo-inositol) to nourish the growing seedling (Lott, 1975; Hayakawa *et al*, 1990).

1.4 - WHEAT BACKGROUND

Since the beginning of civilization, the composition, food quality and uses of the grain have lead wheat to be a valued food source for man. A cereal plant derived from the wild *Triticum* native to the middle east, archeological evidence shows use of wheat grain 16, 000 to 15, 000 years BC (Wendorf *et al* 1979), and abundant evidence of wheat crop cultivation 10, 000 years BC. Current worldwide production of wheat is over 595 million metric tons a year (Nafziger, 1995), providing more protein than any other single cereal crop including

rice, corn, barley or other cereal crops. Wheat productivity has been greatly increased by artificial selection for yield and resistance to various problems such as disease or stem weakness. Major wheat growing regions are throughout temperate regions of Europe, North and South America, and Australia. Wheat is classified into two types, winter and spring. Winter wheat, is grown in warmer climates where the grain is planted in the fall, starts initial growth over the winter season, but makes chief growth early in the next season. Spring wheat is grown in colder climates where seeds are planted in the spring, and growth occurs throughout the warmer summer months. A.C. Reed and Celtic grains used within this study are both spring wheats.

1.5 - GRAIN MORPHOLOGY

Many studies have been done looking at the morphology of the wheat grain. The following is a brief summary from the studies of Bradbury *et al* (1956), Hoseney (1986), Pomeranz (1971), Stevens (1973), Swift and O'Brien (1972) and Winton and Winton, (1932).

General

The wheat kernel is light yellow to red in colour, generally ovulate in shape, and normally 4 to 10 mm. long. A groove runs along the entire ventral surface, and a tuft of fine hair is found at the apex of the kernel. The kernel is a dry, indehiscent, one seeded fruit, consisting of an embryo and endosperm tissue, surrounded by nucellar tissue, seed coat and pericarp.

Pericarp

The pericarp can be broken down into two parts, the inner pericarp and outer pericarp. The pericarp, seed coat and nucellar epidermis comprise the layer of the grain referred to as bran. The thickness of this layer is greatly dependent on the variety of the wheat.

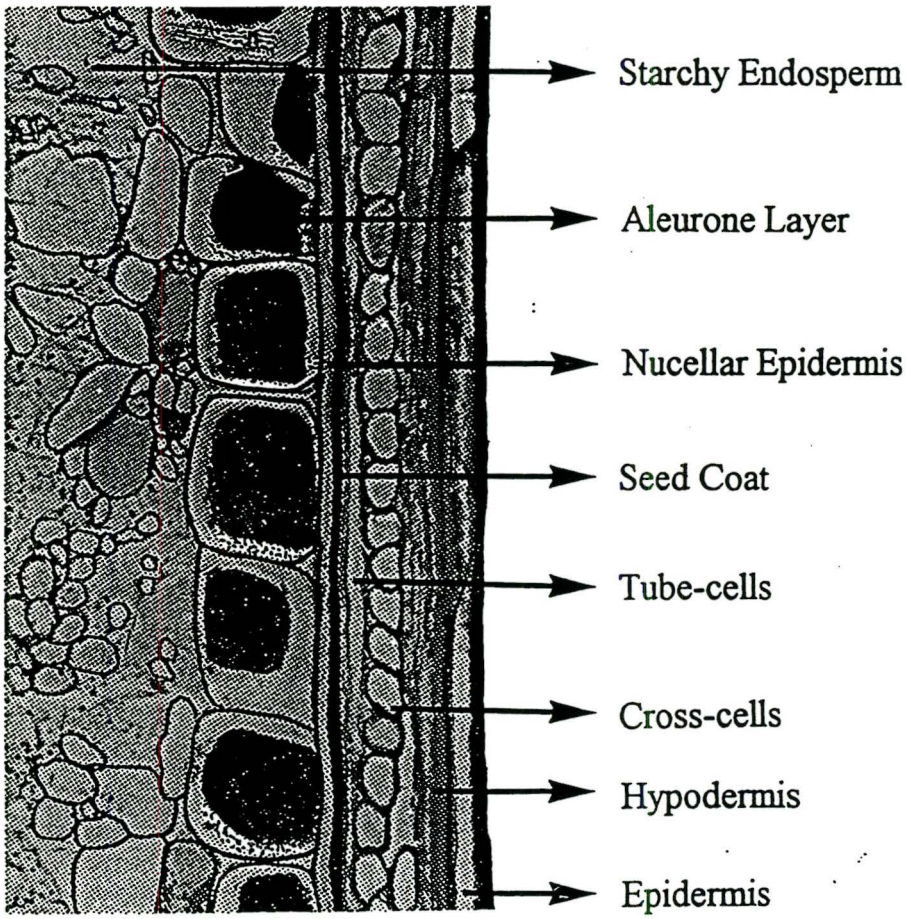
i) Outer Pericarp

The outer pericarp of the kernel consists of epidermis and hypodermis. The epidermis is one cell layer thick and is covered with a thin cuticle layer (Fig 1.1). Stomata are sometimes present, and modifications of cells in the epidermis are responsible for the formation of hairs at the apex of the grain. The hypodermis is also a single cell layer thick, and lies directly under the epidermis (Fig 1.1). Cells of both the epidermis and hypodermis have thickened cell walls ranging in thickness from 3.0 to 5.7 um, and the cells tend to be elongate with very little extracellular space.

ii) Inner Pericarp

The inner pericarp is composed of intermediate cells, cross cells and tube cells. Intermediate cells are absent throughout much of the grain, usually being found only at the apex and around the germ. Cross cells are arranged with their long axes perpendicular to the long axis of the grain, hence the name cross cells (Fig 1.1). These cells are closely joined in rows with very little intercellular space. Tube cells tend to be long and cylindrical, and have their axis running parallel with the long axis of the grain (Fig. 1.1). Like the intermediate cells, tube cells are found only in restricted areas of the pericarp of the grain.

Figure 1.1 - Transection through the pericarp and adjacent tissues of wheat grain. x250. Adapted from Pomeranz, 1971.


Starchy Endosperm
Aleurone Layer
Nucellar Epidermis
Seed Coat
Tube-cells
Cross-cells
Hypodermis
Epidermis

Seed Coat

The seed coat is closely connected to the inner pericarp on the outside and the nucellar epidermis on the inside (Fig. 1.1). Layers of the seed coat consist of an outer and inner cuticle, with a layer of pigment cells running between the two. This pigment layer is responsible for a majority of the colour of the wheat grain.

Nucellar Epidermis

The nucellar epidermis or hyaline layer is a thin layer of crushed cells lying between the seed coat and aleurone layer, and is tightly connected with both (Fig 1.1).

Aleurone Layer

The aleurone layer is the single cell outer layer of the endosperm, but often is more firmly attached to the bran layer. Aleurone cells surround the kernel, are cuboidal to oblong in shape, and have thickened cell walls (Fig. 1.1). Variable thickness of cell size in longitudinal cross section, ranging from 37 to 65μm cause the inner surface of the aleurone layer to have an irregular surface.

Starchy Endosperm

The starchy endosperm makes up the greatest bulk of the grain (Fig 1.1). Three cell types make up the starchy endosperm, peripheral, prismatic, and central. The peripheral cells form a one cell thick layer just inside the aleurone layer. Cells of this layer are similar in size and shape to that of the aleurone layer, except the cell walls are much thinner. The prismatic

cells form a layer one to several cells thick just below the peripheral cells. The shape of the prismatic cells tends to be an intermediate between the regularly shaped, cuboidal peripheral cells, and more irregularly shaped, elongate central cells. The rest of the endosperm is composed of the central cells. Both the prismatic and central cells tend to be more elongate in shape than that of the peripheral cells, and are filled with starch granules surrounded by a protein matrix.

Germ Tissue

The germ or embryo extends along one third of the dorsal surface of the wheat kernel. The germ tissue consists of two major components, the embryo axis and the scutellum.

i) Embryo Axis

The embryo axis is made up of the rudimentary root and shoot. The plumule, or the upper embryo axis, forms the shoot of the plant. The plumule contains several embryo foliage leaves attached to a short stem. These leaves are covered by a protective sheath, the coleoptile. The lower embryo axis is made up of the primary root and root cap, all enclosed by the coleorhiza.

ii) Scutellum

Attached to the embryo axis just below the plumule, the scutellum forms a shield that covers most of the embryo axis, separating it from the endosperm. Most of the scutellum is composed of parenchyma cells abundant in oil droplets ranging from 0.5 to 1.0 μm in

diameter, and large protein bodies with phytate deposits (Swift and O'Brien, 1972). Secreting cells are formed along the outer layer of the scutellum, next to the starchy endosperm.

1.6 - GRAIN COMPOSITION

General

Reserves of the wheat grain consist of 8-15% protein, 1.5-2% lipids, 64-73.5% carbohydrates (starch 60-68%, free sugars 2-3%, cellulose 2-2.5%), and 1.5-2% mineral nutrients (Peterson 1965). There is an uneven distribution of reserves in the wheat grain, with the endosperm comprising a bulk of the starch reserves, the embryo containing a majority of lipid and protein reserves, and the aleurone layer storing most of the mineral nutrient reserves of the grain (Peterson, 1965).

Protein

Protein reserves are found in most of the tissues throughout the grain in specialized vacuoles called protein bodies. In cells of the aleurone layer these protein bodies are often referred to as aleurone grains, or aleurone bodies. In the mature grain, cells of the scutellum, embryo axis, and aleurone layer contain many protein bodies ranging from 2 to 5 μm in diameter. Protein from the endosperm of the mature grain is more of a matrix throughout the entire cell due to the compression and breakage of the protein bodies by the starch granules during dehydration associated with maturation (Parker, 1980; Bechtel, 1985).

Storage, or gluten proteins make up to 75 to 85% of the protein content within the mature wheat kernel. Gluten is a combination of two types of proteins, gliadins and glutelins. The gliadin fraction is a complex mixture of simple polypeptides, and the glutelin fraction is in the form of large disulphide linked molecules or aggregates (Payne *et al* 1985). The four most common amino acids within the storage proteins are glutamine, proline, leucine, and serine, often with glutamine making up to 50% of the total amino acids within the protein molecule (Bernardin, 1978). Work on the wheat grain done to increase the protein levels of the wheat kernel often results in an increase in storage proteins within the grain. Increasing storage protein levels is not always of benefit due to the fact that the wheat is lacking in essential amino acids, and can be considered nutritionally poor (Berardin, 1978). The high glutamine level within storage proteins is partially responsible for interactions that are seen between the proteins. The glutamine molecules form hydrogen bonds with proteins, and with available water. It is this interaction between protein molecules and water that gives the dough made from wheat flour its characteristic elasticity (Bernardin, 1978; Finney, 1978). Lipid molecules are also attracted to the protein molecules. Binding of the lipids to the protein changes the overall charge of the protein molecule, and enhances the protein-to-protein interactions (Bernardin, 1978). Along with storage proteins there are also water soluble enzymatic proteins within the grain. These consist of albumins, globulins, and glycoproteins. The water soluble proteins are responsible for preparing the gluten proteins to enable them to perform their functional properties within the plant (Bernardin, 1978). Protein of the endosperm tends to be high in proline and glutamine, and low in other basic amino acids, whereas protein from the aleurone layer tends to be low in proline and

glutamine, but high in other basic amino acids, especially arginine (Simmons and O'Brien, 1981; Stevens, 1973). The protein content of the aleurone is of importance because 70% of bran protein is actually protein from the adhering aleurone layer, making the bran fraction of the grain a rich source of protein. This bran is often used in animal feed, and other food products (Fulcher *et al* 1972).

Lipids

There is a complex assortment of lipids distributed within the wheat grain. Lipid reserves are in both a free nonpolar and a bound polar form. The nonpolar lipids consist of free fatty acids and triglycerides, and the polar lipids consist of phospholipids and glycolipids. About 70% of whole grain lipids are nonpolar, 20% are glycolipids, and 10% are phospholipids.

The germ of the grain contains the highest lipid content, and also has the highest percentage of phospholipids. Lipids of the endosperm are mainly associated with starch grains, and are also high in phospholipids.

Carbohydrates

Other than cell wall material, large reserves of carbohydrates within the mature grain are stored in starch and phytate.

i) Starch

The highest quantity of starch within the grain is located within the cells of the

endosperm, but starch is also found within the embryo axis, scutellum, and aleurone layers. Starch granules range from 2 μm diameter spherical bodies to 33 μm elongate bodies. The endosperm, being the main reserve for starch, is also the main source of flour. The ratio of starch to protein, and the varying amount of different proteins within the endosperm are the main factors which affect bread quality and use. Due to this relationship intense interest has been put into studies dealing with the levels and types of starch and protein within the wheat endosperm.

ii) Free Sugars

Free sugars such as raffinose and sucrose also contribute to carbohydrate stores within the mature grain. Of these free sugars a majority are stored within the embryo of the grain, and they are utilized during early embryo development (Peterson, 1965).

Phytate and Mineral Nutrients

Phytate is the main component of the globoids which are located within the protein bodies of the cells from the embryo axis, scutellum, and aleurone layer. Although a small store compared to that of protein and starch, phytate is a rich store of myo-inositol, phosphorus, and essential cations (Ockenden and Lott, 1989; Scott and Loewus, 1986). Often 70 to 90% of the grain's phosphorus store is bound in phytate (Boutwell, 1917). Proton microprobes, EDX analysis, and digestive techniques have shown the mature wheat grain also to contain Ca, Cu, Fe, Mg, Mn, K, and Zn within the globoids (Boutwell, 1917; Lott and Spitzer, 1980; Mazzoline *et al* 1985).

1.7 - MORPHOLOGICAL DEVELOPMENT OF WHEAT GRAINS

The time necessary for wheat grain to develop from anthesis to maturity varies substantially depending on many environmental and genetic factors including temperature, rainfall, day length and cultivar (Sofield *et al*, 1974). All influences considered, normal time for grain maturation is approximately 45 to 55 days. During grain ripening there is great translocation of stored reserves from the leaves and stems of the plant into the forming seed tissues, and seed reserves. Most of the requirements of the developing grain have been stored within the plant by the time of anthesis, but photosynthesis continues as long as the plant has green tissue (Peterson, 1965). As reserves are broken down and transported to the developing grain, the leaves of the plant start to die in sequence from the lowest leaves upwards. Chlorophyll is degraded and products are exported from the stem, the leaf and the leaf sheath. While the plant is dying and reserves are being transported, a great deal of change and development occurs within the grain. A brief outline of some of the changes that occur during grain development is summarized below.

Parental Genetic Contributions

Tissues comprising the mature wheat grain come from three locations of the inflorescence of the parent plant. Maternal tissue gives rise to the pericarp layers. The endosperm, both starchy and aleurone, are derived by fertilization of the central cell of the embryo sac. The embryo arises from cells derived from the zygote. Both the endosperm and

the embryo therefore contain genetic material from both the male and female parent.

Embryo Axis Development

The embryo remains quite small until 15-20 days post anthesis. Up to this time the embryo is held in a small out pocketing of the embryo sac. From 15 -30 days post anthesis there is rapid growth of the embryo, and differentiation of the coleoptile, coleorhiza, and the root and shoot primordia (Smart and O'Brien, 1983). By 23 days post anthesis the embryo is about half grown, and a fluid filled space is evident around the embryo. This fluid is the result of the hydrolysis of the endosperm tissue in the area (Smart and O'Brien, 1983). Further development from this point involves the filling of the embryo with storage reserves, growth and expansion.

Scutellum Development

The first signs of scutellar development are 15 days post anthesis (Smart and O'Brien, 1983). From approximately day 23 post anthesis there is an accumulation of reserves into the scutellum starting where the scutellum attaches to the embryo and slowly filling the rest of the scutellum (Smart and O'Brien, 1983).

Endosperm Development

The development of the endosperm takes place in five phases which are outlined below (Simmons and O'Brien, 1981).

i) Phase One - Anthesis to First Triple Fusion Nucleus

Phase one starts when two male nuclei enter the embryo sac and fuse with the egg nucleus and the two polar nuclei. Fusion with the two polar nuclei of the central cell with one of the male nuclei leads to the formation of the primary endosperm nucleus.

ii) Phase Two - Synchronous Free Nuclear Division

Cycles of free nuclear division in the cytoplasm, without the intermediate formation of cell walls, produce nuclei which line the embryo sac.

iii) Phase Three - Division and Enlargement

Phase three starts three days post anthesis and lasts for 24 to 48 hours. In this period there is a great deal of cell division and differentiation, followed by some cell enlargement.

iv) Phase Four - Cell Enlargement and Aleurone Development

Expansion in size during this phase is due to an increase in cell volume, not cell number. In this phase most of the grain storage reserves (protein, starch, and lipids) are accumulated. With the cessation of cell division, the small thin walled outer cells of the endosperm develop into large isodiametric cells with large nuclei and granular contents. By ten days post anthesis some of these cells contain small electron-dense bodies, which are thought to be globoids. Seventeen days post anthesis the electron-dense particles are clearly recognizable as globoids. By day 35 post anthesis the aleurone cells have achieved typical characteristics of the mature grain including: lipid droplets, cytoplasm densely packed with

protein bodies, spherosomes, numerous mitochondria, and plastids. Also in this stage due to asymmetric dorsiventral development of the kernel the two lobes of the grain curve around the groove region.

v) Phase Five - Cell Desiccation

In this phase fresh weight declines as water is lost. There is small loss in dry weight of the grain, this may be due to soluble material moving to other areas of the wheat head, or may be due to respiratory activity.

1.8 - DEVELOPMENT OF GLOBOIDS

Globoids have been detected within the cells of the aleurone layer, scutellum, and embryo axis within the protein bodies (Morrison *et al*, 1975). Each protein body characteristically has one to three large globoids ranging from 1.5 to 2.5 μm, and 15 or more smaller particles, ranging from 0.5 to 1.0 μm (Morrison *et al*, 1975).

Eight to ten days post anthesis the small electron-dense particles first appear within the vacuoles of the cells. These small particles rapidly enlarge to 1 to 2 μm by 14 day post anthesis. Development of the globoids is slowed early after anthesis because cell wall synthesis competes for reserves of myo-inositol (Parker 1980).

1.9 - METABOLISM OF RESERVES DURING GERMINATION AND SEEDLING GROWTH

Mobilization and reallocation of stored reserves of the grain is essential for the establishment of the seedling. Stores of protein, carbohydrates, and mineral nutrients supply the growing seedling with needed requirements, until these requirements can be obtained from the environment or produced by the plant. In cereal plants the mobilization of reserves is stimulated by the release of growth regulators as the radicle starts to elongate during germination (Bewley and Black, 1985).

Protein Mobilization

The mechanism of protein mobilization within the wheat grain is not clearly understood. Products of protein mobilization consist mainly of amino acids, dipeptides, and oligopeptides. Endopeptidases, carbopeptidases, and aminopeptidases are the three main types of enzymes involved in protein breakdown. Early in germination the three mentioned types of enzymes have little effect on reserve breakdown (Bewley and Black, 1985; Kruger and Preston, 1978). Not until the second day of seedling growth does the increased level of proteases correspond to the increased level of storage protein hydrolysis. The two day lag in proteolysis may be due to initial insolubility or compartmentalization of the proteins (Kruger and Preston, 1978).

Lipid Mobilization

Breakdown of lipid reserves within the wheat grain is carried out by a number of lipases. The main products of lipid breakdown consist of glycerol and free fatty acids. Free fatty acids are in turn converted to sucrose through a series of reactions within the glyoxysomes, mitochondria and cytoplasm. Sucrose can then be utilized by the growing embryo (Bewley and Black, 1985).

Carbohydrate Mobilization

i) Starch

In the wheat grain the major starch reserve is located within the starchy endosperm. Small reserves of starch are also found within the embryo and aleurone layer. Starch within the embryo provides a source of respirable substrate for the germinating grain until reserves from the starchy endosperm are mobilized, which is usually not until the third day of seedling growth (Bewley and Black, 1985). The mobilization of the starch from the starchy endosperm begins with the production of alpha-amylase in the region of the scutellum and within the aleurone layer. The synthesis and release of alpha-amylase is controlled by release of gibberellins from the embryo. The major products of starch mobilization are maltose and glucose. Maltose and glucose are converted to sucrose in the scutellum, transferred to and utilized by the growing embryo axis (Bewley and Black, 1985).

ii) Phytate

As phytate is mobilized by phytase, there is the release of monophosphates to hexaphosphates and myo-inositol. Myo-inositol released is incorporated into pectin and hemicellulose-cellulose fractions in the developing embryo axis (Roberts *et al*, 1968). Mineral nutrients released with the breakdown of phytate do not accumulate in the endosperm or scutellum indicating that the embryo axis utilizes the released nutrients at the same rate they are being mobilized (Eastwood and Laidman, 1971).

Wheat Phytase

Phytase is found within wheat grains wherever there is the presence of phytate. Phytase activity is low until the start of germination, when activity rapidly increases due to stimulation by gibberellins released from the embryo (Matheson and Strothers, 1969; Pomeranz, 1971). The increased activity of phytase in the wheat seedling is not due to synthesis of the enzyme but rather it is due to the activation of inactive enzymes (Eastwood and Laidman, 1971). Even though a four fold increase in phytase activity has been observed in wheat aleurone cells during germination, *in vivo* experimentation has shown that phytase level within the grain would be adequate for phytate mobilization, only hydration of the grain is needed, and the increase in phytase activity would be excessive (Eastwood and Laidman, 1971). The degradation of phytate by phytase is of importance for seedling growth due to the need for the stored mineral nutrients and myo-inositol. The activity of phytase has also become of great interest due to the antinutritional effect of phytate. Numerous studies have shown that phytate levels reduce dietary availability of Mg, Ca, Zn, and Fe in breads, and

other cereal products (Bassiri and Nahapetian, 1977; Ranhotra *et al*, 1974). Variations on bread making techniques, and the use of phytase activity stimulators, such as calcium, are techniques being used to decrease phytate levels within cereal products, in hopes of increasing nutritional quality.

1.10 - BIOLOGICAL ROLE OF MINERAL NUTRIENTS

The breakdown of phytate during germination and early seedling growth releases phosphorus, myo-inositol, and other cations. The release of these stores is essential for the establishment of the growing seedling. The mineral nutrient stores are used in various ways throughout the growing plant. The following is a summary of some of the biological roles of the mineral nutrients released as taken from the studies of Hyde *et al* (1963), Anderson and Phyliotis (1969), Mengel and Kirkby (1982)and Glass (1989).

Phosphorus

The most important role of phosphorus in plant tissue is its involvement in energy rich compounds that can release stored energy to cellular processes such as photosynthesis or protein synthesis. Of these high energy compounds, adenosine triphosphate (ATP) is probably the most recognized and essential. Other energy supplying compounds involving phosphorus include; uridine triphosphate, cytosine triphosphate and guanosine triphosphate. Phosphates are also important in compounds such as nucleic acids and glycerol phosphates of membrane phospholipids. Phosphorus deficiency has a great effect on protein and nucleic

acid synthesis resulting in slow growth, and poor fruit or seed set.

Potassium

Potassium is an ion extremely important in the activation of various enzyme systems throughout the plant cell. As well as maintaining osmotic potential, the chemical potential of the water and turgor pressure, the pressure within the cells caused by the movement of water, potassium also plays a key role within the guard cells, adjusting turgor pressure to allow opening and closing of the pore thereby controlling gas exchange and transpiration. Potassium deficiency can lead to the synthesis of toxic amines within the leaves, resulting in slower growth, chlorosis, and decreased turgor within the plant.

Calcium

Calcium has a wide range of biological roles throughout the cell, the most important of which deals with membrane permeability, and cellular integrity. A majority of calcium within the cell is bound as calcium pectate which contributes to cell wall strength. Calcium is also important for the activation of enzyme systems, especially those which are membrane bound, and has been shown to affect cell elongation and division. Lack of calcium will lead to cell permeability leaks and the breakdown of membrane structure, as well as retarded growth and chlorosis of the meristem regions.

Magnesium

Magnesium is an important component of the chlorophyll molecule which accounts

for 20% of the cellular content of magnesium. Magnesium cations are often found associated with mobile (malate and citrate) and insoluble (oxalate and pectate) anions. Magnesium is also a cofactor in enzyme systems involved in phosphorylation processes, forming a bridge between ATP and the enzyme necessary for activity to occur. Deficiency of magnesium may inhibit protein synthesis, causes chlorosis and in severe cases causes necrosis.

Manganese

Like magnesium, manganese plays a role as a cofactor in phosphorylation enzymes forming the bridge between ATP and the enzyme; in most cases manganese can be substituted for magnesium for this role. Manganese is responsible for the oxidation of indole acetic acid (IAA) by the activation of IAA oxidases. Manganese is also involved in the oxidative reduction process in the photosynthetic electron transport system, and is essential in photosystem II where it participates in photolysis of water. Manganese deficiency has a great effect on the chloroplast structure and function. Signs of deficiency are first visible in young leaves showing spots and stripes of chlorosis.

Iron

Iron plays an essential role in many enzyme systems, being an important component of haem, or haemin containing enzymes. Some of these haem containing enzymes are catalase, peroxidase, and cytochrome oxidase. Only a small portion (0.1%) of total iron in the plant is found in the heam systems, a bulk of the remaining is stored as ferric phosphoproteins called phytoferritin, a reserve that is used by developing plastids. Iron is also

found as ferredoxin within the chloroplast. This form of iron is responsible for the transfer of electrons in the oxido-reduction process. Deficiency of iron will slow chlorophyll production in younger leaves, resulting in chlorosis.

Zinc

Zinc is known to be involved in over 80 metallo-enzymes. As well zinc is a cofactor in many other enzyme systems, forming stable complexes with such enzymes as enolase, aldolase, phosphatase, DNA polymerase, RNA polymerase and carbonic anhydrase. Zinc is also needed for the formation of tryptophan, and is involved with nitrogen metabolism. Zinc deficiency will result in leaf chlorosis and abnormal shoot formation and growth.

In the past, vast amounts of research have been done looking at carbohydrate and protein levels within the wheat grain. The goal of the research in this thesis is to present the first comprehensive study of the changes to mineral nutrient levels within globoids during seed set and seedling growth within two cultivars of wheat.

CHAPTER 2 : METHODS AND MATERIALS

2.1 - GRAIN TISSUE AND GROWING CONDITIONS

Grain Tissue

Triticum aestivum grains cv. Celtic and A.C. Reed were supplied by the University of Guelph Crop Science Department, from pooled stock grown in field conditions at the Elora Research Station in 1994 under conditions similar to those described later for 1995 wheat growth.

Germination and Seedling Growth Conditions

Grains of both cultivars were grown in Petri plates on sterile filter paper, moistened with distilled water. Each plate contained ten to twelve grains, and the plates were covered with a clear plastic cup to prevent excessive moisture loss, but still allowing for gas exchange. Growing seedings were kept moist by daily applications of distilled water. Plates of grains were placed in a model E7 Conviron growth chamber and exposed to 16:8 light:dark periods. Light intensity of the chamber was 80 μmol.s^{-1}m^{-2}. A constant temperature of 21 °C was maintained within the chamber both day and night.

Seed Set Conditions

Grains for seed set samples were grown under field conditions at the Elora Research

Station. Grains were planted May 4th, 1995 and reached anthesis by July 15, 1995. Soil was preplant fertilized with 225 lbs/acre of 5-20-20 fertilizer. A broadleaf herbicide was sprayed the first week of July and throughout the growing season for weed control.

2.2 - SAMPLING STAGES

Germination and Seedling Growth

Samples to study changes in mineral nutrient content during seedling growth were selected by coleoptile length. Dry seed, 10 mm and 30 mm coleoptile lengths were chosen to define each of the sampling stages (Table 2.1). A 40 mm coleoptile length was also examined, but no globoids were located within the aleurone, scutellum or the embryonic axes at this stage.

Seed Set

Samples to study changes in mineral nutrient content during grain development were selected on time intervals within the period between grain anthesis and grain maturation (Table 2.1).

Time Limitations

Due to time constraints of the M.Sc. program early seedling growth experimentation had to start in the fall of 1994 with grains that were produced in the summer of 1994. Grains used for seed set experimentation could only be obtained from wheat grown in the summer of 1995.

Table 2.1 - Stages and Collection Times

Seed Set

STAGE	COLLECTION TIME
1	10 DAYS POST ANTHESIS
2	18 DAYS POST ANTHESIS
3	26 DAYS POST ANTHESIS, MATURE DRY GRAIN

Germination and Seedling Growth

STAGE	COLEOPTILE LENGTH
4	DRY GRAIN
5	10 mm
6	30 mm
7	40 mm

- Stages 1, 2 and 3 were taken from grains grown in 1995.
- Stages 4, 5, 6 and 7 were grown from grains produced in 1994.

2.3 - SAMPLING

Sampling for Energy Dispersive X-ray Analysis

For each stage during seed set and seedling growth, ten random grains were used for analysis. From each of these ten grains the aleurone layer, scutellum and embryonic axis were dissected and ten globoids were analysed from each portion, making a total of one hundred globoids analysed for each of the three portions of the grain, for each stage of growth.

Sampling for Whole Grain Analysis

Whole grain P, K, Mg and Ca contents were measured on pooled grains from each of the stages of growth during seed set and seedling growth. Pooled numbers varied from 30 to 100 grains depending on dry weight of the grain at the stage sampled. Whole grain protein content was measured on approximately 10.0 g samples of pooled grains from each stage of growth during seed set and seedling growth.

Sampling for Fresh and Dry Weights

Fresh and dry weights were measured from 3 replicates of 20 random grains from 20 different plants. To obtain dry weights the grains were dried in an oven at 125°C for 3 h (Roberts and Roberts, 1972).

2.4 - EDX ANALYSIS PROCEDURE

Freeze dried aleurone layers, scutella and embryonic axes were separately ground with an alumina mortar and pestle for each of the ten grains from each of the stages of growth. A portion of each ground tissue powder was picked up on carbon-formvar coated, 200 mesh copper grids, which were then recoated with carbon to stabilize the powder and reduce charging within the sample during analysis. Using a JEOL 1200 EX-II scanning transmission electron microscope (STEM) with a PGT model IMIX -II microanalysis system, ten globoids from each of the grain portions were analysed for the following elements P, K, Mg, Ca, Mn, Fe, S, Zn and Cl. X-ray counts for each of the measured elements were obtained at the following window widths, P 1905.3 - 2120.7 eV; K 3193.7 - 3432.3 eV; Mg 1153.7 - 1354.3 eV; Ca 3568.6 - 3813.4 eV; Mn 5758.5 - 6037.5 eV; Fe 6259.9 - 6546.1 eV; Zn 8478.9 - 8795.1 eV; S 2196.6 - 2417.4 eV; Cl 2508.7 - 2735.3 eV. Globoids were analysed for sixty second periods at an accelerating voltage of 80kV. Detector distance, aperture, spot size, and tilt were kept the same for all analyses. Cut off values were required for each of the analysed elements in order to differentiate actual element levels from that of the variation around zero caused by the background modelling program. Cut off values were determined for each element based on the lowest P/B ratio that the analysed element produced. Correction factors for overlapping Ca, Fe and Zn peaks were determined by P. Beecroft and M. West (Beecroft, 1995) and were used within this study to obtain corrected values from the analysis data.

2.5 - ATOMIC ABSORPTION SPECTROSCOPY

Whole grain samples were analysed for K, Mg, and Ca by atomic absorption spectroscopy (AAS) for stages during seed set and seedling growth. Liquid samples were measured by aspirating fine droplets of solution into a 1500 - 3000 °C air-acetylene flame. At these temperatures the sample droplets were converted to ground state ions which absorb light at a characteristic wavelength.

A major problem involved with AAS is chemical interference within the sample. Chemical interference is produced when an element within the sample bonds to the desired sample element causing the element of interest not to be converted to ground state. Therefore it will not absorb light at the expected wavelength, and the sample will show lower concentrations than are actually present (Walsh, 1982). To overcome this interference, elements are added to the sample which will preferentially bond to the interfering element, leaving the desired element free for analysis. When measuring K, Ca and Mg this chemical interference is a concern. To overcome this interference, La was added to samples analysed for Ca and Mg, and Cs was added to samples being analysed for K (Walsh, 1982).

Atomic Absorption Procedure

30 grain pooled samples were finely ground to a homogeneous mixture. A mortar and pestle was used for grinding of the wet samples from stages 1, 2, 5 and 6 (Table 2.1), and an electric blender was used to grind dry samples from stages 3 and 4 (Table 2.1). From each stage duplicate, 0.2 g samples were weighed out from the pooled ground grains for Ca

determination, and 4 samples of 0.07 g were weighed out from the pooled ground grains for Mg and K determination. Samples were placed into 15 ml crucibles and placed on a hotplate with a central temperature of 465 °C to char. Charring was started on the outer edges of the hotplate and as smoking ceased, the samples were moved towards the hotter centre of the hotplate. For the final charring the crucibles were covered with tin foil (Ockenden and Lott, 1986). Charring was completed when the samples no longer produced smoke. Charred samples were placed into a Blue M Electric Company muffle furnace and ashed at 550 °C for 4 h. The ashed sample was then acid treated first with 1:2 v/v Aristar nitric acid/deionized distilled water, using about 1.5 ml of nitric acid. The acid solution was then heated on low temperature to dryness. Samples were then treated with a 1:1 v/v hydrochloric acid/deionized distilled water solution using about 1.5ml hydrochloric acid. Again the solution was heated on low temperature to dryness. The dry acid treated ash was then prepared for AAS by diluting the samples to give a final volume of 0.01g sample tissue/ml (Ockenden and Lott, 1986). K samples were diluted initially with deionized distilled water, and the final volume was made with Cs (1100 ppm) and water. The Mg and Ca samples were diluted with an La/HCl solution (1000 ppm). For each dilution 5ml of the diluting solution was swirled around the crucible, stirred with a glass rod and poured into a volumetric flask with the use of a funnel. This procedure was repeated 3 times with 2ml rinses. Finally the glass rod and funnel were rinsed into the flask, and the flask was brought to volume with the diluting solution. Ca samples were diluted to 25ml. Samples were centrifuged for 2 to 3 minutes, the supernatant was decanted and stored in vials for analysis. The Mg and K samples were initially diluted to 50ml and centrifuged for 2 min between 1500 and 2000 RPM. 1 ml

samples of the supernatant were transferred to 10 ml volumetric flasks, diluted to volume and centrifuged again. The supernatant was then decanted and stored for analysis (Ockenden and Lott, 1986).

Standards of known concentrations were prepared for Ca, Mg and K. K standards of 0.2, 0.8, 1.2, 1.6 and 2.0 ppm were prepared from 100 ppm stock K/Cl solution in 1100 ppm Cs. Ca standards of 1.0, 2.0, 3.0, 4.0 and 5.0 ppm were prepared from stock $Ca/C0_3$ solution in 1000 ppm La/HCl. Mg standards of 0.05, 0.1, 0.2, 0.4, 0.6 and 0.8 were prepared from 100ppm stock Mg solution in 1000 ppm La/HCl. All standand stocks were supplied by VWR Scientific. Atomic absorption readings were taken on the known concentrations so a standard line could be produced. From this line the concentration of the element from the samples could be determined by absorbency values obtained.

2.6 PHOSPHORUS DETERMINATION

Whole grain samples were analysed for phosphorus spectrophotometrically by the molybdenum blue method derived from the AOAC Official Methods of Analysis (Helrich, 1990). Acid digested samples produced a colour reaction relative to the amount of phosphorus present within the sample. Light absorbance values were obtained on a Zeiss PMQ II spectrophotometer.

Phosphorus Analysis Procedure

Fifty grain pooled samples were finely ground to a homogeneous mixture. A mortar and pestle was used to grind wet samples from stages 1, 2, 5 and 6 (Table 2.1), and an electric

grinder was used to grind dry samples from stages 3 and 4 (Table 2.1). Dry ground samples were filtered through a 1mm mesh to remove any larger fragments that remained after grinding. Approximately 0.10 g samples were placed in each of 6 digestion tubes. To each tube 3ml of concentrated HNO_3, 1/2ml of concentrated H_2SO_4 and a small glass bead were added. Digestion tubes were heated with a kjeldahl digestion unit to boiling for approximately 50 min until thick white sulphuric acid fumes were produced within the tube. Throughout approximately the first half hour of boiling the tubes were tapped continuously with a plastic rod to avoid the samples boiling over. Once the thick white smoke was produced the tubes; they were removed from the heat, allowed to cool and approximately 0.75ml of hydrogen peroxide was added. Digestion tubes were again heated until thick white fumes were produced within the tube, they were then removed from the heat, allowed to cool and approximately 10 ml of deionized distilled water was added to each of the tubes. Samples were transferred to 25 ml volumetric flasks, tubes were rinsed 3 times into the 25 ml volumetric flask with approximately 2 ml of water per rinse, and the volumetric flask was filled to volume with deionized distilled water. Two ml samples were transferred into 10 ml volumetric flasks from each of the 25 ml volumetrics. To each 2 ml of sample 1ml of molybdenum (Mb), 1ml of hydroquinone (HQ) and 1ml of sodium sulfite (SO_3) was added and the volumetric was filled to volume with deionized distilled water. Molybdenum, hydroquinone and sodium sulfite were prepared as outlined in the AOAC Official Methods of Analysis (Helrich, 1990). A Blank sample, a 2 ml and 1 ml PO_4 standards were prepared in 10 ml volumetric flasks, adding the 1 ml Mb, 1ml HQ, and 1 ml SO_3. 10 ml samples were allowed to sit for 45 minutes at room temperature while the colour reaction occurred, and

absorbence values were then determined.

2.7 - PROTEIN CONTENT

Whole grain protein content was determined by combustion of approximately 10 g of pooled samples. As samples were combusted the nitrogen gas that was emitted from the samples was measured. Total nitrogen was multiplied by a standard 6.25 to determine whole grain crude protein. Protein evaluation was carried out by Agri-Food Laboratories, Guelph, Ontario.

2.8 - STATISTICAL ANALYSIS

Minintab Statistical Software Version 7.2 was used to carry out analysis-of-variance (AVOVA'S) and Tukey's tests. ANOVA'S determined statistically significant sources of variation and the Tukey's test was used to determine differences within the analysed groups.

CHAPTER 3 : RESULTS

3.1 - MORPHOLOGICAL CHANGES

Seed Set

Grains of A.C. Reed and Celtic demonstrated typical grain growth patterns described by Waldren and Flowerday (1979). Grains of both cultivars were planted May 4, 1995 and had reached anthesis by July 15, 1995. Grains of both cultivars matured and dried by Aug 9, 1995. Grains of A.C. Reed developed and dried approximately 5 days faster than that of grains of Celtic. During the time between anthesis and grain maturation chlorophyll was degraded and mobilized within the plant. This break down of chlorophyll caused the plant to change colour from green to brown. Degradation of the chlorophyll became noticeable within the leaves of the plant 2 weeks post anthesis. Later in growth chlorophyll was degraded within the stem followed by complete breakdown of chlorophyll within the entire plant by maturation (Fig. 3.1).

Early Seedling Growth

Early seedling growth followed the pattern documented by Waldren and Flowerday (1979). Grains of both cultivars germinated by the second day of growth after imbibition. Grains of the A.C. Reed cultivar reached 30mm of coleoptile length by 5 days

Figure 3.1 - Morphological changes of the wheat plant 2 weeks post anthesis and at grain maturation.

Figure 3.1 a) Celtic wheat plant 2 weeks post anthesis.

Figure 3.1 b) Celtic wheat plant at maturity.

Figure 3.1 a)

Figure 3.1 b)

of growth, and grains of the Celtic cultivar reached coleoptile length of 30 mm by 6 days of growth. As the seedlings grew, storage tissue in the starchy endosperm was degraded and the grain cavity was emptied.

Grain Fresh and Dry Weights

Fresh and dry weights were determined for each stage of growth. From these weights the percent moisture of each growth stage could also be determined (Table 3.1). For both cultivars, fresh weight decreased and dry weights increased during seed set. During early seedling growth fresh weight increased throughout each stage for both cultivars. Dry weights for both cultivars increased between stages 4 and 5 and than dropped slightly by stage 6. Celtic grains generally had higher fresh and dry weights than that of A.C. Reed. Stages 3 and 4 were both dry grain stages, stage 3 being from the end of seed set in 1995 and stage 4 the start of seedling growth using grains produced in 1994. Between stages 3 and 4 dry weights were similar, but fresh weights were higher in stage 3 samples. This higher fresh weight caused the percent moisture to be higher in stage 3 grains for both A.C. Reed and Celtic cultivars.

3.2 - SUBCELLULAR CHANGES

Globoids

Globoids that were analysed ranged in size from 0.25 μm to 2.50 μm in diameter. Within both cultivars, globoids found within the aleurone layer tended to be larger in diameter than those found in the scutellum and embryonic axis. Although harder to detect

Table 3.1 - Mean (±SD) fresh weights (g), dry weights (g), and percent moisture of A.C. Reed and Celtic whole grains and seedlings at various stages of growth.

		FRESH WEIGHT			**DRY WEIGHT**			**PERCENT MOISTURE**	
STAGE		REED	CELTIC		REED	CELTIC		REED	CELTIC
SEED SET									
1	MEAN- SD-	0.86 ±0.05	1.10 ±0.08		0.39 ±0.02	0.52 ±0.01		54.89 ±1.23	52.52 ±2.54
2	MEAN- SD-	0.87 ±0.05	1.23 ±0.05		0.60 ±0.03	0.71 ±0.01		31.71 ±1.44	42.35 ±1.98
3	MEAN- SD-	0.72 ±0.03	0.82 ±0.03		0.60 ±0.02	0.71 ±0.02		13.54 ±0.18	12.45 ±2.18
SEEDLING GROWTH									
4	MEAN- SD-	0.58 ±0.01	0.69 ±0.01		0.53 ±0.01	0.63 ±0.01		8.63 ±0.21	8.30 ±0.18
5	MEAN- SD-	1.72 ±0.02	1.88 ±0.02		0.60 ±0.002	0.71 ±0.02		65.15 ±0.20	62.04 ±0.54
6	MEAN- SD-	1.95 ±0.20	0.86 ±0.05		0.56 ±0.003	0.61 ±0.03		71.45 ±0.22	74.49 ±0.81

SD = Standard deviation

at stage 1, globoids were found in all three grain portions analysed throughout seed set. During seedling growth the globoids were absent from the scutellum and embryonic axis by the 10 mm coleoptile stage. Globoids remained within the aleurone until the 30 mm coleoptile stage by which time they were infrequent and difficult to locate. Globoids were not present within the aleurone by the 40 mm coleoptile stage.

Changes in Globoid Element Composition

Changes were similar for both cultivars of wheat. Even before background subtractions were completed, general trends could be seen using the spectra collected. During seed set globoids from the aleurone layer, scutellum, and embryonic axis from each stage had high P, K and Mg peaks. As seed set progressed K peaks in all three grain portions increased and Mg peaks appeared to decrease. No other noticeable changes were observed in the heights of the other peaks (Fig 3.2). During early seedling growth it was obvious from the peaks that K levels were decreasing, while again there was no noticeable changes in the heights of the other peaks of biological origin (Fig 3.3).

Peak-to-Background Ratios

Peak-to-background (P/B) ratios were calculated for P, K, Mg, Ca, Mn, Fe, Zn, S and Cl, from each globoid of the analysed grain portions during seed set and seedling growth. Mean P/B ratios and standard deviations for all elements analysed during seed set are shown in Table A1 in Appendix 1. Mean P/B ratios for P, K, Mg and Ca are shown in table 3.2.

Figure 3.2 and 3.3 - EDX spectra produced from globoids of freeze dried powders of Celtic wheat grains. Spectra produced from A.C. Reed grains generally followed the same trends.

Figure 3.2 - Typical spectra produced from EDX analysis of the globoids from the aleurone, scutellum and embryo axis during seed set.

a) Aleurone Layer
1) Stage 1
2) Stage 2

b) Scutellum
1) Stage 1
2) Stage 2

c) Embryo Axis
1) Stage 1
2) Stage 2

Figure 3.3 - Typical spectra produced from EDX analysis of globoids of the aleurone layer during early seedling growth.

a) Stage 4

b) Stage 6

- X-axis shows the X-ray energy in keV from 0 to 10.
- Cu peaks observed are artifacts produced by the use of copper grids to hold the samples during analysis.

Figure 3.2 - Seed Set

Figure 3.2 a)

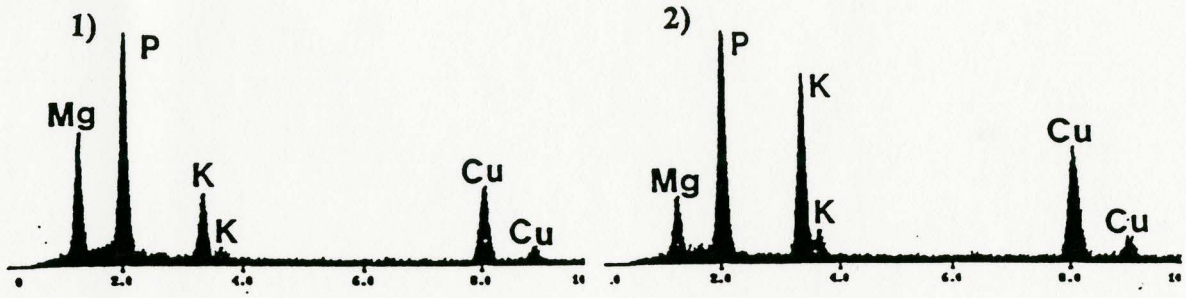


Figure 3.2 b)

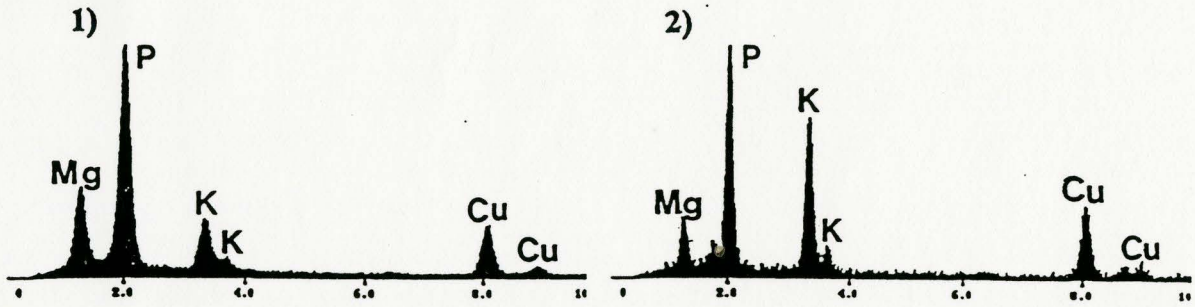


Figure 3.2 c)

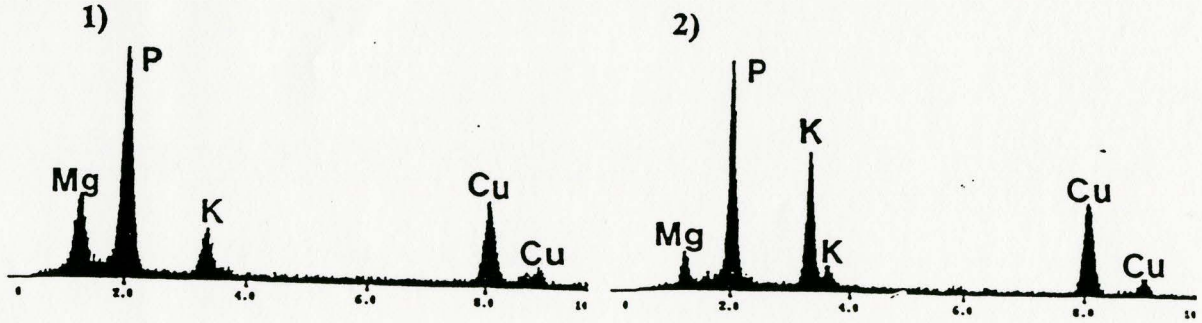


Figure 3.3 - Early Seedling Growth

Figure 3.3 a)

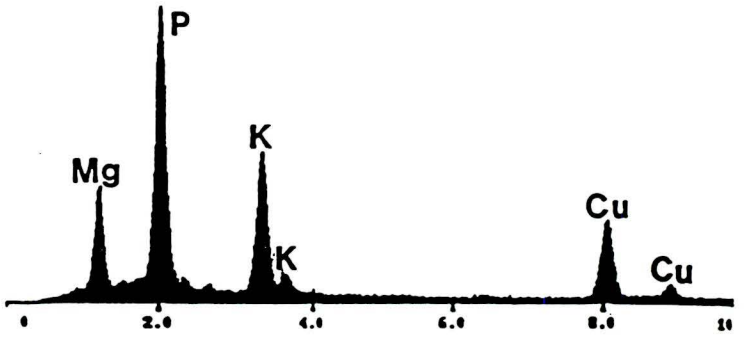


Figure 3.3 b)

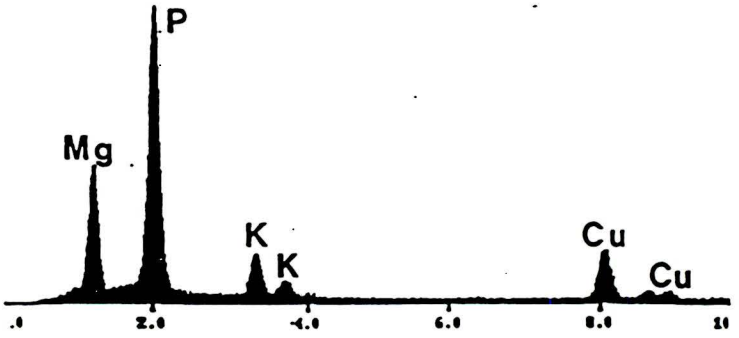


Table 3.2 - Mean peak-to-background ratios of P, K, Mg, and Ca produced from EDX analysis of globoids from the aleurone layers, scutella, and embryonic axes of A.C. Reed and Celtic wheat grains at various stages of growth during seed set.

3.3 a) Phosphorus peak-to-background ratios for A.C. Reed grains
3.3 b) Phosphorus peak-to-background ratios for Celtic grains
3.3 c) Potassium peak-to-background ratios for A.C. Reed grains
3.3 d) Potassium peak-to-background ratios for Celtic grains
3.3 e) Magnesium peak-to-background ratios for A.C. Reed grains
3.3 f) Magnesium peak-to-background ratios for Celtic grains
3.3 g) Calcium peak-to-background ratios for A.C. Reed grains
3.3 h) Calcium peak-to-background ratios for Celtic grains

- Values in the same row followed by the same letter are not significantly different at $P > 0.05$.
- Values in the same column followed by the same number are not significantly different at $P > 0.05$.

Table 3.2 a - A.C. Reed - Phosphorus

	STAGE		
TISSUE	1	2	3
ALEURONE	11.29A1	10.64A1	8.89B1
SCUTELLUM	10.16A2	10.64A1	7.57B1
EMBRYONIC AXIS	8.68A2	8.80A2	6.65B2

Table 3.2 b - Celtic - Phosphorus

	STAGE		
TISSUE	1	2	3
ALEURONE	11.08A1	11.50A1	10.39B1
SCUTELLUM	8.19A2	8.14A2	6.63B2
EMBRYONIC AXIS	10.01A1	9.98A3	7.17B3

Table 3.2 c - A.C. Reed - Potassium

	STAGE		
TISSUE	1	2	3
ALEURONE	4.24A1	9.08B1	12.74C1
SCUTELLUM	5.05A2	8.81B1	11.95C1
EMBRYONIC AXIS	5.50A3	7.04B2	10.77C2

Table 3.2 d - Celtic - Potassium

	STAGE		
TISSUE	1	2	3
ALEURONE	3.82A1	11.08B1	13.45C1
SCUTELLUM	5.43A2	5.92A2	9.13B2
EMBRYONIC AXIS	5.21A2	6.22B2	10.72C3

Table 3.2 e - A.C. Reed - Magnesium

	STAGE		
TISSUE	1	2	3
ALEURONE	5.24A1	4.14B1	2.82C1
SCUTELLUM	3.21A2	2.93A2	2.37B1
EMBRYONIC AXIS	2.39A3	1.91B3	2.78A1

Table 3.2 f - Celtic - Magnesium

	STAGE		
TISSUE	1	2	3
ALEURONE	5.68A1	4.36B1	3.03C1
SCUTELLUM	2.04A2	2.37A2	2.45A2
EMBRYONIC AXIS	3.05A2	3.22A3	3.54A3

Table 3.2 g - A.C. Reed - Calcium

	STAGE		
TISSUE	1	2	3
ALEURONE	0.80A1	0.71A1	0.27B1
SCUTELLUM	0.62A1	1.98B2	1.24C2
EMBRYONIC AXIS	0.57A2	0.23A3	0.59A3

Table 3.2 h - Celtic - Calcium

	STAGE		
TISSUE	1	2	3
ALEURONE	0.71A1	0.33B1	0.42B1
SCUTELLUM	0.57A1	1.14B2	0.61A1
EMBRYONIC AXIS	0.59A1	.055A3	0.59A1

Mean P/B ratios and standard deviations for P, K, Mg and Ca from globoids of the aleurone layer during seedling growth are shown in table 3.3.

Peak-to-Background Ratios During Seed Set

Phosphorus: During seed set phosphorus P/B ratios decreased significantly within globoids from the aleurone, scutellum, and embryo axis of both cultivars of wheat. In all cases this decrease occurred between the second and third stages of growth (Table 3.2 a/b; Fig. 3.4 a-f). In A.C. Reed grains phosphorus P/B levels were highest in globoids from the aleurone and scutellum compared to the embryo axis throughout each stage of growth (Table 3.2 a). In Celtic grains globoids from the aleurone layer also had significantly higher P/B ratios than that of the scutellum, or embryo axis, except for stage one where globoids from the aleurone and embryo axis layer were not significantly different (Table 3.2 b).

Potassium: Potassium P/B ratios increased significantly in globoids from all 3 grain portions examined throughout each stage of seed set, except for Celtic scutellum where an increase was not seen until the third stage of growth (Table 3.2 c/d; Fig. 3.4 a-f). Within A.C. Reed grains potassium P/B ratios were significantly lower in globoids from the aleurone during stage one in comparison to the scutellum and embryo axis globoids. By stage 2 and 3 levels within the aleurone and scutellum were significantly higher than that of the embryo axis (Table 3.2 c). Like A.C. Reed grains, Celtic grains also had significantly lower P P/B ratios in globoids from the aleurone layer at stage one, but ratios within the aleurone were then higher than globoids from the scutellum and the embryo axis by stages 2 and 3 (Table 3.2 d).

Table 3.3 - Mean (±SD) peak-to-background ratios from EDX analysis of globoids from the aleurone layer of A.C. Reed. and Celtic wheat grains at various stages of early seedling growth.

	STAGE		P	K	Mg	Ca	Mn	Fe	Zn	S	Cl
A.C. REED	4	MEAN SD	10.06A 2.38	11.64A 2.97	3.17A 1.28	0.39A 0.34	NS	0.19A 0.11	NS	0.36A 0.27	NS
	5	MEAN SD	12.03B 1.90	10.07B 2.84	4.87B 1.14	0.73B 0.57	NS	0.21A 0.15	0.12B 0.13	0.21B 0.19	NS
	6	MEAN SD	12.45B 2.12	5.66C 1.89	5.27B 1.26	0.61C 0.49	NS	0.24A 0.12	NS	0.17B 0.33	NS
CELTIC	4	MEAN SD	12.09A 2.04	11.02A 3.03	4.71A 1.18	0.46A 0.17	NS	0.16A 0.11	0.13A 0.07	0.07A 0.17	NS
	5	MEAN SD	12.11A 1.76	8.70B 1.97	5.31B 1.06	0.50A 0.32	NS	0.28B 0.28	NS	0.11A 0.15	NS
	6	MEAN SD	12.65A 1.93	6.02C 2.43	5.73B 1.40	0.78B 0.73	NS	0.23A 0.13	NS	0.14A 0.20	NS

- Values in the same row followed by the same letter are not significantly different at $P > 0.05$.
- NS = mean P/B ratios were not greater than the cut off level.

Figure 3.4 - Changes to the mean peak-to-background ratios of P, K, Mg and Ca of globoids from the aleurone layer, scutellum, and embryo axis of A.C. Reed and Celtic wheat grains during seed set.

Figure 3.4 a) A.C. Reed aleurone layer

b) Celtic aleurone layer

c) A.C. Reed scutellum

d) Celtic scutellum

e) A.C. Reed embryo axis

f) Celtic embryo axis

Figure 3.4 a) P/B Ratios
A.C. Reed Aleurone Layer

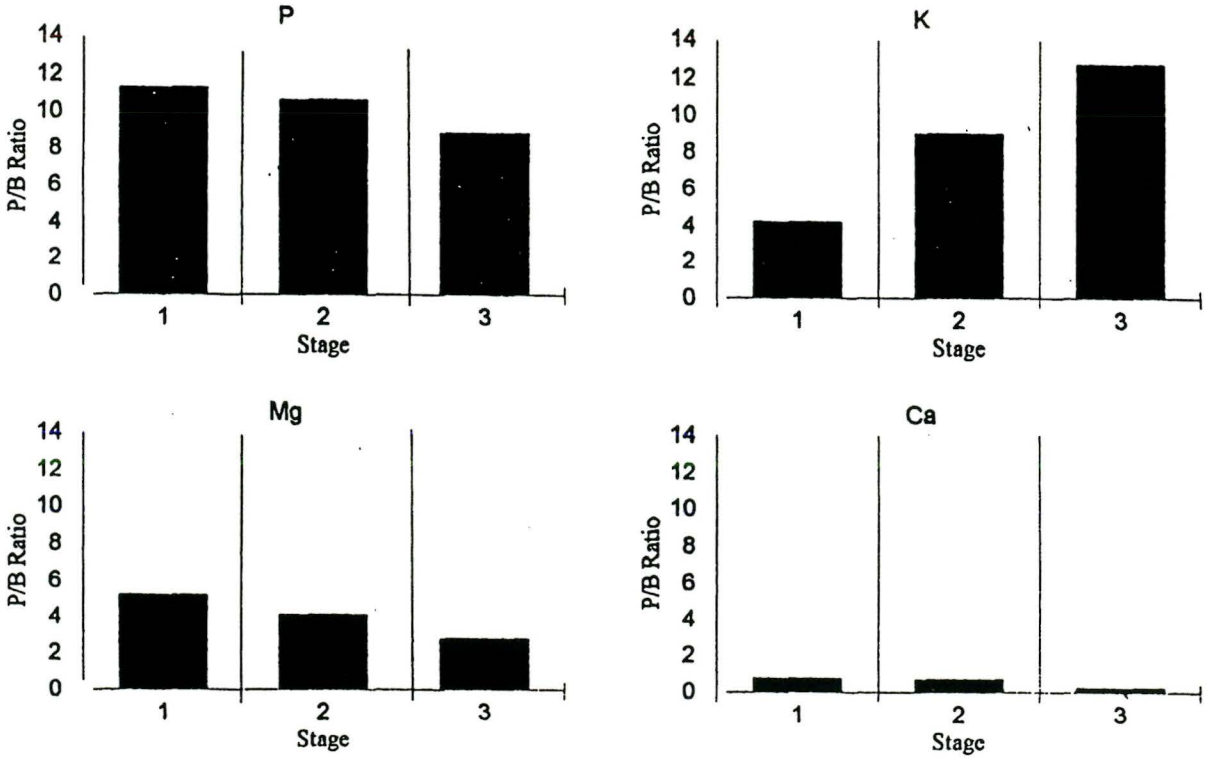


Figure 3.4 b) P/B Ratios
Celtic Aleurone Layer

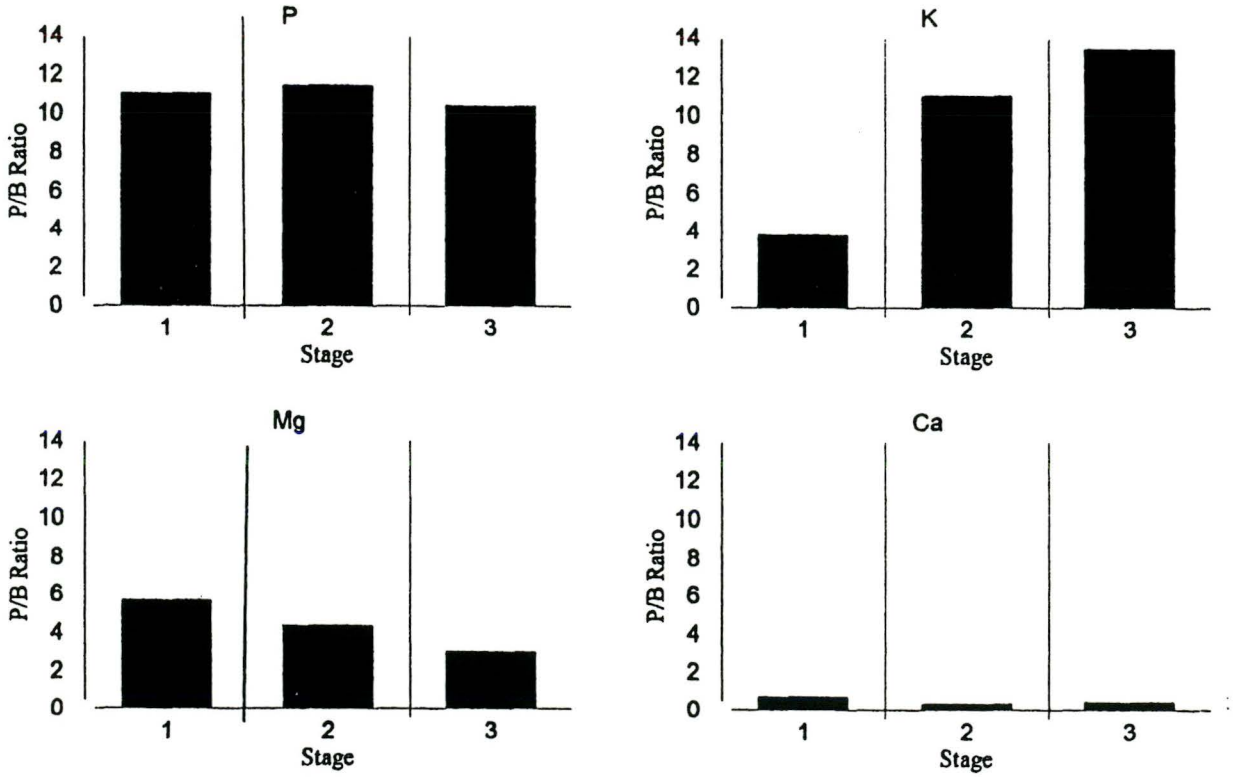


Figure 3.4 c) P/B Ratios
A.C. Reed Scutellum

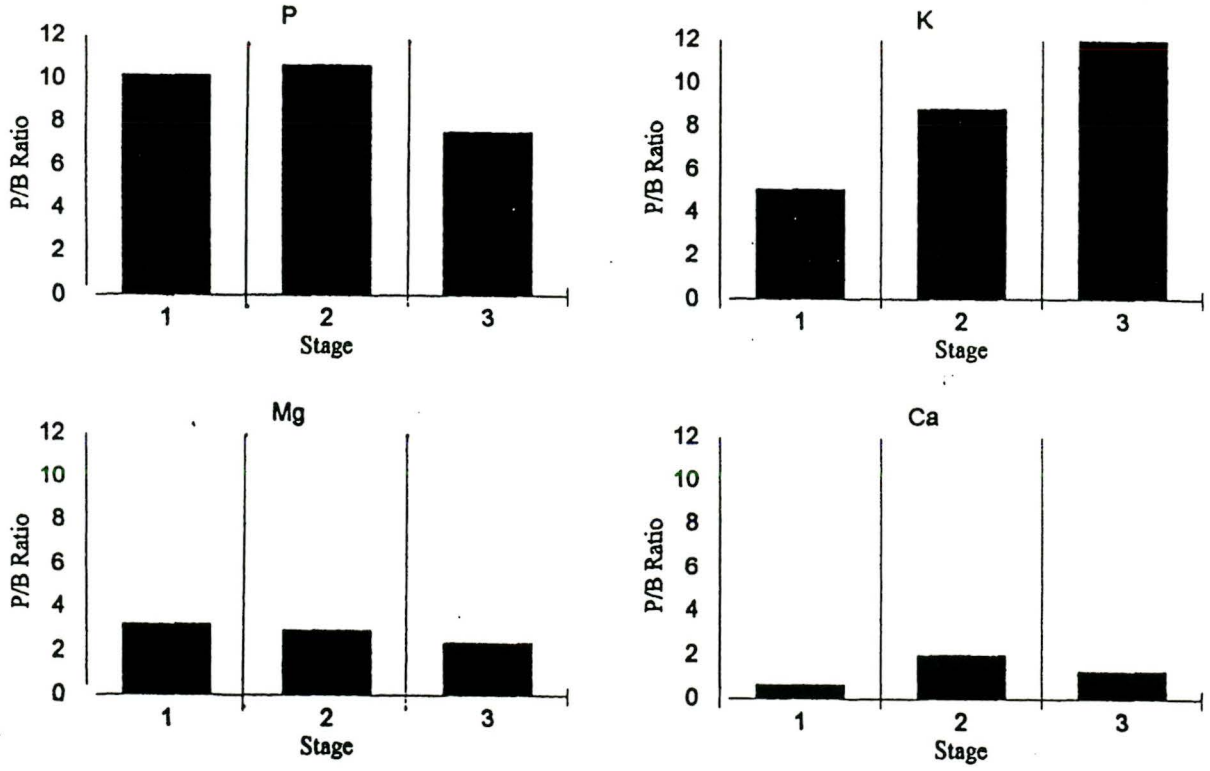


Figure 3.4 d) P/B Ratios
Celtic Scutellum

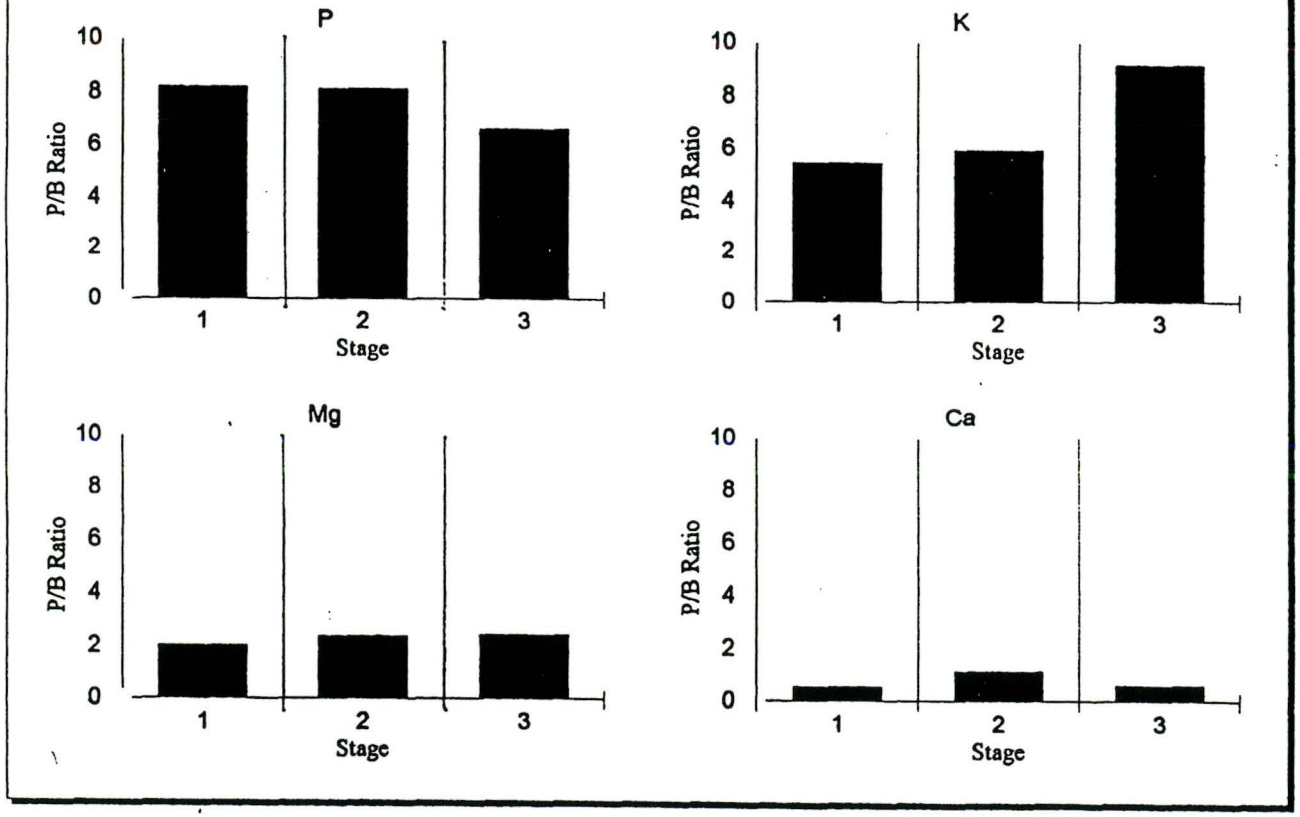


Figure 3.4 e) P/B Ratios
A.C. Reed Embryonic Axis

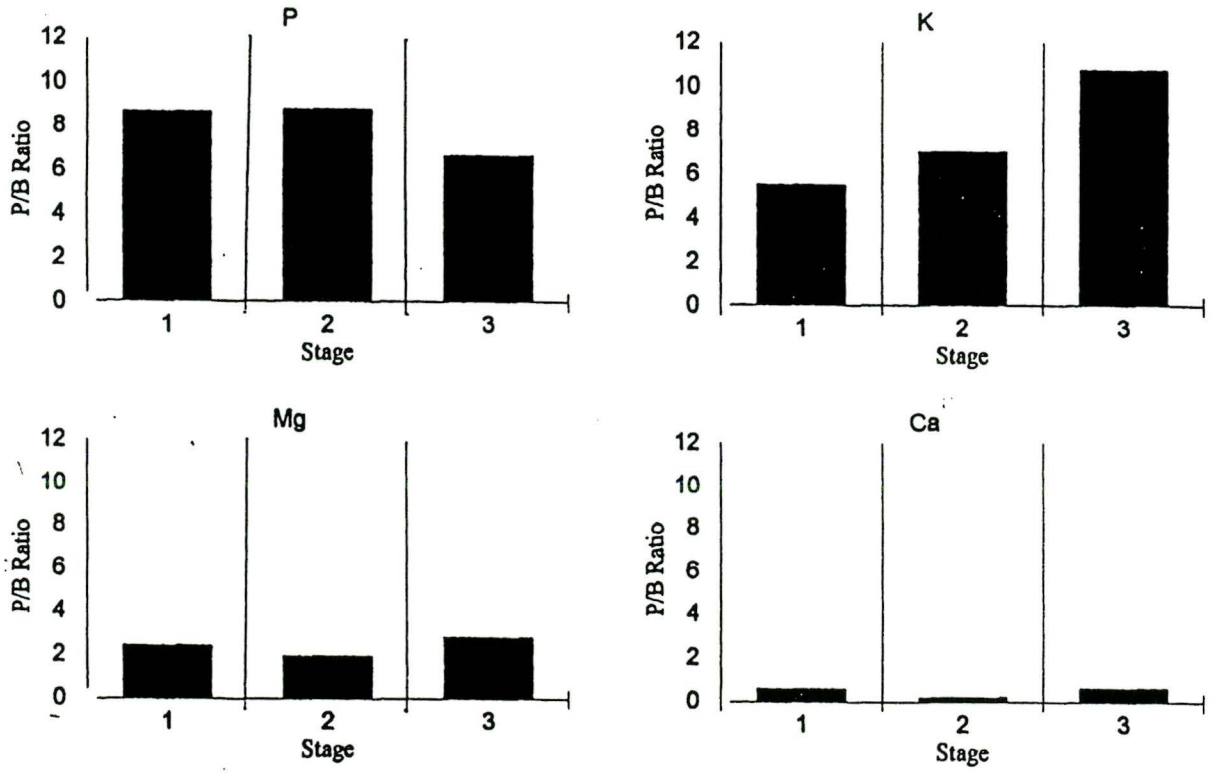
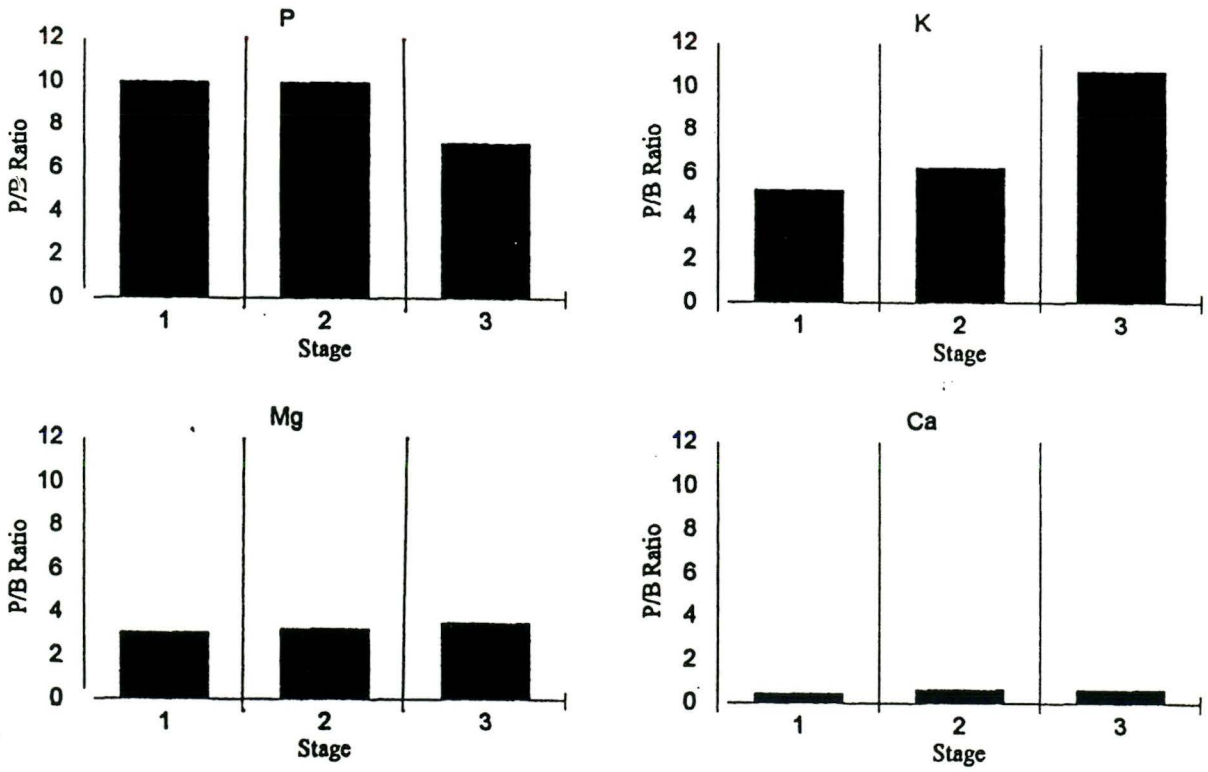


Figure 3.4 f) P/B Ratios
Celtic Embryonic Axis



Magnesium: A.C. Reed and Celtic grains both had a significant decrease in P/B ratios for Mg within globoids of the aleurone layer over each of the stages of seed set (Table 3.2 e/f)(Fig. 3.4 a-f). Globoids from the scutellum and the embryo axis showed little change over this period in Celtic grains. A.C. Reed grains had a decrease in Mg in the embryo axis globoids by stage 2, which was followed by an increase by stage 3 (Table 3.2 e). P/B ratios also increased in globoids from the scutellum between stages 2 and 3 (Table 3.2 e) During stages 1 and 2, A.C. Reed grain had significantly higher P/B ratios of Mg within globoids from the aleurone than that of the scutellum and the embryo axis. P/B values of globoids from the embryo axis were also lower than that of the scutellum in A.C. Reed grains during these stages. By stage 3 there was no significant difference within globoids from the tissues of the A.C. Reed grains. Celtic grains also had higher P/B values within globoids from the aleurone layer than from scutellum and embryo axis globoids over the 3 stages of seed set. Levels within the embryo axis were slightly higher than that of the scutellum over stages 2 and 3 of seed set.

Calcium: In both A.C. Reed and Celtic grains there was a decrease in Ca P/B ratios within globoids from the aleurone during seed set. In globoids from the scutellum of both cultivars a large increase occurred before stage 2, which was followed by a decrease in P/B ratios within both cultivars as the grains matured. The globoids from the embryo axis of both cultivars showed little change throughout the stages of seed set (Table 3.2 g/h; Fig. 3.3 a-f). At stage 1, Ca P/B ratios were similar for globoids from all portions of the grains studied from both cultivars. By stage 2 in both cultivars, Ca levels were significantly higher in globoids from the scutellum than that of globoids from the aleurone

or the embryo axis. A.C. Reed grains still had higher Ca level in globoids from the scutellum at stage 3, but in Celtic grains there was no significant difference within the Ca levels of globoids from each of the grain portions (Table 3.2 g/h).

Manganese and Zinc: Mn and Zn concentration were localized to globoids from the embryo axis. Mn levels increased in Celtic grains between stages 1 and 2 (Table A1 c). Zn levels remained relatively constant during stages of seed set (Table A1 c) .

Iron, Sulphur and Chlorine: Values of Fe, S and Cl were obtained from globoids from the aleurone layer, scutellum, and embryo axis for the two cultivars of wheat (Table A1). These values seemed to follow no biological trend, levels were not detected in all globoids and when they were detected by the P/B program the values often were too low to be considered as anything more than a fluctuation about the background level. Due to these circumstances further discussion of these elements was omitted from this section.

Peak-to-Background Ratios During Early Seedling Growth

Due to the fact that globoids were not detectable within the scutellum and embryo axes by the fourth stage of growth, P/B ratios were obtained only from the aleurone layer of the two cultivars of wheat for stages 4 through 6. Mean P/B ratios and standard deviations for stages of early seedling growth are shown in Table 3.3. Again, due to the fact that no particular trend was observed or because the actual P/B ratios were too small, Mn, Fe, Zn, S, and Cl will not be discussed further in this section.

Phosphorus: Throughout early seedling growth Celtic grains had no significant change in P/B ratios for phosphorus within globoids from the aleurone layer. A.C. Reed grains had

a slight decrease by the fifth stage of growth then remained constant (Table 3.3; Figure 3.5 a/b).

Potassium: In both A.C. Reed and Celtic grains a significant decrease in P P/B levels occurred within globoids from the aleurone through each stage of early seedling growth (Table 3.3; Fig. 3.5 a/b).

Magnesium: In the globoids of the aleurone layer of both cultivars the P/B levels of Mg increased between the fourth and fifth stage of early seedling growth. No significant change was noted within either cultivar between the fifth and sixth stage of growth (Table 3.3; Fig 3.5 a/b).

Calcium: Calcium P/B ratios increased in both cultivars during early seedling growth. A.C. Reed aleurone had increased by the fifth stage of growth, followed by a slight decline by the sixth stage. Celtic aleurone globoids remained constant between stages 4 and 5 and then significantly increased by stage 6 (Table 3.3; Fig 3.4 a/b).

Figure 3.5 - Changes to the mean peak-to-background ratios of P, K, Mg and Ca of globoids from the aleurone layer of A.C. Reed and Celtic grains during early seedling growth.

Figure 3.5 a) A.C. Reed aleurone layer

Figure 3.5 b) Celtic aleurone layer

Figure 3.5 a) P/B Ratios
A.C. Reed Aleurone Layer

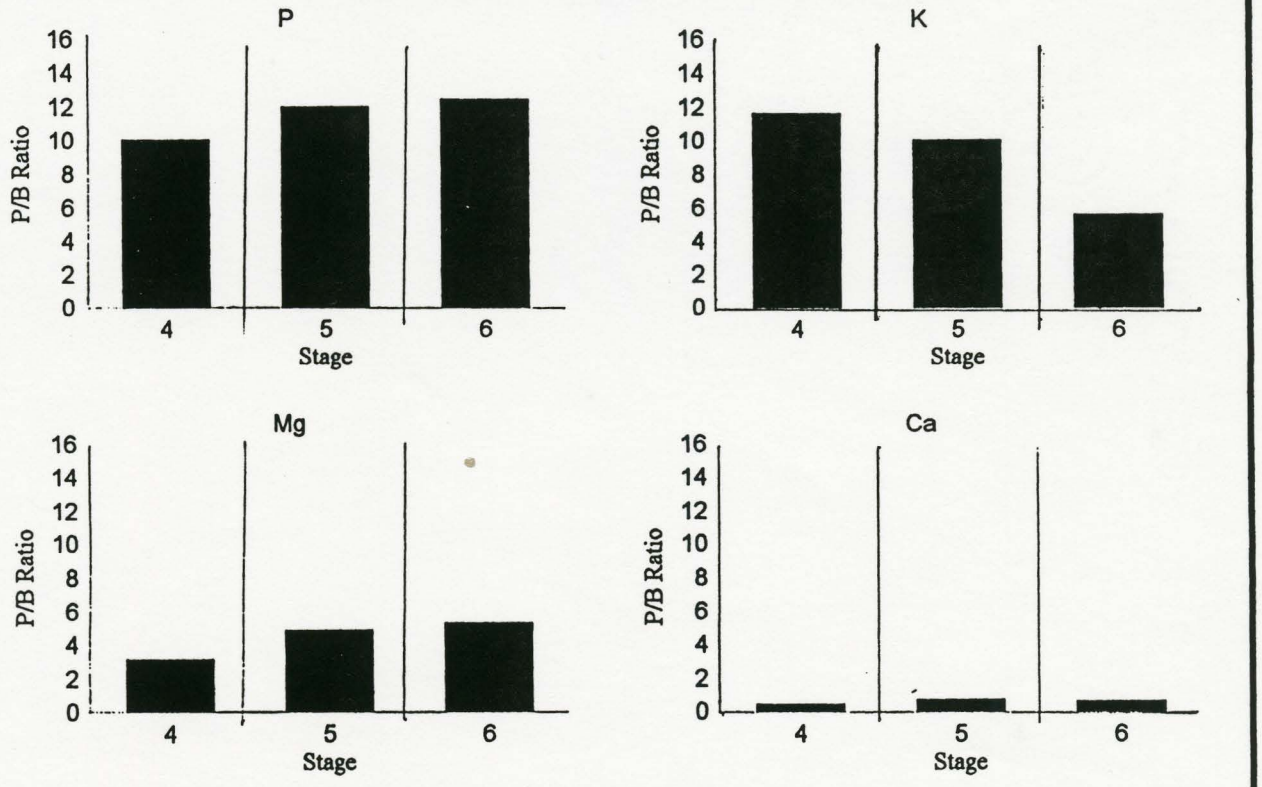
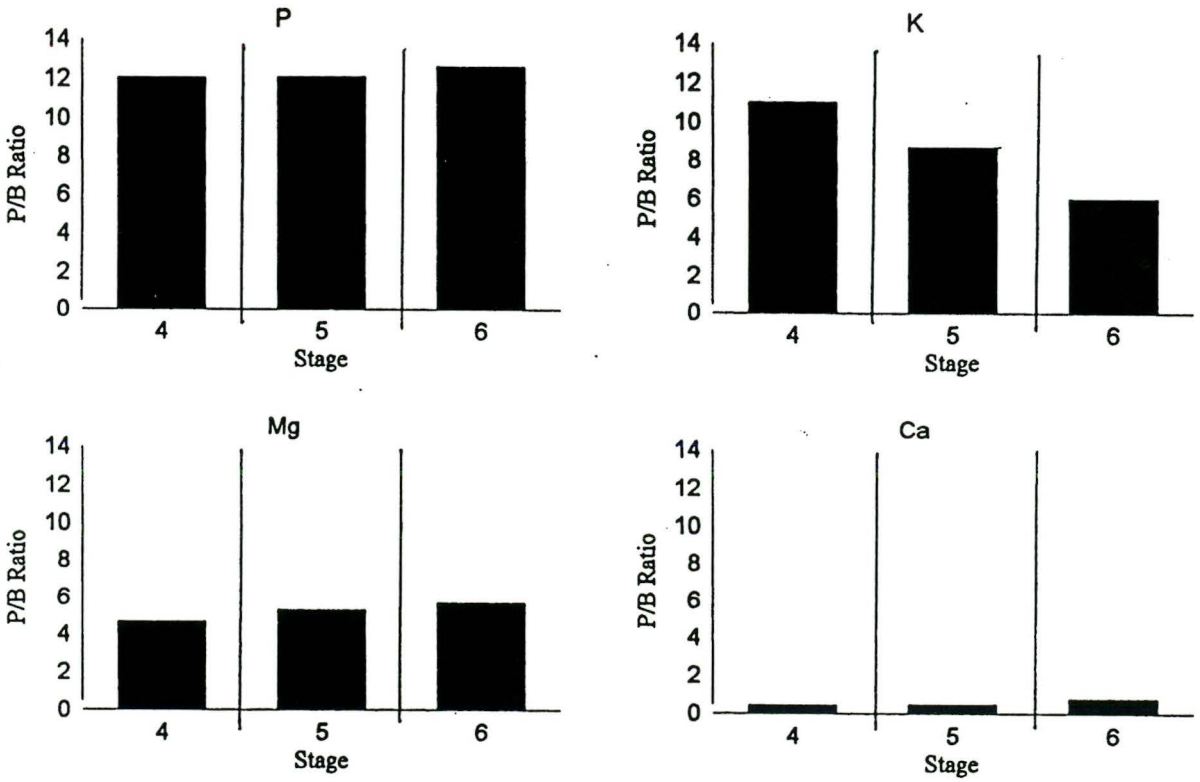


Figure 3.5 b) P/B Ratios
Celtic Aleurone Layer



3.3 WHOLE GRAIN ANALYSIS

Protein

Whole grain percent protein per gram dry weight for each stage of growth for each cultivar are shown in Table 3.4. Throughout seed set, in both cultivars there is a very slight increase in percent protein on a dry weight basis. During early seedling growth stages a slight increase is also noted for the percent protein per gram dry weight for both cultivars. In both cultivars, protein concentrations were slightly higher in grains grown in 1995 by the end of seed set than in the dry grains used for stage 1, grown in 1994. Celtic cultivar had higher protein concentrations than that of A.C. Reed throughout seed set and early seedling growth.

Phosphorous

Whole grain phosphorus concentrations for each stage of seed set and seedling growth for each of the 2 cultivars are presented in table 3.5. Throughout seed set stages there is a decrease in phosphorus per grain within both cultivars. During early seedling growth stages phosphorus concentrations remain relatively constant in both cultivars. P concentrations were generally slightly higher in Celtic grains than in A.C. Reed grains.

Potassium

In both A.C. Reed and Celtic cultivars whole grain K concentrations increased throughout seed set, and remained relatively constant during stages of early seedling

Table 3.4 - Whole grain percent protein per gram dry weight for A.C. Reed and Celtic wheat grains at various stages of growth during seed set and early seedling growth.

		PERCENT PROTEIN	
STAGE		REED	CELTIC
SEED SET			
1	MEAN- SD-	13.70 ±0.14	14.95 ±0.02
2	MEAN- SD-	14.13 ±0.62	14.98 ±0.47
3	MEAN- SD-	14.15 ±0.19	16.13 ±0.45
SEEDLING GROWTH			
4	MEAN- SD-	13.94 ±0.30	15.98 ±0.30
5	MEAN- SD-	14.27 ±0.64	17.56 ±0.48
6	MEAN- SD-	15.23 ±0.55	18.82 ±0.50

Table 3.5 - Whole grain percent phosphorus per grain for A.C. Reed and Celtic grains at various stages of seed set and early seedling growth.

		PERCENT PHOSPHORUS	
STAGE		REED	CELTIC
SEED SET			
1	MEAN- SD-	0.15 ±0.01	0.19 ±0.00
2	MEAN- SD-	0.15 ±0.00	0.20 ±0.01
3	MEAN- SD-	0.10 ±0.00	0.14 ±0.01
SEEDLING GROWTH			
4	MEAN- SD-	0.13 ±0.01	0.16 ±0.01
5	MEAN- SD-	0.12 ±0.00	0.17 ±0.01
6	MEAN- SD-	0.13 ±0.01	0.17 ±0.00

growth (Table 3.6 a). K concentrations throughout seed set and early seedling growth were higher in the higher protein Celtic grains than that of the lower protein A.C. Reed grains.

Magnesium

Mg concentrations in whole grain A.C. Reed and Celtic cultivars increased throughout seed set and remained relatively constant throughout stages of early seedling growth (Table 3.6 b). Mg concentrations were similar in both A.C. Reed and Celtic grains throughout seed set and early seedling growth.

Calcium

Throughout seed set in both A.C. Reed and Celtic cultivars Ca concentrations decreased by the second stage of seed set followed by an increase by the third stage of seed set. During early seedling growth Ca concentrations remained relatively constant (Table 3.6 c).

3.4 - METEROLOGICAL CONDITIONS

Mean temperature and rainfall in 1994 and 1995 for the months of May, June, July and August are shown in table 3.7. Conditions were supplied by Environment Canada, Atmospheric Meteorological Summary, Hamilton Airport, Ontario.

Table 3.6 - Whole grain atomic absorption analysis for K, Mg and Ca concentrations (ppm/grain) in A.C. Reed and Celtic whole grains for stages during seed set and early seedling growth.

Table 3.6 a - Potassium

	Seed Set			Seedling Growth		
Stage	1	2	3	4	5	6
A.C. Reed	22.19 ±1.81	50.26 ±.074	98.76 ±10.54	69.45 ±1.77	71.94 ±3.86	62.01 ±4.52
Celtic	40.80 ±1.13	66.09 ±1.26	158.28 ±3.62	117.57 ±3.80	133.34 ±4.72	117.55 ±3.16

Table 3.6 b - Magnesium

	Seed Set			Seedling Growth		
Stage	1	2	3	4	5	6
A.C. Reed	27.34 ±1.60	33.89 ±2.09	38.51 ±3.25	31.99 ±2.51	34.16 ±1.49	32.94 ±3.08
Celtic	20.79 ±0.81	31.11 ±1.05	36.30 ±0.72	30.51 ±0.99	33.02 ±0.34	31.49 ±0.87

Table 3.6 c - Calcium

	Seed Set			Seedling Growth		
Stage	1	2	3	4	5	6
A.C. Reed	19.81 ±.061	14.40 ±0.99	18.80 ±0.18	18.11 ±1.00	17.91 ±0.23	18.47 ±0.13
Celtic	18.08 ±0.24	12.70 ±2.27	18.71 ±0.37	16.14 ±0.54	17.39 ±0.69	16.08 ±.019

Table 3.7 - Mean temperature and rainfall in 1994 and 1995 for the months of May, June, July and August. Supplied by Environment Canada, Atmospheric Environment Service, Hamilton, Ontario.

	1994		1995	
	Mean Temperature (°C)	Mean Rainfall (mm)	Mean Temperature(°C)	Mean Rainfall (mm)
May	11.4	108.6	12.7	57.1
June	18.7	67.8	19.8	44.7
July	20.9	98	21.6	52.3
August	18.5	67.6	21.8	41

CHAPTER 4 : DISCUSSION

4.1 - MORPHOLOGICAL AND SUBCELLULAR CHANGES

Seed Set

Grown in field conditions, the grains used for seed set studies followed a growth pattern described by Waldren and Flowerday (1979). Grains increased in size as carbohydrates, protein and mineral nutrients accumulated in the grain until late in maturation when the grains started to dehydrate and decrease in size. Globoid particles were present in each of the tissue regions and in all stages studied during seed set. Although the concentration of mineral nutrients within the globoids varied, the globoid structure was detected early after anthesis as noted by Parker (1980). As seed set progressed globoids became increasingly more difficult to locate in powdered tissue due to the increase in starch and protein that was accumulating within the developing cells.

Photosynthesis within the flag leaf and ear of the wheat plant during maturation provides the majority of carbohydrates that are stored within the grain (Bewley and Black, 1985; Thorn, 1965). Thorn (1965) showed that 17-30 % of the grain's sugars were

produced by photosynthesis within the ear of the plant, but the ear also uses greater amounts of sugar in respiration than it produces during this time. Due to respiration within the ear the flag leaf actually has to contribute 110-120 % of the total carbon stored in the grain. Since the ear of the grain stays greener longer than the leaves of the plant, photosynthesis and respiration continue later in maturity within the ear. This can result in loss of grain weight late in maturation because respiration often exceeds photosynthesis (Bewley and Black, 1985).

A majority of the nitrogen that is used for protein synthesis is obtained by the remobilisation of proteins from the senescing organs of the plant. Asparagine and glutamine are the major imported amino acids, others are assimilated within the grain and not imported (Bewley and Black, 1985).

Sugars, amino acids and nitrogen are thought to travel to the developing grain via the plant's phloem, through transfer cells in the nodal region of attachment, to the grain's vascular bundle which lies along the crease of the grain. Assimilates are transported from the grain's vascular bundle into the endosperm through nucellar projections and spread from this point down the concentration gradient (Bewley and Black, 1985; Longnecker and Robson, 1993). Mineral nutrients follow a similar pattern of transport to arrive at the grain, but little is known about the pathways in which mineral nutrients move from the grain's vascular bundle into the rest of the grain (Pearson and Rengel, 1994). It was thought that mineral nutrients probably followed the same pathways as the other assimilates (Bewley and Black, 1985; Longnecker and Robson, 1993), but work by Pearson *et al* (1996) with Mn and Zn pathways raises debate with

regard to this theory due to the segregation of mineral nutrients to particular areas of the grain and the slower distribution of mineral nutrients to the grain compared to that of other assimilates.

Early Seedling Growth

During early seedling growth mineral nutrients reserves, carbohydrates and protein were mobilized in all areas of the grain and transported to the growing seedling. Seedlings maintained a well described growth pattern during the study period (Waldren and Flowerday, 1979) indicating that the stored mineral nutrients, carbohydrates and protein were sufficient to meet the needs of the growing seedling without the addition of an external source of nutrients. Mineral nutrients were mobilized and utilized quickly within the scutellum and the embryonic axis. This rapid mobilization is indicated by the lack of globoids within the scutellum and embryonic axis by the time the coleoptile had reached 10 mm in length. Rapid mobilization of phytate in the embryo has also been observed by Mukhtar and Laidman (1982) who document that the limited reserves of mineral nutrient within the scutellum and embryo axis were depleted within two days of early seedling growth.

Globoids within the aleurone layer became harder to locate as seedling growth progressed. This is likely due to the gradual mobilization of the globoids. All globoids were not broken down within the aleurone at the same time, but were gradually mobilized throughout the stages of early seedling growth. This loss of most of the globoids made it exceedingly difficult to locate globoids at later stages of growth. Beyond the 30mm

coleoptile stage used in this study, globoids were not detected. The lack of globoid particles by this stage of growth illustrates that all globoids were digested during early stages of seedling growth when plants were not supplied with an exogenous mineral nutrient source.

Protein body fusion and protein mobilization followed a pattern noted by Hara and Matsubara (1980), and Lott and Vollmer (1973). As the aqueous content increased within the cells protein bodies fused and became less dense and protein and starch were eventually mobilized. Although dealing with crushed samples the decrease in protein and starch volume and density could be detected by the ease in which the globoids were located. Dry seed tissue and early germination samples were often quite densely packed with protein and starch. Although present in greater numbers at these stages, the globoids were often harder to locate within these early growth stages due to the density of protein and starch. In later growth stages globoids became easier to detect because they were not surrounded as much by protein and starch as they were in earlier stages of growth.

4.2 - CHANGES IN GLOBOID COMPOSITION

EDX analysis of globoids from both cultivars in each of the tissue regions at all stages of growth revealed relatively high concentrations of P, K, and Mg. Ca was also detected in most samples but at lower levels. Mn was detected within globoids of the embryo axis but not in the aleurone layer or scutellum. This localization of globoids that contained Mn corresponds to work by Lott and Spitzer (1980) in which Mn was detected

only within globoids from the radicle of the embryo axis. Zn levels were also observed mainly within globoids from the embryo axis. How and why this partitioning of mineral nutrients occurs is still not clearly understood. Fe concentrations were detected within some of the samples but levels were often too low to be accurately measured above background noise, and there was no regularity to the levels, or location of this trace mineral nutrient. S levels detected are most likely due to protein surrounding the globoids, hence these values were also very irregular and followed no specific trend.

Both A.C. Reed and Celtic cultivars had significant change in nutrient levels within the globoids throughout the stages of early seedling growth and seed set. In both cultivars the changes that occurred generally followed the same trends as detailed below.

i) Seed Set

Changes to mineral nutrient levels within the globoids were examined from 3 separate tissues, during the stages of seed set. In both cultivars and in all 3 of the tissues K levels increased significantly and P, Mg and Ca levels decreased slightly.

The most dramatic change that was observed was the increase in K levels within the globoids of all tissue regions. The change to K levels can most likely be explained by the decreasing aqueous content of the cell during seed set, and the effect this decrease would have on the solubility of the globoid complex. In both cultivars, during the period between stage 1 (10 days post anthesis) and grain maturation the wheat grains percent moisture decreased to approximately one quarter of the moisture at anthesis. K^+ ions free within the cells during early grain formation when water content was higher would now

bind to the globoid complex as water levels decreased. As K^+ ions were bound to the globoids, K P/B ratios would thus increase.

The slight drop in P, Mg and Ca within globoids from some of the tissues was unexpected. For these P/B ratios to be decreasing the globoids would either have to become less dense or would have to be losing valuable mineral nutrients that have a high affinity for the globoid complex. A possible explanation for this may be the increased K levels within the globoid. Levels of P, Mg and Ca were at peak levels early after anthesis. The high affinity that Mg and Ca have for the globoid would have caused the globoid to be a compact highly associated unit. The binding of K^+ ions to the globoid during seed set may have caused a slight disassociation of the compact globoid unit, increasing the space between Mg, Ca and P. The electron beam used for EDX analysis would have detected this change in structure as changing elemental levels within the globoids. Early during seed set the interactive beam volume hitting the globoid would have come into contact with higher levels of P, Mg and Ca. As K^+ ions bound to the globoid and increased the space between the elements less P, Mg and Ca would have been within the same interactive beam volume. Although levels of P, Mg and Ca would not have actually decreased within the entire globoid the amount of each of these elements within the beam field would have decreased, and it would appear that P, Mg and P levels would have been decreasing.

The actual structural aspects of the packaging of phytate in a globoid is still poorly understood and the structural changes that occur within the globoid during seed set have never been documented. The above proposed explanation of events that occur during

seed set have not been proposed before, but seems like a valid explanation for the decline in P, Mg, and Ca P/B levels during seed set.

ii) Early Seedling Growth

Within globoids from the aleurone layer little change was noted in P levels, there was a significant decrease in K levels, and levels of Mg and Ca both increased slightly.

The exchange of ions that occurred within the globoids during early seedling growth can possibly be explained by the change in the aqueous content of the cells and the effect that this would have on the solubility of the globoid. During early seedling growth the grains imbibed a large amount of water increasing the percent moisture of the grain approximately 8.5 times. As the grains became imbibed, K^+ ions, being easily dissociated were mobilized from the globoid. Mg^{++} and Ca^{++} ions that are free within the vacuoles would have a high affinity for phytate which, at that time, would have open bonding sites due to the dissociation of K^+ ions. Di-valent and tri-valent ions have a stronger bond to phytic acid than that of mono-valent ions (Brown *et al*, 1961), so as the contents of the vacuoles became increasingly more hydrolysed throughout early seedling growth K P/B ratios would decrease as K^+ ions were mobilized, and Mg and Ca P/B ratios would increase as Mg^{++} and Ca^{++} ions joined the globoid. The lack of change of P levels within the globoids during early seedling growth indicates that although changes are occurring to the levels of the cations, the phytate structure of the globoid is relatively unchanged. The increased linking of di-valent and tri-valent ions within the globoid may also increase the stability of the globoids until the stored nutrients would be required later

in growth.

The activity of phytase probably had little effect on the ion exchange which occurred within the globoids since this exchange is more likely due to the aqueous environment and the mobility of the ions involved. The final breakdown and mobilization of the phytate backbone is more likely influenced by phytase activity. The reported gradual increase in phytase levels during early seedling growth (Eastwood and Laidman, 1971) may explain the sequential breakdown of the globoids.

As the ions are exchanged within the globoid the question arises as to what is happening to the ions that are mobilized, and where are the ions that are being incorporated into the globoid coming from. K is very important to many cellular activities ranging from enzyme activation, to maintaining turgor pressure within the cell. It is likely that K mobilized from the globoid would be transported out of the vacuole and utilized within the cell cytoplasm. It is also likely that the excess K mobilized within the aleurone layer may be transported out of the cells of this layer to other growing areas of the seedling. Increased levels of Mg and Ca within the globoids could possibly have come from globoids that had been previously broken down within the cell, may have been transported from the cytoplasm into the vacuole, or may have been transported from other cells by active transport. By storing these free nutrients in the globoids the plant would have a source of ions later in growth when needed.

4.3 - WHOLE GRAIN ANALYSIS

Protein

In both A.C. Reed and Celtic cultivars whole grain protein concentrations increased during seed set on a dry weight basis. This increase was expected as the grain would accumulate storage protein to be utilized in the following year of growth. Unexpectedly, concentration of protein also increased during stages of early seedling growth on a dry weight basis. This increase in protein concentration during early seedling growth may possibly be caused by the compartmentalization of storage protein (Kruger and Preston, 1978) or may have been caused by the unequal timing of mobilization of starch and protein reserves early in seedling growth. Grain storage proteins do not start to be mobilized by protease activity until the second day of seedling growth. During these first two days of growth, starch reserves are rapidly broken down and utilized as a respiratory substrate for the germinating seed and growing seedling (Bewley and Black, 1985; Kruger and Preston, 1978). Due to the fact that protein levels were measured on a dry weight basis, early seedling growth samples would have contained relatively high concentrations of starch and protein on a dry weight basis, compared to that of later samples in which starch levels would be greatly reduced compared to that of protein levels. Because protein was measured on a dry weight basis, early samples would thus contain higher starch to protein concentrations than later seedling growth samples which would have had lower starch to protein concentrations. This change in dry weight concentrations of starch and protein would have made it appear that protein levels were

actually increasing.

Another factor that may have caused the increase in protein levels on a dry weight basis during early seedling growth may be the compartmentalization of the storage proteins within the dry seed (Kruger and Preston, 1978). Due to this compartmentalization of the protein, incomplete extraction may have produced lower protein values at early stages of seedling growth than are actually present. Protein concentrations were higher in Celtic grains compared to that of A.C. Reed which was expected because Celtic grains are known to have a higher protein content.

Whole grain protein analysis was carried out by Agri-Food Laboratories, Guelph, Ontario. Analysis data that was obtained by Agri-Food Labs. was only on a dry weight basis and no per grain data could be determined. Although the data did show that Celtic grains have a higher protein content then that of A.C. Reed grains, repetition of the protein analysis would be useful in order to determine the changes that occurred on a per grain basis. Analysis data on a per grain basis would be beneficial in this study in order to relate protein levels to other whole grain analysis on P, K, Mg and Ca, and may also give a clearer picture in the actual changes that occurred to protein content during seed set and early seedling growth.

Phosphorus

Whole grain P concentrations declined slightly on a per grain basis during stages of seed set indicating that some of the P within the grain was transferred out of the grain back to the glume. This decrease in P concentrations was also noted in a study by Despo

(1994) in which P concentrations peaked 1 week post anthesis and declined slightly until grain maturity. However in most studies, P concentrations per grain remain relatively constant from the time between anthesis and maturity (Despo, 1994; Karlen and Whitney, 1980)

Throughout early seedling growth P concentrations remained relatively constant within both cultivars. This was expected due to the fact that no additional P had been supplied to the plant during stages of early seedling growth.

P concentrations were higher in Celtic grains compared to that of A.C. Reed grains. This may be an influence of the higher protein concentrations of the Celtic grains.

Potassium and Magnesium

As expected whole grain K and Mg concentrations increased throughout seed set on a per grain basis and remained relatively constant throughout stages of early seedling growth in both cultivars. The main point of interest here is the relationship between the protein levels of the cultivars and the relationship to potassium levels. Blevin (1985) noted the correlation between high protein levels and high K levels. This correlation held true in whole grain samples from this study. Celtic grains, which had higher protein concentrations, also had higher K concentration throughout stages of seed set and early seedling growth. To the authors knowledge this correlation between protein and K has never been noted before within two similar cultivars of the same genus and species.

Calcium

During seed set an unexpected drop in whole grain Ca was seen within both cultivars by the second stage of growth, followed by an increase by the third stage of growth. In other studies, the normal pattern for wheat whole grain Ca concentrations during seed set was for a relatively constant level from anthesis to maturity (Karlen and Whitney, 1980).

During the stages of early seedling growth Ca levels remained relatively constant as was expected without the addition of any Ca sources to the growing seedling.

4.4 - COMPARISON OF A.C. REED AND CELTIC GRAINS

In whole grain concentrations of elements and EDX analysis of globoids from the various tissues, the changes noted within mineral nutrient levels were similar for both A.C. Reed and Celtic cultivars. Although protein and mineral nutrient levels differed between the two cultivars this did not seem to effect the timing of accumulation or mobilization which was generally the same between the two cultivars. Grown under identical conditions, the levels of stored reserves were different between the two cultivars at the same stage in both whole grain and globoid analysis. Protein, P, K, Mg and Ca levels were generally higher in Celtic grains compared to that of A.C. Reed grains. These higher levels in Celtic grains indicate that the specific plant cultivar does have control over sequestering and storing reserves within the globoid, and throughout the whole grain.

4.5 - YEARLY CHANGES

There are many seasonal influences both before and after anthesis that may effect the growth patterns of grains between two differing years of growth. Effects on grain growth patterns would then in turn have an influence on the accumulation of storage reserves with in a particular cultivar. Rainfall, temperature, illuminance and mineral nutrient availability during plant growth and seed set are particularly important factors that can influence protein, carbohydrate and mineral nutrient levels within the grain (Wardlaw, 1970; Sofield *et al*, 1974). Not only are rainfall and temperature important factors in grain production, but the timing of these two elements is also of importance. For maximum grain yield, in relation to protein and starch levels, it is ideal for the plant to have an adequate mixture of sun and rain during the time between germination and anthesis, this promotes strong and healthy plant growth, inturn increasing reserves stored within the plant. From anthesis to grain maturity it is beneficial for the plant to have mainly dry and warm conditions. Warm dry conditions during seed set have been shown to increase grain yield (Sofield *et al*, 1974).

An interesting finding within this study was the difference in mineral nutrient and protein levels in dry grains between two different years of growth of grains produced on plants grown at the same location. Grains used for seed set samples were obtained from plants grown in the summer of 1995. Grain used for early seedling growth samples were obtained from grains grown in the summer of 1994. Stage 3 of seed set and stage 4 of early seedling growth were both dry grain stages, the only difference being the year in

which the dry grains were produced (stage 3 being from 1995 and stage 4 being from 1994). Looking at whole grain values obtained from each of these stages, dry grains from the summer of 1995 generally had a higher protein, P, K, Mg and Ca levels than did dry grains grown in the summer of 1994. Looking at the rainfall and temperature conditions for the months of May, June, July and August for the years of 1994 and 1995 (Table 3.7) a correlation between the meteorological conditions and grain content can be noted. Plants from both 1994 and 1995 had sufficient rainfall and temperatures between germination and anthesis to promote strong plant growth. During the time between anthesis and grain maturation plants grown in 1995 had warmer temperatures and less rainfall. Lower rainfall and higher temperatures would have promoted higher protein content within the grain (Pomeranz, 1971; Sofield *et al*, 1974) which was true for plants grown in the summer of 1995. The higher protein levels may have in turn influenced higher mineral nutrient levels which were also noted within the grains grown in the summer of 1995.

EDX analysis of the globoids from the aleurone showed that K levels were higher in 1995 dry grains then 1994 grains but P, Mg and Ca levels were lower in the grains from 1995 then the grains from 1994. Therefore P, K and Mg levels were higher within the whole grain but individual globoid accumulations were lower. This may be an indication that globoid accumulation of mineral nutrients is not directly related to whole grain accumulations.

4.6 -RELATIONS TO OTHER STUDIES

Although little work has been done looking at the composition of the globoid throughout seed set and early seedling growth, it was generally accepted that cation levels remained unchanged within the globoids as the phytate reserves were formed and in turn utilized. The work presented here on two monocot cultivars, work by Beecroft (1995) on two dicot *Cucurbita* species and work by Lott *et al*, (1995) on *Ricinus communis* (castor bean) has shown that there are in fact great changes occurring to the cation levels within the globoids during seed set and early seedling growth and that these changes may be widespread throughout all phytate storing seeds. The most obvious of the trends within each of these studies is the great drop in K and the slight increase in di-valent cations within the globoids during stages of early seedling growth.

4.7 - BASELINE FOR ANALYSIS

In a study such as this in which events occurring within the seed are followed throughout seed set and early seedling growth, one of the major difficulties which arises is the establishment of a baseline to use for analysis. In many studies analysis is based on fresh weight or dry weight of the tissue being studied. In this study fresh and dry weights presented a problem because neither remained constant throughout the stages of the study.

During seed set the grain started with a relatively high moisture content and a low dry weight, but by the time the grain had reach maturity the dry weight had increased greatly as reserves were accumulated in the grain and fresh weight had decreased greatly as the grain desiccated. During early seedling growth fresh weights increased as the plant grew and took in water and dry weight remained relatively constant as the plant produced reserves through photosynthesis and lost reserves through respiration. For whole grain analysis the best baseline decided upon was a per grain basis. This baseline was decided upon because it takes into account the overall changes that occurred within the grain and was relatively easy to visualize. All whole grain analyses were carried out on a per grain basis except for protein content which was done on a dry weight basis by an independent lab as explained earlier.

4.8 - KEY FINDINGS

In the past extensive studies have focused on protein and carbohydrate levels within the wheat grain but little work has been done looking at mineral nutrient levels of the grain. This study is the first looking at the actual changes in the concentrations of mineral nutrients within the globoid throughout seed set and early seedling growth in a cereal crop.

During the stages of seed set and early seedling growth P/B ratios indicated that there were significant changes occurring to mineral nutrient levels within the globoids of the aleurone layer, scutellum and embryo axis. Changes occurring within the P/B ratios

were similar in A.C. Reed and Celtic cultivars. Throughout seed set within the aleurone, scutellum and embryo axis, levels of P, Mg and Ca generally declined slightly, or remained relatively constant, K levels increased significantly within all three grain portions studied. During early seedling growth P levels remained constant, K levels decreased, and Mg and Ca levels increased slightly. Changing nutrient levels within the globoids during both seed set and early seedling growth can probably be attributed to the changing aqueous content of the cells during the various stages of growth.

Whole grain analysis was performed for protein, P, K, Mg and Ca. In both A.C. Reed and Celtic grains protein concentrations increased during seed set and seedling growth. The unexpected increase in protein during seedling growth being explained by the changing proportion of starch to protein and the effect that this would have on dry weight measurements. P, K, Mg and Ca concentrations showed an overall increase during seed set and remained relatively constant during early seedling growth stages in both A.C. Reed and Celtic cultivars.

Although there was no major difference between grain reserve filling time, there was a definite difference in the level of protein, P, K, Mg and Ca reserves within the cultivars grown in the same field conditions, at the same period of time. Celtic grains had higher whole grain protein, P and K levels than that of A.C. Reed. Celtic globoids also appear to have higher concentrations of P, K, Mg and Ca than did A.C. Reed. A yearly variation was noted within both of the wheat cultivars. Dry grains grown in the summer of 1995 had higher levels of whole grain protein, P, K, Mg and Ca then did those of grains grown in the summer of 1994.

4.9 - FUTURE DIRECTIONS

This study has looked at the changes that occur within the globoids of the wheat grain during stages of seed set and early seedling growth. Although many questions have been answered throughout this work many more have been raised, leaving open many future research directions that could be pursued, some of which are listed below:

1) This work, the work by Beecroft (1995) and the work of Lott *et al*, (1995) have looked at relatively lipid rich tissues of both monocot and dicot species. Changes in ion levels that have occurred within the globoids during early seedling growth have been similar for both monocot and dicot species. Further EDX analysis, similar to the present work, on other monocot and dicot species may reveal if the changes found within these studies are widespread within all phytate storing seeds.

2) The actual changes that are occurring within the vacuole and within the globoid itself during seed set and early seedling growth are not clearly understood. Further studies into factors such as pH within the vacuole during the time of globoid formation and degradation may explain more fully why cation levels within the globoids are changing.

3) Relatively large concentrations of K and smaller concentrations of Mg and Ca are

interchanged within the globoid during stages of seed set and early seedling growth. Many questions arise as to where the ions are coming from, or going to when they are exchanged within the globoid. Are these ions coming from the vacuole in which the globoid is located? Were they transported into or out of the vacuole of that cell, or into or out of surrounding cells? Have these ions been mobilized from other globoids within the cell, or were they stored elsewhere within the plant?

CHAPTER 5 : LITERATURE CITED

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APPENDIX 1 : MEAN (±STD) PEAK-TO-BACKGROUND RATIOS

Table A1 - Mean (±STD) peak-to-background ratios for EDX analysis of globoids from the aleurone layer, scutellum and embryo axis of A.C Reed and Celtic grains at various stages of growth during seed set for all elements analysed.

Table 3.2 a) Aleurone Layer
Table 3.2 b) Scutellum
Table 3.2 c) Embryonic Axis

- NS = Mean P/B ratios are not greater then the cut off level.

Table A1 a) Peak to Background ratios for the aleurone layer analysis

	STAGE		P	K	Mg	Ca	Mn	Fe	Zn	S	Cl
A.C. REED	1	MEAN STD	11.29 ±2.43	4.24 ±2.26	5.24 ±1.54	0.80 ±1.12	NS	0.28 ±0.23	NS	0.19 ±0.24	NS
	2	MEAN STD	10.64 ±1.84	9.08 ±1.99	4.14 ±1.15	0.70 ±0.74	NS	0.24 ±0.11	NS	0.24 ±0.14	NS
	3	MEAN STD	8.89 ±2.31	12.74 ±2.19	2.82 ±0.71	0.27 ±0.12	NS	0.25 ±0.12	NS	0.31 ±0.19	NS
CELTIC	1	MEAN STD	11.08 ±2.17	3.82 ±1.89	5.68 ±2.42	0.70 ±0.79	NS	0.25 ±0.14	NS	NS	0.19 ±0.35
	2	MEAN STD	11.50 ±1.20	11.08 ±2.38	4.36 ±0.81	0.33 ±0.21	NS	0.26 ±0.12	NS	0.23 ±0.11	NS
	3	MEAN STD	10.39 ±1.52	13.45 ±2.61	3.03 ±0.82	0.42 ±0.14	NS	0.26 ±0.14	NS	0.20 ±0.16	NS

Table 3.2 b) Peak to background ratios for scutellum analysis

	STAGE		P	K	Mg	Ca	Mn	Fe	Zn	S	Cl
A.C. REED	1	MEAN STD	10.16 ±2.63	5.05 ±1.85	3.21 ±1.62	0.62 ±0.08	NS	0.45 ±0.33	NS	0.32 ±0.26	0.14 ±0.13
	2	MEAN STD	10.64 ±1.69	8.81 ±1.99	2.93 ±1.14	1.98 ±0.07	NS	0.29 ±0.17	NS	0.15 ±0.09	1.15 ±0.08
	3	MEAN STD	7.57 ±2.38	11.95 ±2.82	2.37 ±1.10	1.24 ±0.99	NS	0.26 ±0.17	0.25 ±0.43	0.49 ±0.34	NS
CELTIC	1	MEAN STD	8.19 ±1.68	5.43 ±1.46	2.04 ±0.92	0.57 ±0.63	NS	0.49 ±0.37	NS	0.45 ±0.14	0.13 ±0.12
	2	MEAN STD	8.14 ±2.11	5.92 ±1.43	2.37 ±1.38	1.14 ±0.45	NS	0.48 ±0.30	NS	0.28 ±0.16	0.20 ±0.11
	3	MEAN STD	6.63 ±3.13	9.13 ±1.91	2.45 ±0.93	0.61 ±0.60	NS	0.28 ±0.30	0.92 ±0.52	0.05 ±0.17	NS

Table 3.2 c) Peak to background ratios for embryonic axis analysis

	STAGE		P	K	Mg	Ca	Mn	Fe	Zn	S	Cl
A.C. REED	1	MEAN STD	8.68 ±2.68	5.50 ±2.10	2.39 ±1.19	0.57 ±0.36	NS	0.29 ±0.29	0.22 ±0.33	0.44 ±0.15	NS
	2	MEAN STD	8.80 ±2.23	7.04 ±2.65	1.91 ±1.65	0.23 ±0.11	0.33 ±0.57	0.12 ±0.15	0.24 ±0.24	0.18 ±0.17	NS
	3	MEAN STD	6.65 ±2.89	10.77 ±2.87	2.78 ±1.40	0.59 ±0.73	0.37 ±0.70	0.16 ±0.15	0.26 ±0.37	0.19 ±0.35	0.30 ±0.28
CELTIC	1	MEAN STD	10.01 ±1.96	5.21 ±1.68	3.05 ±1.09	0.59 ±0.44	0.24 ±0.44	0.25 ±0.18	0.39 ±0.51	0.04 ±0.09	NS
	2	MEAN STD	9.98 ±2.30	6.22 ±2.02	3.22 ±1.93	0.55 ±0.51	0.53 ±0.90	0.18 ±0.16	0.23 ±0.37	0.25 ±0.18	NS
	3	MEAN STD	8.17 ±2.28	10.72 ±3.43	3.54 ±1.16	0.90 ±0.64	0.56 ±0.75	0.33 ±0.29	NS	0.29 ±0.22	NS