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Screening Methodologies and the Genetics of Aluminum Tolerance in Spring Wheat

By

Janice Zale

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Screening Methodologies and the Genetics of Aluminum Tolerance in Spring Wheat. Submitted by Janice Zale in partial fulfillment of the requirements for the degree of Master of Science in Plant Breeding.

Supervisor .....

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Date: 25/8/87

Dedication

To my mother,

Mary

Abstract.

The Canadian Spring wheat collection was assessed for aluminum (Al) tolerance using hematoxylin ratings and relative root length indices (with and without Al). Relative root lengths indicated a continuous distribution of phenotypes. HY 320 and Vernon ranked as highly tolerant and were both derived from imported germplasm. Thatcher and most of its derivatives were moderately sensitive to sensitive in reaction to Al. Incorporation of the Al tolerance trait in Canadian wheat has depended upon imported germplasm. Hematoxylin ratings gave similar tolerance classifications, with some differences.

Root biomass for 20 cultivars screened in Al toxic soil in greenhouse pot tests was correlated with the relative root length index developed from the nutrient solution studies. Preliminary whole root Al assays suggested that Al tolerant cultivars accumulated more Al on a whole root basis than intolerant cultivars.

Eight wheat cultivars were characterized for Al tolerance and plant-induced pH changes in mixed N solutions. Aluminum tolerant cultivars (Alondra 's', Atlas 66, PF 7748 and Kenya Kongoni) alleviated Al toxicity by rapidly increasing pH, but sensitive cultivars (Garnet, Park and Thatcher) maintained lower root microzone pH values. Cultivar tolerance was correlated with the rate of the pH increase, the final pH and the negative log of the mean H^+ concentration.

A six parental half diallel crossing scheme was designed to investigate the genetics of Al tolerance. Root length evaluation of F_2 and backcross lines revealed monogenic inheritance with dominance of the tolerant phenotypes in some crosses. Varying degrees of dominance in the F_1 suggested that multiple alleles for the same locus were responsible for much of the observed tolerance. Root regrowth evaluation of the 15 F_2 lines detected monogenic and digenic inheritance with ambidirectional tolerance classes within some families. The root regrowth technique appeared sensitive to heterogeneity within crosses, but inheritance data may be obscured by this technique.

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CHAPTER I
Introduction

Soil acidity is a major factor limiting crop growth on some Canadian soils. In Alberta and the Peace River region of British Columbia there are an estimated 328,000 ha of strongly acid (pH<5.5) cultivated soils (Penney *et al.* 1977). Penny *et al.* (1977) also estimated that there was an additional 1,600,000 ha of cultivated soil in the pH range of 5.6 to 6.0. In many acid mineral soils Al toxicity becomes deleterious to cereal growth as pH values decline below 5.0 (Foy 1974). Aluminum levels as high as 7.4 me $100g^{-1}$ soil have been recorded in the Peace River region (Penney *et al.* 1977). The continual use of soil acidifying fertilizers (Hoyt and Henning 1981; McCoy and Webster 1977; Mahler *et al.* 1985; Perl and Webster 1982) and the deposition of acid rain will enlarge the problem. Soil amelioration with lime or fertilizer is not always feasible or economical.

Fortunately wheat cultivars are known to display considerable differential aluminum tolerance and the trait is heritable. Incorporation of aluminum tolerance into Canadian wheats has not yet been consciously implemented. The purpose of the work reported here was to screen the Canadian spring wheats for aluminum tolerance, to establish a reference set of Canadian cultivars of known differential reaction to aluminum, and to compare screening methodologies in nutrient solution and in aluminum toxic Silver Valley soil. The genetic control of aluminum tolerance was also investigated.

A. Literature Review

1. Evolution of Metal Tolerance

The ability of a plant species to colonize metal contaminated soil requires the possession or evolution of an adaptive tolerance mechanism (Antonovics *et al.* 1971; MacNair 1981). Tolerant germplasm may already exist within the population or tolerance may be dependent upon adaptive mutations (Antonovics *et al.* 1971). Selection for an adaptive gene at low frequency within an outbreeding species can theoretically change a metal intolerant population to a tolerant population within one generation (MacNair 1981). Continued selection for minor genes within the same population would gradually increase the tolerance expressed in each generation, producing multiple shifts in gene frequency (MacNair 1981). Wu *et al.* (1975) demonstrated that on copper polluted sites the 70 year old lawns of *Agrostis stolonifera* were characterized by tolerant genotypes and the young 5

year old lawns also showed considerable tolerance. Strong selection pressure induced by the stressful environment promoted evolution of tolerant genotypes (Wu *et al.* 1975)

The genetics of metal tolerance in self-pollinated plants with higher levels of ploidy is more complicated. An inbreeding species carrying a metal tolerant gene approaches homozygosis by the rapid fixation of alleles. Allelic interaction is eventually abandoned, but duplications and nonallelic interactions become important sources of genetic variability (McKey 1964). Increasing levels of ploidy will augment gene duplications, and disomy in a self-pollinated polyploid (e.g. wheat) will introduce another form of heterozygosity vis-a-vis the formation of homeologous loci. Homeologous tolerance alleles may mutate repeatedly and these homomeric alleles are capable of diversified function and interaction (McKey 1964). The evolution of tolerance in an autogamous disomic polyploid is also dependent on selection pressure (e.g. man-made toxic waste spills or inherent soil toxicities as a result of gradual soil degradation). The genetic system governing tolerance may be more complex due to genome duplications.

The fitness of a particular tolerant genotype may be reduced on noncontaminated soil as is the case in copper tolerant *Agrostis tenuis* (MacNair 1981). Relatively little is known about the fitness of metal tolerant crop plants on noncontaminated soil (Foy 1983), but aluminum has been reported to stimulate growth in specific genotypes of tea, wheat, rice and corn. (Foy *et al.* 1978).

2. Distribution of Aluminum in Soil

Aluminum is the third most abundant element in the soil next to oxygen and silicon. Aluminum occurs in the aluminosilicate minerals, intergrade minerals, sesquioxides, and can be complexed with organic matter (McLean 1976; Hargrove and Thomas 1982). In the layer silicates, aluminum conveniently fits into the octahedral pores forming a six-fold coordination with oxygen. Aluminum is sometimes substituted for silicon in the tetrahedral units forming a four-fold coordination with oxygen. Oxides of iron and aluminum are usually the end result of intensive weathering and are relatively stable. Jackson (1963) postulated that in igneous rocks aluminum shares an oxygen bond with silicon in a four-fold coordination framework structure (as in the feldspars). As weathering proceeds aluminum gradually acquires a six-fold coordination as in the layer silicates such as kaolinite and montmorillonite. Hence with leaching, feldspars gradually hydrolyze to montmorillonites which if severely leached will eventually hydrolyze to gibbsite complexes (Jackson 1963).

Schnitzer and Skinner (1963) demonstrated that humic and fulvic acids have carboxyl groups which bind to hydroxy ions of iron and aluminum; and the average

composition of aluminum-hydroxy complexes were $Al(OH)^{2+}$. These complexes induced much weaker acid behavior than was expected for a pure carboxyl system.

Aluminum is gradually released upon intensive leaching and weathering. Organic matter decomposition and the production of organic acids, fertilizer amendents and the deposition (wet and dry) of acids also increase acidification. Depositions of cations displace adsorbed aluminum causing the aluminum to hydrolyze and sequentially release its protons which in turn increases acidity (Tisdale and Nelson 1975). Sulfides within the soil can oxidize to sulphate and produce acid which also increases exchangeable aluminum.

3. Chemistry of Soil Aluminum

The chemistry of soil aluminum below pH 5.0 is similar to pure solution chemistry. Aluminum exhibits both covalent and ionic character in bonding (McLean 1976) and can bind to oxygen in a wide array of functional groups. Once aluminum is displaced from the surface colloid it becomes a coordination compound with six water ligands (McLean 1976; Bohn *et al.* 1979; Barnhisel and Bertsch 1982). The coordinate convalent bond exhibits ionic character as the bond seems to be contributed wholey by the ligand. The monomeric coordinate compounds are designated as hexa-aqua aluminum ions ($Al(H_2O)_6^{3+}$ -Al for brevity) and are exchangeable (Bohn *et al.* 1979; Barnhisel and Bertsch 1982). Aluminum hexahydrate acts as a weak acid with a dissociation constant of 1.08×10^{-5} (McLean 1976) and it sequentially loses protons as the pH of the system increases. The hydrolysis products can form monomers, polymers or copolymers of iron and aluminum (Rengasamy and Oades 1978).

In mineral soils Al predominates at pH<4.7, and $Al(OH)_2^+$ predominates between pH 4.7 and 6.5 (McLean 1976). Gibbsite ($Al(OH)_3^0$ precipitates throughout a wide range in pH once the solution is saturated and the solubility product is exceeded (Bohn *et al.* 1979). Soluble Al is minimal between pH 6.5 - 8.0; $Al(OH)_4^-$ is soluble above pH 8, but due to its negative charge is repelled from colloidal surfaces and does not significantly contribute to adsorption (McLean 1976).

Hydroxy-Al species (notably $Al(OH)^{2+}$ and $Al(OH)_2^+$) polymerize at pH 4.7 - 6.5 and form coatings on mineral surfaces or they form continuous or discontinuous islands on the interlayers of silicate minerals (Bohn *et al.* 1979; McLean 1976). These polymerized species are only partially neutralized and lower the net negative charge on the soil colloid (exchange sites are blocked and the cation exchange capacity (CEC) is lowered) (McLean 1976). In organic soils, the $Al(OH)^{2+}$ adsorbed is not readily exchangeable and exchange sites are blocked until an increase in pH precipitates gibbsite (Hargrove and Thomas 1982). These principles are important aspects of the pH dependent charge in both mineral and

organic soils. Aluminum and iron oxides and hydroxys can specifically adsorb phosphate through ligand exchange rendering phosphate unavailable for plant use.

In many agricultural soils Al toxicity is particularly severe below pH 5.0, however, soil composition (organic matter, type of clay, mineral status) also influence the severity of Al toxicity in crop plants (Foy 1983). Kaolinitic soils at pH 5.5 may show severe Al toxicity (Foy 1983) due to pH dependent charge. Mineral status in soils (Ca: Mg ratios, N,P) will also influence toxicity since some species and cultivars are more efficient nutrient utilizers (Foy 1983; Gerloff 1976). Hue *et al.* (1986) determined that short-chained carboxylic acids present in the soil acts as Al detoxifiers. Citric, oxalic, and tartaric acids were considered strong detoxifiers and detoxification was related to the relative positions of COOH/OH groups on the main C chain which favored stable 5 or 6 bond ring structures with Al.

Since there is no direct relationship between pH, extractable Al and plant toxicity for all soils several researchers have proposed using Al activities rather than Al concentrations as a measure of toxicity (Adams 1971; Sposito 1984). Long and Foy (1970) have suggested using plant genotypes as indicators of Al toxicity. Several regional laboratories and research centers have conducted field trials to determine which Al assay procedures are best indicative of critical Al levels and crop responses to lime (Macleod and Jackson 1967; Hoyt and Nyborg 1971; Reeve and Sumner 1971; Sheppard and Floate 1984).

Traditionally at pH values below 5.0 monomeric Al was thought to be the dominant Al species toxic to most plants. Kerridge (1967) and Wagatsuma and Ezoe (1985a) have presented experimental results indicating that root elongation in wheat was more inhibited at pH 4.5 than at pH 4.0 in the presence of Al. Hydroxy-Al species polymerize at higher pH than at lower pH. Bartlett and Riego (1972b) demonstrated that a 1mM hydroxy-Al polymer solution at near neutrality was more harmful to maize seedlings in comparison with the control. In contrast, Blamey *et al.* (1983) indicated that monomer Al ions were more toxic to soybean root elongation and that the toxicity was alleviated with the addition of OH- which induced hydroxy-Al polymers to form. Growth was closely related to the sum of the monomeric Al species (Alva *et al.* 1986). Differences in Al analytical procedures have been ascribed as the cause of these discrepancies. Differential crop species' reaction to differences in Al speciation complicates the matter (Wagatsuma and Ezoe 1985a).

4. The Genetics of Aluminum Tolerance in Plants

The genetics of metal tolerance in crop plants is important in developing breeding strategies to transfer a trait from donor to recipient lines. If the trait is controlled by a single

gene with discrete effects it can be readily transferred into recipient cultivars by backcrossing. If the trait is controlled by polygenes continual selection after the F_2 generation is necessary to ensure selection of tolerant phenotypes.

Qualitative examination of F_2 and F_3 segregation patterns from diallel crosses of tolerant and sensitive barley cultivars indicated that Al tolerance was controlled by a single dominant gene (Reid 1969). Maternal effects were not implicated in the reciprocal F_1 population. Rhue *et al.* (1978) qualitatively evaluated F_2 and backcross segregation patterns in maize crosses and determined that Al tolerance was controlled at a single locus with multiple alleles. Cytoplasmic effects were not implicated in the inheritance. Magnavaca (1982) concluded that Al tolerance determined by root length in another series of American inbreds was quantitative, but that the great differences in Al tolerance between the American and Brazilian lines did not preclude major gene control for the additional tolerance.

Camargo (1984b) determined that the broad sense heritability estimates for Al tolerance were high in F_2 populations of sensitive x tolerant rice crosses. Partial dominance was observed for Al sensitivity and no maternal effects were observed in this study. Hanson and Kamprath (1979) determined that Al tolerance in soybean was heritable (0.67) with responses in each cycle of selection. The response to selection was indicative of quantitative inheritance. In F_1 hybrids of sorghum the genetic control of Al tolerance determined by root length appeared to be complex (Furlani 1981). Visual examination of the Al pretreated roots tended to give somewhat contradictory results.

A review of the genetics of Al tolerance in wheat is given in Chapter 5.

5. Symptoms of Aluminum Toxicity

Symptoms of Al toxicity are not always easily discernable (Foy 1983) but the symptoms often appear as P or Ca^{2+} deficiencies. The roots are characteristically discolored and swollen with undeveloped laterals, and cell division and root elongation is inhibited (Clarkson 1965). Aluminum induced drought may be a consequence of the decreased surface area in root absorption (Foy 1983). Ohki (1986) determined that Al toxicity in wheat decreased photosynthesis, chlorophyll concentration and transpiration.

On the cellular level, Al injury resulted in the progressive vacuoalation of the root cap cells and disorganization of the cytoplasm (Bennet *et al.* 1985b). Plasma membrane structure and function may become impaired (Foy 1983). Prolonged Al stress caused alterations in the endoplasmic reticulum, and the migration of the Golgi apparatus secretory vesicles (Bennet et al. 1985b). Aluminum inhibited DNA synthesis in a sensitive wheat cultivar (Wallace and Anderson 1984), and Al was strongly bound to nucleic acids in tea plants (Matsumoto *et al.* 1976).

6. Physiology of Aluminum Tolerance

MacNair (1981) speculated that metal tolerance controlled by a major gene would likely involve internal sequestering of the metal within the plant (cellular accumulation or exclusion, cellular transport and direct detoxification within the cells). Polygenic control of tolerance would likely implicate more complex physiological and biochemical processes or the evolution of Al tolerant enzymes. Al tolerance may also be related to plant nutrition.

a. Plant Nutrition

Differences in plant nutrition (ameliorating effects of P and Ca, N preference, rate of nutrient uptake) and metabolism have been implicated as mechanisms conferring tolerance in many plant species (Foy 1983). Tolerance to Al has been related to the plant's ability to utilize P in the presence of Al. Aluminum tends to coprecipitate P and accumulate within the roots of many sensitive species (Foy *et al.* 1978). A wide array of biologically active forms of P have been reported to be decreased in the presence of Al in some species (Foy 1983). Aluminum increased P absorption in barley (Clarkson 1966), soybean (Sartain and Kamprath 1977) and in wheat (Foy *et al.* 1974) but Al generally decreased the P transported to plant tops (Clarkson 1966; Foy *et al.* 1974).

Aluminum generally decreases Ca^{2+} uptake and transport in most crop species (Foy *et al.* 1974; Clarkson and Sanderson 1971; Mugwira *et al.* 1980), and Ca^{2+} is known to have an ameliorating effect on plant growth (Wagatsuma 1983b; Furlani and Clark 1981; Aniol 1983; Rhue 1979). Alva *et al.* (1986) reported that increased Ca^{2+} concentrations had beneficial effects on root elongation in the presence of Al in alfalfa, clover, soybean and sunflower. The beneficial effect was postulated to be due to amelioration of Al induced Ca^{2+} deficiency.

Aluminum treatments have been shown to decrease Mg^{2+} and K^{+} accumulations in rye, wheat and triticale (Mugwira *et al.* 1980), in corn (Clark 1977), and in snapbean (Foy 1972). Magnesium has also been shown to protect plant growth from Al injury (Aniol 1983). Mineral interactions with iron and silicon have also been reported in plants (Foy 1983).

A review of the N preference hypothesis and associated plant-induced pH changes is given in Chapter 3.

b. Internal Sequestering

Bennet *et al.* (1985a) demonstrated that the peripheral cells of the root cap were the primary sites of Al uptake, and they proposed that Al uptake was a function of the acid

mucopolysaccharides in the cells involved. Huett and Menary (1979) used energy dispersive x-ray analysis and found Al distributed uniformly across the roots of three different species, and they concluded that Al entered the meristematic cells and the symplasm from the cortex (nonmetabolic uptake). Wagatsuma (1983b) speculated that Al is immobilized in the pectic substances of the cell wall. When the cell wall becomes saturated with Al, the Al diffuses into the cells and binds with the various nucleic acids and phosphate compounds in the cell.

The plasma membrane has also been implicated as a barrier to Al entry into the cell (Wagatsuma 1983a). Aluminum has been demonstrated to decreases membrane fluidity in *Thermoplasma acidophilium* (Viestra and Haug 1978), and Al appeared to change membrane structure in corn (Suhayda and Haug 1986)

Organic acids have also been implicated as internal detoxifying agents. Bartlett and Riego (1972a) demonstrated that the toxicity of Al was alleviated by citrate, EDTA, and soil organic matter. Suhayda and Haug (1986) determined that an Al tolerant corn hybrid maintained higher levels of malic and trans-aconitic acid under Al stress than a sensitive hybrid. The intolerant cultivar was proposed to have lower organic acid levels because of Al induced injury and leakage of metabolites out of the root. Haug and Caldwell (1984) speculated that organic acids facilitate Al chelation to prevent Al induced inactivation of calmodulin.

c. Plant Biochemical and Physiological Processes

Various researchers have reported either increasing or decreasing activities of membrane bound ATPases in the presence of Al (Foy *et al.* 1978; Matsumoto and Yamaya 1986). In peas, membrane associated Mg-dependent ATPase was competitively inhibited by Al with respect to ATP, and Matsumoto and Yamaya (1986) suggested that Al bound to ATP causing a decrease in available ATP.

The evolution of metal tolerant enzymes have been postulated as biochemical tolerance mechanisms. Acid phosphatases have been implicated as enzymes enabling Al stressed plants to extract P from organic sources, but acid phosphatase activity in three ecotypes of *Agrostis tenuis* decreased under stress. Additional research is required to identify whether other enzymes may relieve metal stress.

CHAPTER II

Hematoxylin Ratings and Relative Length Indices

A. Introduction

Hematoxylin is the colorless form of the dye hematin (Jensen 1962) and it is used extensively in botanical microtechnique. An aqueous solution of hematoxylin must be oxidized to hematein by exposure to air before it can stain tissue, and the hematein will only bind to the tissue after the tissue has been mordanted by ferric ions (Jensen 1962). These ferric ions attach to the negative binding sites in the tissue (e.g., chromosomes, certain proteins) and then the metals chelate the dye (Jensen 1962).

McLean and Gilbert (1927) boiled tissue specimens in saturated solutions of ammonium carbonate with hematoxylin to demonstrate that Al accumulated in the root cortex of sensitive crop species. Wright and Donahue (1952) used Al pretreatments to mordant barley tissue and hematoxylin stain to indicate the Al distribution within the cortical root regions. Henning (1975) used hematoxylin to determine the course of entry of Al into wheat root tips and the cellular disorganization resulting from the Al stress.

Polle *et al.* (1978) developed a nondestructive hematoxylin method for visually estimating tolerance to Al in wheat. This screening method detects differences in Al accumulation in the root tip, and the root tips are visually assessed for stainability. Sensitive cultivars are thought to accumulate more Al in the root tips (Wallace *et al.* 1982). Aluminum apparently mordants tissue and the hematein will bind to the Al. Tolerance ratings are based on the amount and intensity of the stain. Polle *et al.* (1978) reported that tolerance ratings given by the hematoxylin method were well correlated with Al tolerance ratings determined by root elongation and field trials. Wallace *et al.* (1982) reported that the hematoxylin method could distinguish between Al sensitive and tolerant genotypes (as verified by cessation of root elongation) after only two hours of Al pretreatment, whereas quantitative analyses of whole roots did not discern differences in Al accumulation.

Quantitative methods for evaluating differential genotype tolerance to Al have focussed on root dry matter production or root length comparing control and Al levels. A relative tolerance index (root biomass in Al /root biomass in control) has been utilized by many researchers (Taylor and Foy 1985a; Mugwira *et al.* 1981). This index corrects for genotypic differences in germination and growth rate. The relative growth index essentially expresses all of the growth in the treatment as a proportion of the growth in the control.

The great majority of commercially grown Canadian spring wheats have never been characterized for Al tolerance. The few published reported in the literature which have characterized Canadian cultivars included entries that are either of historical interest only (Anonymous 1967), are economically unimportant, or the varieties have been classified for tolerance to soil acidity *per se* (Mesdag and Slootmaker 1969).

The objectives of these experiments were to:

1a. Conduct a preliminary screening of the Canadian spring wheats using the hematoxylin procedure (Polle *et al.* 1978).

b. Determine whether age differences in the experimental material (4 days vs 10 days) affected the hematoxylin ratings.

c. Determine whether the hematoxylin ratings were well correlated with tolerance ratings derived from root measurements.

2. Design a screening system capable of detecting differential tolerance in a large number of segregating progenies for use in later genetic studies.

B. Materials and Methods

Experiment 1a

Sixty-six spring wheat cultivars encompassing all of the licensed, approved and historical wheats previously grown in Canada were screened for Al tolerance in nutrient solution. In addition, ten imported cultivars with diverse origins were also included as reference cultivars representing a range of Al previously reported in the literature (Namwila 1985; Rajaram *et al.* 1981; CIMMYT 1983; Polle *et al.* 1978; Nychiro and Briggs 1985, Taylor and Foy 1985a). The pedigrees and origins of the cultivars are given in Appendix Table 1.

The nutrient culture system was similar to Polle *et al.* (1978). On day one of the experiment the seed was pregerminated in the dark at room temperature (22 ± 2°C) for 24 hours. The pregerminated seed was then sown crease down on the plastic mesh bottoms of styrofoam planting trays with holes drilled to accommodate individual cultivars.

Two seedling trays per 10 l tub of solution were used to test age differences in the hematoxylin procedure. The first batch of pregerminated seeds (designated as age 1) were sown entirely to one tray. Seedling trays were floated in tubs with distilled water for 24 hours and then transferred to 10 l of nutrient solution (Polle *et al.et al.* 1978). The nutrient solution was changed daily.

On day six, the second batch of seeds (designated as age 2) were pregerminated in the dark at 22± 2°C for 24 hours, and the pregerminated seed was sown into seedling trays

and floated on 10 l of distilled water for 24 hours. On the eighth day, both seedling trays were transferred to the same treatment tubs supplied with nutrient solution for another 30 hours. Al in the form of $AlCl_3$ was supplied with the nutrient solution for the last 18 hours of growth. The composition of the nutrient solution is given in Table 2.1. The nutrient tubs were vigorously aerated at all times.

Table 2.1 Composition of nutrient solution used in experiments 1a and 1b.[1, 2]

	mM
$CaCl_2$	4.0
KNO_3	6.5
$MgCl_2 \cdot 6H_2O$	2.5
$(NH_4)_2SO_4$	0.1
NH_4NO_3	0.4
$AlCl_3$ [2]	0.18, 0.36, 0.72, 1.40

1 Nutrient solution after Polle *et al.* (1978).
2 pH was initially adjusted to 4.0 with 0.25 M HCl.

Since the seedling tray floated directly on the solution, gaps between the seedling tray and the solution allowed dust to enter the system. Plastic skewers were pierced into the styrofoam trays to create a support for a canopy of clear polyethylene which hung loosely over the trays. This prevented dust entry. The canopy was removed for the duration of the Al treatments (Polle *et al.* 1978).

Root washing and hematoxylin staining were performed according to Polle *et al.* (1978), however the hematoxylin staining period was reduced to ten minutes duration. The seedlings were fixed in 95% ethanol and stored in the refrigerator until assessments for root staining could be performed.

The seedlings were grown in a growth chamber at 22°C. Irradiance was provided by 26 fluorescent (1500 mA) and 45 (40 W) incandescent lamps, and it measured 322 µmol $m^{-2}sec^{-1}$ 1.5 m from the light source. A 16 hour light cycle was used during the initial growth period and continuous lighting was provided during the Al treatments. Relative humidity was maintained at 60%. No attempt was made to control the solution temperature which averaged 22 ± 2°C.

Experiment 1b

A separate experiment was designed to elucidate genotype x Al interaction, as measured by root length. The seventy-seven spring wheat cultivars (mostly the same as those in experiment 1a) were screened for Al tolerance in nutrient solution for 10 days.

The nutrient culture was similar to that described in experiment 1a, with the following modifications. Polystyrene planting trays were cut to fit the treatment tubs at the desired volumetric level so that the seedling trays were not floating on the nutrient solution. The pregerminated seeds were sown into the seedling trays and the trays were positioned over 8 l of nutrient solution to grow on for 4 days. The nutrient solution was replaced on the third day, but refilled to volume daily. The Al treatments (O, 360μM) were applied on the fifth day of the experiment.

The seedlings were grown in a growth chamber at 22°C. Irradience was provided by 26 fluorescent (1500mA) and 30 (40W) incandescent lamps at it measured 348 μmol m^{-2} sec^{-1} 1.0m from the light source. A continuous light cycle was used for the duration of the experiment. Relative humidity was not controlled but averaged 68%. After 10 days of growth, the seedlings were preserved in 95% ethanol until the length variables could be determined.

C. Plant Measurements and Calculated Variables

Experiments 1a and 1b

Assessments for hematoxylin root staining were similar to Takagi *et al.*(1983) with the following modifications. Primary seminal roots from every plant were assessed for staining. Seedling that did not stain apically scored "3" for every Al treatment. Primary seminal root tips that stained continuously around the root cap (greater than 4mm from the root apex) scored "1". Intermediate and indistinct banding of root tips scored "2".

Age 2 seedlings were also measured for total seminal root length (RL), longest seminal root (LSR) and shoot length (SL), and root and shoot length indices (RLI, SLI and RLI, respectively) similar to the root tolerance index utilized by Foy and co-workers (Mugwira *et al.* 1981; Taylor and Foy 1985a) were developed by dividing the length variables at each treatment level by the corresponding control values (+Al/-Al).

In experiment 1b, only the root and shoot length variables were studied. Seed weight was determined from an average of 100 randomly selected seeds from each of the 77 cultivars.

D. Statistical Design and Analyses

Experiment 1a

The treatments in the hematoxylin staining procedure were 5 Al levels (0, 180, 360, 720, and 1400μM), 2 ages and 76 wheat genotypes. The experimental design was a split-split plot with Al levels allocated as subplots and genotypes allocated to subsubplots. Genotypes were scored on the average of 4 plants per subsubplot. Due to space and labor

requirements the three replications were performed over time. The analysis of variance was performed for a fixed effects model.

The raw data for hematoxylin score indicated that the distribution of the rankings was not normal but tended to a Poisson where the variance is equal to the mean. The analysis of variance was therefore performed on the square root transformation of hematoxylin scores. Adjustments $\sqrt{score+0.5}$ were made for an abundance of small values (Steele and Torrie 1980). The transformed distribution was improved somewhat and the correlation with RLI also improved, therefore the transformed values were used in the analyses of variance.

Analyses of variance for all of the measured and calculated variables from age 2 seedlings was performed for a fixed effects model of a split-plot with Al levels as whole plots and genotypes randomly allocated to subplots. The sum of squares for Al levels was partitioned into its linear, quadratic and cubic sums of squares for the relative root and shoot length variables.

Multiple comparisons using Duncan's Multiple Range Test were performed on all main effect variables of interest (mean comparisons for transformed hematoxylin score (Table 2.4) were presented on the original scale to emphasize the differences). LSD values were calculated and used in comparisons between genotype treatment means at the same Al level. Simple Pearson correlations for the variables studied were performed.

Experiment 1b

The treatments included 2 Al levels (0, 360 μM) and 77 wheat cultivars. The experimental design was a 77 x 2 factorial arrangement of treatments arranged in randomized complete blocks. There were 4 replications performed over time. The variables measured were RL, SL and LSR. The analyses of variance was performed for a fixed effects model calculated from the mean of 4 plants per plot.

The calculated variables RLI, SLI, and LSRI were used in a one-way analysis of variance and multiple comparisons with Duncan's Multiple Range Test were used to compare genotype tolerance ratings. Simple effect interactions and simple Pearson correlations were calculated as outlined in Experiment 1a. Spearman rank correlations between the tolerance values of Experiment 1a and 1b were used to determine competence in ratings. Missing plot data was calculated by the method outlined by Gomez and Gomez (1984).

E. Results and Discussion

Experiment 1a

i. Hematoxylin Ratings:

Sixty-six Canadian spring wheat cultivars and ten imported cultivars were screened in nutrient solution at 5 Al levels and at 2 ages. The analysis of variance for hematoxylin score (Table 2.2a) indicated the existence of significant genotypic differences, significant age x Al levels, and significant Al level x genotype interactions. The results of the hematoxylin staining were in general agreement with other published works for the same reference cultivars (Polle *et al.* 1978; CIMMYT 1983). Treatment means averaged over replicates and ages for 6 representative cultivars are presented in Table 2.3.

Table 2.2a Analysis of variance for the square root transformation of hematoxylin score for two ages of 76 wheat cultivars grown in nutrient solution at 5 Al levels. (experiment 1a)

	Mean Squares	
Source of Variation	df	Hematoxylin Score
Replications	2	0.049
Al levels	4	28.351 **
Error a	8	0.039
Age	1	0.137 ns
Age x Al levels	4	0.078 *
Error b	10	0.018
Cultivars	75	0.258 **
Cultivars x Al levels	300	0.099 **
Cultivars x Age	75	0.009 ns
Cultivars x Age x Al levels	300	0.010 ns
Error c	1500	0.009
Total	2279	
CV (%)		6.4

* Statistical significance at α=0.05

** Statistical significance at α=0.01

Table 2.2b Analysis of variance for the square root transformation of hematoxylin score for 76 young wheat cultivars grown in nutrient solution at 5 Al levels.

Source of Variation	df	Mean Squares Score	
Replications	2	0.010	
Al levels	4	14.317	**
Error a	8	0.043	
Cultivars	75	0.139	**
Cultivars x Al levels	300	0.055	**
Error b	750	0.008	
Total	1139		
CV (%)		6.3	

* Statistical significance at α=0.05

** Statistical significance at α=0.01

Table 2.3 Treatment means (averaged over replicates and ages) for transformed hematoxylin score in experiment 1a. (Cultivars are representative of the 77 entries)

	Hematoxylin Score					
Al µm	0	180	360	720	1400	ave.
Cultivar						
Kenya Kongoni	1.87	1.85	1.81	1.40	1.22	1.63
Garnet	1.87	1.76	1.77	1.44	1.22	1.61
PF 7748	1.87	1.87	1.77	1.28	1.24	1.61
Alondra 's'	1.87	1.87	1.64	1.27	1.24	1.58
Thatcher	1.87	1.38	1.38	1.28	1.27	1.44
Park	1.87	1.24	1.41	1.33	1.32	1.43

$LSD_{0.05}$ for comparing cultivars at the same Al level is 0.11. The LSD value was derived from the results of experiment 1a (See Table 2.2a).

The data for the age 2 seedlings (young seedlings) indicated a heterogeneous response to different levels of Al (Table 2.2b). Increasing levels of Al generally increased root tip staining in most cultivars, however the less readily stained cultivars tended to exhibit less root tip staining, but more banding in the region of elongation (greater than 4mm from the root apex).

In general, the imported cultivars and the Canadian cultivars derived from imported germplasm (e.g., HY 320, Pitic 62, and Norquay) were most tolerant. Aluminum tolerant genotypes have likely been bred on soils with toxic quantities of Al (Foy *et al.* 1974; Mugwira *et al.* 1981; Takagi *et al.* 1983). Selection for adapted cultivars may have unintentionally and indirectly incorporated genes for Al tolerance on native soils (Foy *et al.* 1974). Brazilian and CIMMYT derived lines exhibited a preponderance of tolerant genes. Most of the economically important Canadian wheats ranked intermediate to low in hematoxylin scores (Table 2.4) at all Al levels. This indicated that selection for tolerance had not been an important adaptive character as determined by the hematoxylin test.

The significant age x Al level interaction (Table 2.2a) indicated that the genotypes responded differently to varying Al levels. The direction of the response measured by root tip staining was the same, and although the magnitude of the response varied between the 2 ages at 360 μM of Al the difference was unimportant. The averaging of simple effects over the main effects of age obscured significant age differences.

The assessment of hematoxylin tolerance ratings was simplified at the young seedling stage. At this age, only the root tips stained, but at the mature seedling stage less of the root apex stained while the remainder of the root discolored complicating the hematoxylin assessment.

Table 2.4 Means for hematoxylin score, root length index (RLI) and root length (RL) for age 2 seedlings of 76 wheat cultivars (experiment 1a)

Cultivar	RLI	RL	Hematoxylin Score
Redfife	0.843 a	8.04 n-f_2	1.47 g-j
Chuckar 's'	0.841 ab	8.39 k_2	2.12 a-g
Romany	0.806 abc	10.57 b-e	2.09 a-h
Preston	0.796 a-d	9.31 f-m	1.40 j
Alondra 's'	0.781 a-e	11.02 a-d	2.01 a-j
Vernon	0.777 a-e	7.93 o-f_2	1.85 a-j
Chester	0.766 a-f	7.73 s-f_2	1.53 e-j
Opal	0.762 a-g	8.25 k-c_2	1.88 a-j
Pioneer	0.757 a-h	7.25 x-g_2	1.59 c-j
Renown	0.752 a-i	9.46 e-j	1.58 c-j
Cinquentenario	0.751 a-i	8.79 h-r	2.33 a
Concorde	0.746 a-j	8.58 k-w	1.67 a-j
Huron	0.745 a-j	9.42 e-j	1.99 a-j
Lake	0.739 a-j	8.03 n-f_2	1.59 c-j
Canthatch	0.730 a-k	7.09 b_2-g_2	1.47 g-j
Milton	0.726 a-l	7.41 w-f_2	1.97 a-j
Early Red Fife	0.723 a-m	8.17 l-d_2	1.75 a-j
Canuck	0.722 a-m	7.14 a_2-g_2	1.45 h-j
Pitic 62	0.720 a-m	9.41 e-m	2.13 a-g
Regent	0.720 a-m	8.51 k-x	1.49 g-j
Bananaquit	0.720 a-m	9.17 g-o	2.20 a-d
Renfrew	0.719 a-m	11.17 abc	2.18 a-e
Kenya Kongoni	0.713 a-m	8.65 k-w	2.20 a-d
Chinook	0.712 a-m	6.15 g_2	1.42 j
Reliance	0.711 a-n	6.94 e_2-g_2	1.42 j
Fielder	0.710 a-n	6.85 f_2-g_2	2.32 ab
Cascade	0.710 a-n	10.45 b-e	1.85 a-j
Reward	0.709 b-n	7.89 r-f	1.47 g-j
Springfield	0.709 b-o	7.56 r-f_2	1.73 a-j
Lee	0.704 c-o	7.57 u-f_2	1.45 h-j
Leader	0.700 c-o	7.66 t-f_2	1.51 f-j
Norquay	0.699 c-p	9.45 e-j	2.10 a-i
Kota	0.698 c-p	7.91 p-f_2	1.47 g-j
PF 7748	0.695 c-p	9.91 d-h	2.16 a-f
Ruby	0.692 c-p	8.35 k-p	1.47 g-j
Laval 19	0.689 c-p	8.15 m-e_2	1.90 a-j
Thatcher	0.685 c-p	8.83 h-u	1.53 e-j
Prelude	0.682 c-q	9.89 d-i	1.93 a-j
Manitou	0.682 c-q	8.24 k-d_2	1.57 c-j
Glenlea	0.676 c-q	9.09 h-q	1.69 b-j
HY 320	0.674 c-q	11.77 a	2.27 ab
Stanley	0.665 d-r	8.45 k-y	1.45 h-j
Red Bobs	0.665 d-r	7.79 r-f_2	1.43 j
Red Bobs 222	0.661 e-r	9.27 f-n	1.49 g-j
Rescue	0.658 e-r	7.81 r-f_2	1.54 d-j
Hard Red Calcutta	0.658 e-r	7.56 r-f_2	1.58 c-j
Kenhi	0.658 e-r	7.12 a_2-g_2	1.66 a-j
Napayo	0.656 e-r	7.03 c_2-g_2	1.42 j

Dundas	0.654	e-r	6.99	c_2-g_2	1.70	a-j
Sonora 64	0.646	e-s	6.85	f_2-g_2	2.20	abc
Columbus	0.646	e-s	8.71	h-v	1.49	g-j
Benito	0.640	f-t	7.09	b-g_2	1.57	c-j
Garnet	0.638	f-t	11.47	ab	2.16	a-e
Katepwa	0.637	f-t	7.86	r-f_2	1.48	g-j
Lemhi 53	0.634	f-u	9.04	h-r	1.53	e-j
Park	0.628	g-u	8.21	k-d_2	1.54	d-j
Acadia	0.627	g-u	7.57	u-f_2	1.47	g-j
Ceres	0.625	h-u	9.27	f-n	1.47	g-j
Kitchener	0.625	h-u	9.19	g-n	1.49	g-j
Neepawa	0.621	h-u	7.63	u-f_2	1.52	e-j
Lemhi 62	0.620	i-u	8.96	h-s	1.75	a-j
Saunders	0.613	j-u	8.15	m-e_2	1.57	c-j
BH 1146	0.611	j-u	9.59	e-j	2.25	ab
Quality A	0.610	j-u	8.37	k-a_2	1.40	j
Coronation II	0.602	k-u	9.03	h-r	1.45	h-j
Ladoga	0.591	l-u	10.31	c-f	1.46	h-j
Redman	0.590	m-u	7.77	r-f_2	1.42	j
Selkirk	0.576	n-u	7.23	y-g_2	1.42	j
Cypress	0.574	o-u	6.97	d_2-g_2	1.40	j
Canus	0.564	p-u	6.13	g_2	1.55	c-j
Bishop	0.548	q-u	8.92	h-t	1.54	d-j
Maringa	0.534	r-u	8.97	h-s	2.29	ab
Marquis	0.521	s-u	8.37	k-a_2	1.45	h-j
Pembina	0.516	s-u	8.15	m-e_2	1.47	g-j
Apex	0.509	t-u	7.57	u-f_2	1.44	ij
Sinton	0.504	u	6.90	e_2-g_2	1.45	h-j
Mean	0.6836		8.4032		1.45	

1 Means followed by the same letter within a column are not significantly different at the 5% level of significance by Duncan's Multiple Range Test.

ii. Comparisons Between the Root and Shoot Length Indices and Hematoxylin Ratings

All three relative length variables calculated from measurement of the young seedlings after 18 hours of exposure at Al were capable of detecting cultivar differences (Table 2.5). However none of these variables were sensitive enough to detect a significant genotype x Al level interaction. These analyses therefore assume that the entire population of genotypes reacted similarly to the Al stress.

Partitioning the Al levels sum of squares for the root and shoot length variables revealed the significant linear response to Al levels for all of the variables. The cubic response for the variable RLI was significant at the 5% level, and this response can be explained by the observation that 720 μM Al was sufficient to completely inhibit root elongation in most cultivars. Above this concentration, Al had no additional inhibitory effect. The significant quadratic response at the 5% level for the variable LSRI indicated that 360 and 720 μM Al were optimal concentrations for inhibiting root elongation in most cultivars.

The means of the RLI at each Al level (Table 2.6) revealed that most of the genotypes responded similarly to increasing concentrations of Al. Interaction effects were negligible. The coefficient of variation was high for this experiment repeated in time and the interaction effects may have been obscured, or the method (in particular, duration of Al treatment) was too insensitive to detect significant interactions.

Although the correlation coefficient for RLI and hematoxylin score was significant ($r=0.356$, $\alpha=0.01$; Table 2.7), the number of pairs (74) involved in the correlation lend doubt to the biological significance of this observation. Only 12.7% of the observed variability can be accounted for by the relationship between transformed hematoxylin score and RLI. 40.8% of the observed variability in rating was accounted for by the relationship between actual root length (RL) values and nontransformed hematoxylin score. Wider distributions for hematoxylin score and RL variables may explain this increase in correlation.

The hematoxylin test was able to detect differences in cultivar response after only 18 hours of Al treatment, however, assessments of differential tolerance by root length measurements were not facilitated after this treatment duration. Polle *et al.* (1978) reported a correlation (no data shown) between root elongation and hematoxylin staining after 18 hours of Al pretreatment. The present study does support this result, but the low correlation indicated a weak relationship.

Table 2.5 Analyses of variance for root length index (RLI), shoot length index (SLI), and longest seminal root index (LSRI) for 76 wheat seedlings grown in nutrient solution (experiment 1a).

Source of Variance	df	Mean Squares RLI	SLI	LSRI
Replications	2	0.053	1.017	0.225
Al levels	3	1.830 **	0.833 *	1.082 **
Al linear	1	1752.314 **	174.025 **	1530.667 **
Al quadratic	1	0.026 ns	1.311 ns	9.102 *
Al cubic	1	7.674 *	1.866 ns	0.348 ns
Error a	6	0.026	0.119	0.015
Cultivars	75	0.067 **	0.240 **	0.044 **
Cultivars x Al Levels	225	0.010 ns	0.039 ns	0.009 ns
Error b	600	0.017	0.089	0.020
Total	911			
CV(%)		19.5	28.7	18.9

* Statistical significance at α=0.05

** Statistical significance at α=0.01

Table 2.6 Means for root length index (RLI), shoot length index (SLI), and longest seminal root index (LSRI) for 76 wheat seedlings grown in nutrient solution 4 Al^{3+} levels. (Experiment 1a).

	RLI				SLI				LSRI			
Cultivar Al^{3+} µm	180	360	720	1400	180	360	720	1400	180	360	720	1400
Acadia	0.767	0.718	0.485	0.537	0.996	0.828	0.674	0.754	0.906	0.870	0.648	0.808
Alondra 's'	0.873	0.882	0.740	0.628	0.836	1.130	1.098	0.846	0.936	0.887	0.718	0.657
Apex	0.602	0.452	0.475	0.507	0.784	0.895	0.860	0.772	0.723	0.633	0.599	0.660
Bananaquit	0.940	0.684	0.683	0.572	1.202	1.066	1.301	1.168	1.044	0.732	0.775	0.713
Benito	0.822	0.697	0.569	0.471	0.933	0.771	0.680	0.637	0.816	0.751	0.707	0.630
BH 1146	0.781	0.680	0.528	0.454	0.917	0.839	0.867	0.925	0.849	0.746	0.680	0.633
Bishop	0.619	0.552	0.537	0.484	0.847	0.837	0.850	0.903	0.670	0.651	0.652	0.667
Canthatch	0.729	0.779	0.752	0.659	1.060	1.086	0.999	1.163	0.818	0.853	0.839	0.713
Canuck	0.878	0.793	0.598	0.618	1.052	0.890	0.913	0.886	0.814	0.838	0.622	0.664
Canus	0.678	0.582	0.516	0.483	1.533	0.991	0.914	0.856	0.697	0.647	0.617	0.484
Cascade	0.884	0.693	0.667	0.594	0.915	0.794	0.967	0.896	0.878	0.740	0.661	0.632
Ceres	0.743	0.679	0.568	0.512	1.061	1.060	0.841	0.929	0.821	0.747	0.706	0.635
Chester	0.896	0.801	0.764	0.604	1.188	0.849	1.085	0.869	0.874	0.801	0.772	0.632
Chinook	0.796	0.793	0.740	0.519	1.039	1.300	1.276	0.705	0.806	0.751	0.698	0.542
Chuckar 's'	0.783	0.914	0.848	0.816	0.964	1.151	1.101	0.939	0.837	1.007	0.907	0.871
Cinquentenario	0.868	0.861	0.718	0.557	1.045	1.037	1.044	1.050	0.788	0.782	0.697	0.586
Columbus	0.653	0.681	0.697	0.556	0.907	1.015	1.003	0.868	0.714	0.748	0.791	0.638
Concorde	0.822	0.818	0.713	0.631	1.164	1.070	1.083	1.053	0.893	0.861	0.801	0.740
Coronation II	0.714	0.602	0.521	0.572	1.126	0.801	0.721	0.902	0.786	0.724	0.696	0.728
Cypress	0.629	0.563	0.567	0.538	0.976	0.965	0.947	0.860	0.777	0.698	0.728	0.607
Dundas	0.876	0.710	0.565	0.466	1.142	1.036	0.933	0.945	0.960	0.849	0.706	0.554
Early Red Fife	0.970	0.728	0.598	0.596	0.890	0.803	0.887	0.783	0.989	0.843	0.745	0.777
Fielder	0.851	0.717	0.675	0.598	0.953	0.758	0.730	0.747	0.853	0.745	0.747	0.753
Garnet	0.754	0.715	0.552	0.532	0.915	0.801	0.826	0.863	0.835	0.780	0.632	0.638
Glenlea	0.861	0.688	0.625	0.531	1.351	1.150	0.867	0.889	0.826	0.798	0.748	0.623
Hard Red Calcutta	0.662	0.690	0.609	0.670	0.954	1.172	0.867	1.081	0.752	0.775	0.658	0.664
Huron	0.820	0.781	0.779	0.660	0.993	0.853	1.047	0.934	0.909	0.764	0.776	0.613
HY 320	0.799	0.629	0.727	0.540	1.060	0.827	1.097	1.029	0.820	0.671	0.719	0.661
Katepwa	0.734	0.646	0.630	0.538	1.082	0.687	0.920	0.889	0.718	0.694	0.651	0.629

Kenhi	0.814	0.723	0.570	0.523	1.129	1.422	1.224	0.878	0.834	0.787	0.654	0.567
Kenya Kongoni	0.857	0.753	0.648	0.593	1.086	1.075	0.913	1.071	0.925	0.808	0.679	0.633
Kitchener	0.725	0.587	0.586	0.603	1.476	1.292	1.178	1.276	0.996	0.780	0.826	0.603
Kota	0.805	0.658	0.686	0.643	1.277	1.147	1.019	1.035	0.741	0.778	0.717	0.659
Ladoga	0.704	0.637	0.583	0.441	1.182	0.940	1.068	0.908	0.728	0.736	0.759	0.664
Lake	0.923	0.737	0.701	0.593	1.152	1.178	0.970	0.822	0.916	0.830	0.662	0.597
Laval 19	0.712	0.748	0.662	0.632	0.894	0.986	0.820	0.962	0.775	0.847	0.15	0.748
Leader	0.800	0.679	0.675	0.647	1.022	1.144	1.096	0.990	0.798	0.770	0.691	0.699
Lee	0.793	0.744	0.677	0.602	1.103	1.050	0.962	0.770	0.840	0.806	0.707	0.656
Lemhi 53	0.805	0.663	0.522	0.546	1.166	0.931	0.891	0.857	0.851	0.654	0.587	0.617
Lemhi 62	0.774	0.580	0.599	0.529	1.084	0.880	0.832	0.904	0.831	0.638	0.662	0.643
Manitou	0.722	0.715	0.613	0.676	1.002	0.940	1.178	1.065	0.812	0.733	0.639	0.713
Maringa	0.747	0.590	0.502	0.298	0.724	0.498	0.606	0.531	0.663	0.659	0.631	0.471
Marquis	0.645	0.510	0.437	0.490	0.879	0.973	0.760	0.832	0.749	0.731	0.624	0.640
Milton	0.817	0.748	0.724	0.613	0.868	1.060	0.991	0.759	0.837	0.818	0.727	0.691
Napayo	0.833	0.618	0.627	0.549	1.020	0.902	0.814	0.677	0.742	0.630	0.727	0.692
Neepawa	0.760	0.655	0.569	0.502	1.393	1.115	1.097	0.848	0.769	0.629	0.613	0.547
Norquay	0.777	0.758	0.691	0.570	0.886	1.210	1.146	0.934	0.996	0.802	0.702	0.676
Opal	0.833	0.827	0.777	0.608	1.265	1.039	1.271	1.122	0.973	0.875	0.824	0.732
Park	0.704	0.509	0.635	0.664	1.133	0.864	0.937	0.994	0.790	0.615	0.697	0.707
Pembina	0.541	0.542	0.494	0.486	1.023	1.023	0.939	1.085	0.730	0.749	0.661	0.647
PF 7748	0.796	0.740	0.660	0.582	0.927	0.993	0.816	0.889	0.820	0.855	0.720	0.650
Pioneer	0.942	0.749	0.700	0.635	1.145	1.052	1.048	1.037	0.885	0.699	0.748	0.588
Pitic 62	0.830	0.656	0.699	0.697	1.054	0.831	0.820	0.946	0.839	0.696	0.740	0.760
Prelude	0.760	0.691	0.719	0.560	1.019	1.034	1.014	0.834	0.854	0.834	0.788	0.749
Preston	0.885	0.908	0.657	0.701	1.444	1.406	1.100	0.953	0.870	0.874	0.658	0.748
Quality A	0.707	0.569	0.569	0.494	1.139	1.102	0.844	0.924	0.761	0.829	0.668	0.649
Red Bobs	0.809	0.624	0.642	0.670	1.054	1.034	1.052	0.948	0.918	0.712	0.697	0.648
Red Bobs 222	0.788	0.618	0.627	0.610	1.130	1.058	0.973	1.210	0.793	0.666	0.691	0.708
Red Fife	0.870	0.838	0.837	0.826	1.212	1.165	1.080	0.963	0.836	0.767	0.852	0.850
Redman	0.715	0.633	0.559	0.452	1.463	1.205	1.407	1.049	0.776	0.796	0.629	0.598
Regent	0.815	0.762	0.671	0.634	1.282	1.158	1.250	1.034	0.924	0.824	0.788	0.711
Reliance	0.688	0.667	0.756	0.731	1.073	1.046	1.239	1.087	0.716	0.670	0.706	0.646
Renfrew	0.848	0.750	0.666	0.610	1.390	1.312	1.389	1.290	0.956	0.827	0.810	0.804
Renown	0.810	0.731	0.732	0.734	1.074	1.254	1.067	1.187	0.835	0.844	0.854	0.781
Rescue	0.775	0.663	0.597	0.598	1.183	1.182	0.985	0.984	0.693	0.757	0.657	0.674

Reward	0.786	0.730	0.684	0.638	1.095	0.964	0.999	0.977	0.858	0.894	0.793	0.754
Romany	0.990	0.837	0.720	0.676	1.144	1.157	1.046	0.905	0.954	0.869	0.793	0.800
Ruby	0.859	0.674	0.613	0.622	1.332	1.180	0.955	1.137	0.843	0.743	0.638	0.663
Saunders	0.743	0.624	0.570	0.515	1.130	0.791	0.818	1.067	0.842	0.771	0.629	0.672
Selkirk	0.709	0.541	0.566	0.489	1.221	1.009	0.840	0.949	0.859	0.728	0.747	0.653
Sinton	0.523	0.489	0.515	0.488	0.806	0.536	0.750	0.748	0.612	0.574	0.604	0.576
Sonora 64	0.823	0.623	0.608	0.533	0.947	0.753	0.763	0.848	1.184	0.721	0.743	0.687
Springfield	0.908	0.710	0.646	0.572	0.796	0.881	0.818	0.678	0.954	0.799	0.741	0.675
Stanley	0.827	0.708	0.641	0.486	1.414	1.834	1.267	1.038	0.782	0.742	0.742	0.657
Thatcher	0.831	0.668	0.669	0.574	0.952	1.143	1.031	1.121	0.877	0.710	0.716	0.663
Vernon	0.950	0.832	0.732	0.592	1.165	0.935	1.082	0.847	0.972	0.711	0.808	0.713
Mean	0.[illegible]	0.693	0.638	0.577	1.080	1.015	0.983	0.936	0.832	0.763	0.712	0.673

Table 2.7 Simple correlations among root length (RL), shoot length (SL), longest seminal root (LSR), the root and shoot length indices (RLI and SLI, respectively) and hematoxylin scores (experiment 1a).

	RL	SL	LSR	RLI	SLI	Hematoxylin Score	Transformed Score
SL	0.455**						
LSR	0.892**	0.439**					
RLI	0.650**	0.321**	0.536**				
SLI	0.255**	0.584**	0.248**	0.438**			
Hematoxylin Score	0.639**	0.029	0.576**	0.333**	-0.012		
Transformed Score	0.422**	0.003	0.312**	0.356**	-0.011	0.998**	
LSRI	0.518**	0.305**	0.648**	0.755**	0.429**	0.264**	0.266**

** Indicates statistical significance at the 1% level.

b. Experiment 1b

i. Root length Index

Seventy-seven spring wheat cultivars were screened for Al tolerance in nutrient solution at 2 Al levels (0, 360μM). Seedlings grew well in this arrangement however missing values were reported for Chinese Spring, Maringa, Red Fife, Lemhi 53 and Kenhi. Missing values were caused by either a loss of experimental material during handling or due to a contamination by *Rhizopus*. The soft white spring wheats (Lemhi 53 and Kenhi) were slow to develop on the nutrient solution, and endosperm breakdown was precipitated as the seed imbibed solution promoting invasion by *Rhizopus*. Surface sterilization of the seed did not improve this condition.

The variables RL and LSR, but not SL, were capable of detecting differential genotype interaction at different treatment levels (tables 2.8a and 2.8b) and the magnitude of these interactions are shown in Table 2.9. There was a high correlation ($r=0.853$, $\alpha=0.01$; Table 2.10) between the RL and LSR variables. This association indicated that the average longest seminal root was a good indication of the average root length, but both of these average values do not arithmetically correct scalar differences in growth potential (e.g., the shoot or root length of semi-dwarf varieties in comparison to tall varieties).

Table 2.8a Analyses of variance for root length (RL), shoot length (SL), and longest seminal root (LSR) for 77 wheat cultivars grown in nutrient solution at 2 Al levels (experiment 1b).

		Mean Squares		
Source of Variation	df	RL	LSR	SL
Replications	3	485.2	15.3	19.2
Al levels	1	83840.1 **	4596.4 **	188.3 **
Cultivars	76	184.0 **	8.0 *	15.7 **
Cultivars x Al levels	76	92.8 **	5.3 **	1.1 ns
Error	454	34.5	1.2	3.7
Total	610			
CV(%)		21.1	14.2	15.4

* Statistical significance at α=0.05

** Statistical significance at α=0.01

Table 2.8b Analyses of variance for RLI, LSRI, and SLI (relative values +Al/-Al) for 77 wheat cultivars grown in nutrient solution (experiment 1b).

		Mean Squares		
Source of Variation	df	RLI	LSRI	SLI
Replications	3	0.044	0.024	0.116
Cultivars	76	0.055 **	0.035 **	0.049 ns
Error	223	0.020	0.009	0.052
Total	302			
CV(%)		33.4	21.3	24.4

* Statistical significance at α=0.05

** Statistical significance at α=0.01

Table 2.9 Treatment means for root length (RL), shoot length (SL) and longest seminal root (LSR) for 77 wheat cultivars grown in nutrient solution at 2 Al levels (experiment 1b)

	RL (cm plant-)		SL (cm plant-)		LSR (cm plant-)	
Cultivar µm Al	0	360	0	360	0	360
Acadia	35.21	10.60	13.19	10.78	9.88	3.54
Alondra 's'	43.24	28.29	12.21	17.75	11.17	6.72
Apex	36.23	12.93	15.56	10.35	9.29	4.02
Atlas 66	26.43	19.98	13.18	11.80	9.95	5.55
Bananaquit	35.67	17.62	10.99	8.98	9.02	4.42
Benito	27.84	11.88	10.64	10.89	8.52	3.88
BH 1146	61.10	31.41	16.10	12.68	14.28	6.56
Bishop	53.33	14.29	13.88	10.89	13.49	4.64
Canthatch	41.52	13.69	13.02	11.08	10.48	4.13
Canuck	39.91	12.27	12.81	9.76	10.02	4.83
Canus	28.52	12.26	10.68	11.73	9.19	4.04
Cascade	39.53	17.85	11.01	9.91	11.33	5.45
Ceres	42.23	12.48	12.75	12.24	10.87	4.07
Chester	45.78	15.25	12.96	10.99	11.86	4.75
Chinese Spring	29.85[1]	10.77[1]	9.07[1]	8.12[1]	7.95[1]	3.95[1]
Chinook	25.99	12.96	12.73	12.37	7.15	3.94
Chuckar 's'	36.56	18.46	9.94	10.33	8.90	5.10
Cinquentenario	36.95	21.63	14.25	13.53	9.15	5.91
Columbus	44.34	14.04	13.15	12.86	12.38	4.49
Concorde	41.98	16.61	11.41	11.50	11.39	4.60
Coronation II	59.51	13.37	15.38	10.51	15.07	4.25
Cypress	30.35	11.45	11.53	11.44	8.75	3.79
Dundas	36.55	11.33	13.04	12.17	9.42	3.44
Early Red Fife	37.40	14.54	11.61	10.59	9.81	4.64
Fielder	31.56	17.33	6.64	7.94	8.15	4.47
Garnet	48.95	16.89	13.11	12.49	14.46	4.67
Genlea	45.54	17.29	13.08	12.46	10.93	5.50
Hard Red Calcutta	42.17	12.14	10.82	9.67	11.49	4.06
Huron	35.36	16.25	12.82	10.22	9.05	4.88
HY 320	42.32	28.73	9.41	10.03	10.67	6.94
Katepwa	42.94	13.72	13.19	11.06	11.12	4.54
Kenhi	31.13[1]	10.55[1]	11.59[1]	9.87[1]	8.36[1]	2.80[1]
Kitchener	26.21	15.85	10.54	10.50	7.76	4.03
Kenya Kongoni	40.24	25.58	10.72	11.68	10.19	5.59
Kota	45.56	15.03	15.42	14.12	11.50	4.37
Ladoga	43.46	16.83	11.28	11.62	11.87	4.78
Lake	39.66	18.42	14.75	13.67	11.51	5.31
Laval 19	29.90	11.59	11.76	11.49	8.13	3.38
Leader	41.48	12.98	14.07	12.61	11.10	3.96
Lee	39.63	12.58	13.95	12.85	11.28	4.38
Lemhi 53	33.56[1]	13.26[1]	10.91[1]	9.66[1]	9.55[1]	3.87[1]
Lemhi 62	34.93	13.91	12.99	13.09	10.44	4.22
Manitou	36.16	12.43	13.22	8.56	9.89	3.97
Maringa	37.61[1]	24.07[1]	8.58[1]	8.80[1]	8.56[1]	6.20[1]

Marquis	37.22	18.63	12.40	11.04	10.14	5.19
Napayo	36.24	13.19	11.57	10.21	9.78	4.11
Neepawa	41.21	11.14	12.66	12.00	10.79	3.59
Norquay	34.78	18.87	9.54	8.35	9.01	5.47
Opal	34.63	14.21	12.17	10.58	8.79	4.03
Park	45.17	10.48	13.49	10.82	11.28	3.35
Pembina	43.15	14.14	13.43	12.04	11.29	4.45
PF 7748	49.43	31.03	15.83	12.24	11.92	7.22
Pioneer	31.99	11.22	13.44	12.04	9.14	3.50
Pitic 62	33.45	18.74	10.33	12.37	8.78	4.80
Prelude	38.33	18.26	13.27	13.03	11.17	5.34
Preston	45.44	14.30	14.15	11.65	11.89	4.49
Quality A	41.61	14.02	12.33	11.03	11.34	4.41
Red Bobs 222	37.04	16.98	10.44	10.86	12.69	4.97
Red Bobs	46.65	15.46	13.17	13.10	13.37	5.11
Red Fife	31.34[1]	10.64[1]	10.37[1]	10.33[1]	8.51[1]	3.64[1]
Redman	40.38	14.31	11.81	11.44	10.50	4.95
Regent	44.83	13.47	14.85	12.77	11.67	4.06
Reliance	35.99	13.90	12.00	10.70	9.94	4.26
Renfrew	54.38	21.10	14.21	11.82	12.98	5.25
Renown	45.49	23.72	13.90	13.67	12.83	5.85
Rescue	36.73	14.30	14.74	14.34	11.62	4.77
Reward	40.65	14.06	15.48	13.75	11.73	4.48
Romany	37.67	24.01	10.80	10.97	9.73	6.09
Ruby	48.27	16.00	13.91	13.04	12.18	4.56
Saunders	34.19	12.27	11.90	12.03	8.68	4.30
Selkirk	40.00	12.84	12.48	11.84	10.57	3.91
Sinton	35.32	11.75	10.68	9.17	9.81	3.88
Sonora 64	38.72	21.93	11.06	11.40	8.43	5.22
Springfield	33.74	12.54	11.40	10.01	9.82	3.93
Stanley	38.62	12.58	12.35	9.88	11.01	4.11
Thatcher	39.61	20.59	11.83	10.70	10.46	4.51
Vernon	26.12	16.00	11.29	8.61	7.74	4.45
Mean	39.13	15.95	11.27	12.50	10.45	4.61
LSD 0.05	8.13	8.13	1.52	1.52	2.67	2.67

1 Treatment means with missing plot values (treatment totals averaged over four plots).

Table 2.10 Simple correlations among root length (RL), shoot length (SL), longest seminal root (LSR), the root and shoot length indices (RLI and SLI, respectively) and hematoxylin scores (experiment 1b).

	RL	SL	LSR	RLI	SLI
SL	0.211 **				
LSR	0.853 **	0.268 **			
RLI	0.731 **	0.212 **	0.607 **		
SLI	0.202 **	0.599 **	0.231 **	0.416 **	
LSRI	0.624 **	0.080	0.739 **	0.791 **	0.394 **

** Indicates statistical significance at 1% level.

Both of the variables RLI and LSRI, but not SLI were able to detect significant genotypic differences (Table 2.8b). Most of the research utilizing root length as a measure of Al tolerance utilizes measurements on the longest seminal root (Moore *et al.* 1976; Mugwira *et al.* 1978; Lafever and Campbell 1981; Namwila 1985) or the two longest seminal roots (Kinraide *et al.* 1985). The tolerance indices determined by the measurement of total seminal root length is postulated to be more sensitive to differential Al tolerance in comparison to ratings based only upon the longest roots. The ratings of the former reflect the differential elongation rates of the entire root system rather than the limit of the genetic potential represented by the primary root. In the present experiment, the LSRI accounted for 62.6% of the variation observed in the RLI measurements (Table 2.10). The LSRI is capable of detecting broad cutivar differences.

Based upon Duncan's Multiple Range Test groupings and the relative inhibition of root elongation, the RLI was divided into six somewhat arbitrary tolerance classes (ranging from very tolerant to very sensitive (Table 2.11)) which facilitated cultivar classification and comparison. The RLI ratings of the imported varieties were in consistent agreement with published reports in the literature (Konzak *et al.* 1976; Polle *et al.* 1978; Rajaram *et al.* 1981; CIMMYT 1983; Mesdag and Slootmaker 1969; Namwila 1985).

Alondra 's' performed remarkably well as measured by both RLI and LSRI. This is in direct contrast to a published report that Alondra 's' has no Al tolerance 'per se' in nutrient solution, but has the capacity for increased and more efficient uptake of phosphorus under field conditions (CIMMYT 1983). The nutrient solution in this experiment supplied no phosphorus, therefore the seedlings were entirely dependent on seed reserves which were adequate for the ten days of growth (no deficiencies observed). This report (CIMMYT 1983) did not specify how Al tolerance was assessed (root weights, root lengths, hematoxylin staining, etc.), and other differences in protocol (nutrient solution composition, Al exposure time) could also account for this discrepancy.

In the present study, concentrations of Ca^{2+} and Mg^{2+} were relatively high in comparison to the Al levels and these divalent cations (particularly Ca^{2+} can alleviate the toxic effects of Al (Haug 1984; Wagatsuma 1983b). Perhaps some of the discrepancies with published reports of Al tolerance indicate the differential capabilities of plants to alleviate Al stress (e.g., efficient Ca^{2+} utilizers) with other compensatory cations in solution.

There were other deviations from the published literature (Anonymous 1967; Mesdag and Slootmaker 1969) however these discrepancies emphasize the imprecise nature of screening techniques and the problem of comparing results from different experiments. Mesdag and Slootmaker (1969) screened wheat in acidified soil and visually assessed Al

toxicity, whereas Anonymous (1967) measured root and shoot yield of cultivars grown in nutrient solution. Most deviations from these results were only of minor importance. It should be noted that the 2 lines of Red Bobs (each line obtained from different seed sources) reacted differently to the Al stress. The tolerance of Benito was the result of variable root growth in the control study.

The correlations between seedweight and RLI, average root length in Al, average longest seminal root in Al and the shoot length in the control were all significant (Table 2.12), however the correlation coefficients were so low that little biological relevance could be attached to the observation that bigger seeds generally produced more vigorous seedlings in nutrient solution with or without Al stress. The root length index was positively correlated with the average root length in Al and the average of the longest seminal roots in Al . The correlation coefficient for the former ($r=0.697$, $\alpha=0.01$) indicated that the measurement of total root length in Al alone can define broad tolerance categories particularly in genetic trials where seed is limited to replicate over controls. Although 360 µM Al was effective in screening a diverse population, this concentration depressed the growth of the most tolerant genotypes by 31.3%. A lower Al concentration may have improved the correlation between the RLI and total root length in Al.

Average shoot length in the control was positively correlated ($r=0.524$, $\alpha=0.01$) with the average root length in the control, however only 27.4% of the observed variability could explain this relationship. After ten days of growth, the genotypes exhibited genetic differences in shoot length, and shoot length was unreliable as an indicator of Al tolerance. The addition of Al further decreased the reliability of the shoot length variables in detecting tolerance classes as is evident from the analysis of variance (Tables 2.8a and 2.8b). The Spearman rank correlation coefficient between the RLI of experiment 1b and the hematoxylin score of experiment 1a was significant ($r=0.595$, $\alpha=0.01$). However, only 35.4% of the observed variability could be accounted for by this relationship.

Table 2.11 Means of RLI, LSRI, SLI (relative values +Al/-Al), and tolerance ratings of 77 wheat cultivars grown in nutrient solution.

Cultivar	RLI	LSRI	SLI[2]	Tolerance Rating[3]
HY 320	0.687 a[1]	0.654 ab	1.145	VT
Alondra 's'	0.686 a	0.613 a-d	1.050	VT
Romany	0.673 ab	0.635 abc	1.052	VT
Kenya Kongoni	0.642 abc	0.557 b-l	1.131	VT
Maringa	0.637 a-d	0.740 a	1.019	VT
PF 7748	0.631 a-d	0.607 a-e	0.796	VT
Vernon	0.622 a-e	0.573 b-h	0.746	VT
Benito	0.609 a-f	0.474 c-q	1.080	NA
Cinquentenario	0.602 a-g	0.653 ab	0.956	T
Kitchener	0.586 a-h	0.514 b-n	1.021	T
Pitic 62	0.572 a-i	0.559 b-	1.221	T
Sonora 64	0.566 a-k	0.620 a-d	1.041	T
Atlas 66	0.570 a-j	0.575 b-g	0.903	T
Norquay	0.560 a-l	0.609 a-e	0.899	T
BH 1146	0.549 a-m	0.469 c-r	0.801	T
Fielder	0.546 a-m	0.565 b-i	1.185	T
Chinook	0.530 a-m	0.561 b-j	0.986	T
Renown	0.524 a-m	0.454 d-r	0.991	T
Chuckar 's'	0.516 a-n	0.580 b-e	1.046	MT
Marquis	0.502 a-n	0.507 b-o	0.907	MT
Prelude	0.500 a-n	0.485 b-q	1.008	MT
Bananaquit	0.493 a-n	0.488 b-q	0.818	MT
Lake	0.474 a-o	0.463 a-r	0.924	MT
Huron	0.473 a-o	0.546 b-m	0.815	MT
Cascade	0.471 a-o	0.491 b-p	0.953	MT
Red Bobs 222	0.470 a-o	0.403 g-s	1.090	MT
Rescue	0.439 a-o	0.415 f-s	0.922	MT
Canus	0.431 c-o	0.441 e-s	1.094	I
Opal	0.419 c-o	0.463 d-r	0.882	I
Early Red Fife	0.415 c-o	0.486 b-q	0.978	I
Lemhi 62	0.405 c-o	0.404 g-s	1.012	I
Concorde	0.397 c-o	0.404 g-s	1.025	I
Lemhi 53	0.394 c-o	0.409 f-s	0.904	I
Reliance	0.392 d-o	0.435 f-s	0.900	I
Laval 19	0.390 d-o	0.421 f-s	0.971	I
Renfrew	0.389 d-o	0.408 g-s	0.833	I
Glenlea	0.387 d-o	0.507 b-o	0.968	I
Cypress	0.380 e-o	0.432 f-s	0.990	I
Chinese Spring	0.379 e-o	0.502 l-p	0.917	I
Ladoga	0.376 e-o	0.400 i-s	1.034	I
Springfield	0.375 e-o	0.406 g-s	0.888	I
Garnet	0.371 e-o	0.337 o-s	0.925	I
Canthatch	0.370 f-o	0.407 g-s	0.866	I
Pioneer	0.365 g-o	0.384 m-s	0.899	MS
Napayo	0.363 g-o	0.423 f-s	0.878	MS
Saunders	0.362 g-o	0.504 b-p	1.009	MS
Apex	0.359 g-o	0.434 f-s	0.680	MS
Redman	0.354 g-o	0.475 c-q	0.974	MS

Manitou	0.352 g-o	0.411 f-s	0.667	MS
Reward	0.351 g-o	0.385 l-s	0.892	MS
Red Bobs	0.343 h-o	0.389 k-s	0.995	MS
Red Fife	0.342 h-o	0.428 f-q	1.012	MS
Sinton	0.342 h-o	0.399 i-s	0.870	MS
Pembina	0.339 h-o	0.398 i-s	0.906	MS
Chester	0.349 h-o	0.401 h-s	0.841	MS
Quality A	0.339 h-o	0.389 j-s	0.900	MS
Katepwa	0.331 i-o	0.414 f-s	0.853	MS
Thatcher	0.330 i-o	0.432 f-s	0.925	MS
Kota	0.329 i-o	0.382 m-s	0.913	MS
Lee	0.329 j-o	0.390 j-s	0.928	MS
Ruby	0.329 j-o	0.374 m-s	0.940	MS
Stanley	0.322 j-o	0.371 n-s	0.794	MS
Selkirk	0.321 j-o	0.375 m-s	0.981	MS
Dundas	0.320 j-o	0.368 n-s	0.937	MS
Preston	0.319 j-o	0.378 m-s	0.828	MS
Columbus	0.318 k-o	0.364 n-s	0.979	MS
Leader	0.315 k-o	0.361 n-s	0.904	MS
Canuck	0.309 l-o	0.497 b-p	0.768	S
Acadia	0.307 l-o	0.361 n-s	0.827	S
Regent	0.304 mno	0.354 n-s	0.877	S
Hard Red Calcutta	0.300 mno	0.352 n-s	0.942	S
Kenhi	0.296 mno	0.316 q-s	0.866	S
Ceres	0.296 mno	0.374 m-s	0.970	S
Neepawa	0.271 no	0.333 p-s	0.973	S
Bishop	0.269 no	0.375 n-s	0.775	S
Park	0.233 o	0.298 r-s	0.800	S
Coronation II	0.233 o	0.290 s	0.685	S
Mean	0.4202	0.4526	0.9294	

1. Means followed by the same letter within a column are not significantly different at the 5% level of significance as determined by Duncan's Multiple Range Test.

2. SLI means not significant at α=0.05 (See Table 2.8b)

3. VT = very tolerant
   T = tolerant
   MT = moderately tolerant
   I = intermediate
   MS = moderately sensitive
   S = sensitive

Table 2.12 Simple correlations among the root and shoot length variables (experiment 1b).

	Root length in control	Shoot length in control	Longest seminal root in Al	Root length in Al	RLI
Shoot length in control	0.524 **				
Longest seminal root in Al	0.377 **	0.020			
Root length in Al	0.265 *	-0.060	0.895 **		
RLI	-0.376	-0.399 **	0.536 **	0.697 **	
Seed weight	-0.055	-0.230 *	0.295 *	0.311 **	0.359 **

** Indicates statistical significance at the 1% level.
* Indicates statistical significance at the 5% level.

F. Conclusions

Experiment 1a and 1b:

The results of Experiment 1a indicate that the hematoxylin procedure is capable of detecting differential cultivar responses at each level of Al after only 18 hours of Al treatment. The hematoxylin ratings of the young seedlings are most easily assessed and reproduced, however this method is only recommended as a general screening method capable of detecting gross differences in Al tolerance as visually assessed by root tip staining, reflecting differences in Al accumulation. The advantages of this method are its rapidity, convenience and nondestructiveness.

The RLI developed from experiment 1b is postulated to be a more precise indication of Al tolerance based upon root length inhibition in Al proportionate to the control. The measurement of root elongation more accurately reflects the direct stress of Al in the rhizosphere than does a subjective visual assessment of root tip staining and Al accumulation. The main disadvantage of the RLI is the time required for root measurements, however, if the treatments are staggered in time, roots can be measured in batches. The measurement of the longest seminal root and the development of an LSR index is more convenient and not as time-consuming. From these experiments it was determined that 360 µM of Al is satisfactory in determining tolerance ratings for a diverse population of entries. 300 µM may provide better cultivar differentiation by inhibiting the most tolerant cultivars to a lesser extent.

Major discrepancies between the RLI and hematoxylin ratings persist. Garnet and Renfrew repeatedly scored in the tolerant range in the hematoxylin test but were only intermediate in reaction based on the RLI. Kitchener and Chinook consistently scored

sensitive with hematoxylin yet were classified as tolerant by the RLI. It is not yet known which species of Al hematoxylin binds with in the root tip. Continued research into methodology may elucidate these cultivar x Al interactions.

The Canadian spring wheats and imported reference cultivars were screened for Al tolerance using hematoxylin and relative root length indices. Most of the tolerant cultivars (HY 320, Romany, Kenya Kongoni, Maringa, Cinquentenario and PF 7748) were all derived from Brazilian or Mexican cultivars. CIMMYT derived germplasm appeared to contribute the Al tolerance in Pitic 62, Fielder and Norquay. Thatcher and most Thatcher derivatives were relatively sensitive in reaction to Al.

CHAPTER III
Plant-Induced pH Changes

A. Introduction

Plant induced pH changes due to N preference have been implicated in Al tolerance of wheat (Taylor and Foy 1985 a,b,c,d, Fleming 1983, Foy and Fleming 1982, Dodge and Hiatt 1972). Dodge and Hiatt (1972) indicated that plant-induced pH changes were due to anion-cation uptake differences. If cation uptake exceeds anion uptake the pH will decrease due to the release of H^+, however if anion uptake is greater than cation uptake, the pH increases due to the release of HCO_3^- or OH^- (Dodge and Hiatt 1972). Nitrate reductase activity was found to be lower in wheat cultivars grown in solutions with NH_4^+ than in solutions with NO_3^- alone (Dodge and Hiatt 1972). Foy and Fleming (1982) detected differences in plant-induced pH increases (presumably due to differences in NO_3^- absorption) and in nitrate reductase activities between an Al-tolerant cultivar and an Al-sensitive cultivar grown in a mixed N solution with Al stress. Fleming (1983) determined that Al treatments with NH_4^+ inhibited NO_3^- uptake, decreased the rate of pH increase and injured an Al sensitive cultivar more than an Al-tolerant cultivar. Aluminum injury was intensified by increasing NH_4^+ concentrations in solution (Fleming 1983). Mugwira and Patel (1977) indicated that Al tolerance in wheat, but not in triticale, was consistently related to plant-induced pH changes in 1/5 strength Steinberg solution (Foy *et al* 1967) in the absence of Al. Aluminum tolerant cultivars induced higher pH values than sensitive cultivars. Mugwira and Patel (1977) also noted a close relationship ($r=0.731$, $\alpha=0.01$) between plant-induced pH changes and ion uptake in wheat and triticale grown in solutions of KNO_3 and $Ca(NO_3)_2$ but the wheat and triticale cultivars could not be separated into Al tolerance groups in these solutions.

Root cation exchange capacity values for tolerant wheat cultivars have generally been reported as lower than sensitive cultivars (Foy *et al.* 1967; Mugwira and Elgawhary 1979). Sensitive cultivars are thought to absorb NH_4^+ more rapidly (Taylor and Foy 1985 c,d; Foy and Fleming 1982) thereby maintaining a low root microzone pH with a consequent increase in exposure time and toxicity from Al. Tolerant cultivars of wheat, rye, barley and triticale are known to raise the pH of the root microzones and alleviate Al toxicity (Foy *et al.* 1967; Dodge and Hiatt 1972; Foy and Fleming 1982; Fleming 1983; Taylor and Foy 1985 a,b,c,d; Mugwira and Patel 1977). Al tolerant cultivars are thought to utilize NO_3^- more rapidly (Fleming 1983; Taylor and Foy 1985 c,d).

Taylor and Foy (1985 a,b,c,d) characterized both winter and spring wheat cultivars for plant-induced pH changes and determined that cultivar differences in the rate of the pH decline were negatively correlated to an Al tolerance index. The pH decline coincided to depletion of NH_4^+ from solution and the pH decline per unit rate of NH_4^+ absorption was also correlated to an Al tolerance index in winter wheat (Taylor and Foy 1985d). Taylor and Foy (1985d) also suggested that differential NO_3^- depletion from a mixed N solution with Al was related to Al tolerance. This hypothesis supported published reports that tolerant wheat cultivars grown in mixed N solutions generally induced a higher final pH in the presence of Al (Foy and Fleming 1982; Fleming 1983; Taylor and Foy 1985c) and in the absence of Al (Mugwira and Patel 1977).

In contrast, differential cultivar tolerance to Al was not related to plant-induced pH changes in 2 snap bean cultivars or in 2 soybean cultivars (Foy *et al.* 1972; Foy *et al.* 1978; respectively). Wagatsuma and Yamasaku (1985b) concluded that for 5 barley cultivars, plant-induced pH changes for each cultivar were determined by the N source, but that cultivar differences in Al tolerance persisted regardless of N source.

The primary objective of this experiment was to characterize the cultivars to be used in later genetic studies for plant-induced pH changes in mixed N solutions and to determine whether differential Al tolerance was associated with these pH changes.

B. Materials and Methods

Six spring wheat cultivars (Garnet, Park, Thatcher, PF 7748, Kenya Kongoni and Alondra 's') representing a range of Al tolerance and to be used in later genetic studies were selected to monitor plant-induced pH changes in nutrient culture. In addition, the winter wheat cultivars, Atlas 66 and Scout (the seed supplied by Dr. G.T. Taylor) were included as reference cultivars to standardize the experiment with previously published literature (Taylor and Foy a,c,d).

The nutrient culture was similar to that of Taylor and Foy (1985 a,b). The seeds were germinated in petri dishes in the dark at room temperature (22 ± 2°C) for 24 hours. The seedlings were established and elongated in plexiglass trays equipped with nylon mesh bottoms suspended over 10 l of aerated dilute nutrient solution (dilute nutrient solution after Taylor and Foy 1985 a,b,c,d (See Table 3.1a)) in a growth cabinet at 22°C with a 16 hour light cycle. The light intensity was 440 µmol m^{-2} sec^{-1} at 1.2 m from the light source. Relative humidity was controlled at 60%.

Ten uniform nine day old seedlings of each cultivar were wrapped individually at the stem root interface in polyurethane plugs (previously soaked for 1 week in 95% ethanol and thoroughly washed with distilled water (Wheeler *et al.* 1985)). The seedlings were

individually inserted into 10 holes drilled in the lids of 5 l plastic buckets. An additional hole permitted aeration and pH determination. The composition of the complete nutrient solution initially adjusted to pH 4.5 is given in Table 3.1b (nutrient solution after Taylor and Foy (1985a) and Foy *et al.* (1967), except for iron source which is indicated in Table 3.1b).

Table 3.1a Dilute nutrient solution[1]

	mM
Ca^{2+}	1.27
Mg^{2+}	0.27
NO_3^-	3.32
NH_4^+	0.24

1 Composition after Taylor and Foy (1985a,b,c,d)

Table 3.1b Complete nutrient solution[1 2]

	mM	µM
NO_3^-	3.71	
NH_4^+	0.30	
Ca^{2+}	1.27	
K^+	0.75	
Mg^{2+}	0.27	
SO_4^{2-}	0.12	
HPO_4^{2-}	0.10	
Cl^-		58.5
Na^+		53.9
Fe^{2+} [3]		17.9
B^{3+}		6.6
Mn^{2+}		2.4
Zn^{2+}		0.6
Cu^{2+}		0.1
Mo		0.1
Al [4]		74.0

1 Nutrient composition after Foy *et al.* (1967) and Taylor and Foy (1985 a,b,c,d) except for iron source.
2 pH initially adjusted to pH 4.5 with HCl.
3 Fe added as Fe EDTA made from solutions of $FeSO_4$ and Na_2 EDTA.
4 Al supplied as $AlK(SO_4)_2 \cdot 12H_2O$.

The plants were grown in the greenhouse in the first 2 weeks of May, 1986. A 16 hour light cycle was used and the greenhouse temperature averaged 22 ± 3°C. The relative humidity was not controlled but averaged at 70 ± 4%. Solution temperature was not

controlled but averaged at 21° ± 2°C. pH measurements were taken every other day by withdrawing 25 ml aliquots of solution from each container. The experiment was terminated after 14 days of growth in the greenhouse and after the last pH readings were taken. The plants were doused with 95% ethanol to inhibit growth and temporarily stored in the refrigerator until biomass yields could be determined. The plants were washed thoroughly in tap water, divided into roots and shoots, and dried to a constant weight at 60°C.

C. Plant Measurements and Calculated Variables

The root and shoot dry matter weights for each cultivar were determined and used to construct root and shoot weight indices (RWI and SWI, respectively) by dividing the biomass in the aluminum treatment by the biomass in the corresponding control treatment (Taylor and Foy 1985 a,b,c,d; Mugwira *et al*. 1981).

The values from the Al treatments were used to construct pH variables (Taylor and Foy 1985 a,b). The pH decline was evaluated by determining the rate of the pH decline for the first 10 days of growth and the rate of the plant-induced pH increase was determined for the last 4 days of growth. The minimum pH induced, the negative log of the mean H^+ ion concentration (for the entire 14 days of growth) and the final pH induced were also determined.

D. Statistical Design and Analyses

The treatments were 2 Al levels (0 and 74 μM Al) and 8 wheat cultivars. The experimental design was a 8 x 2 factorial with treatments arranged in randomized blocks with 3 replications of each block. The pots were randomized after 1 week within blocks on the greenhouse bench to decrease variability from uncontrolled sources. The analyses of variance were performed for fixed effects models from the mean of 10 plants per plot. The average root and shoot weight values and the pH variables were used in two-way ANOVAS to detect cultivar response. RWI and SWI were used in one-way ANOVAS to determine cultivar rankings. Simple interactions were studied by determining LSD values for each treatment level. To determine whether functional relationships existed between RWI and the pH variables, regression analyses were performed with RWI as the independent variable and each of the pH variables as dependent variables. The pH variables used in the regression analyses were derived from the Al treatments since differences did exist for some of the variables between treatments (the negative log of the mean H^+ concentration, rate of pH increase, and final pH).

E. Results and Discussion

The plants grew well in the arrangement however, Powdery Mildew from adjacent greenhouse trials contaminated shoot growth of several cultivars (particularly Scout, Thatcher, Kenya Kongoni, and PF 7748). This was noticeable on day 10, however, termination of the experiment on day 14 avoided severe fungal damage. Chlorotic spotting was associated with fungal invasion. Nutrient deficiency symptoms on the shoot growth in the Al treatments were not clearly defined or pronounced, but the shoots were thinner and more flaccid with chlorotic shoot tips than in the control treatments. Roots grown in Al were stubby and discoloured with undeveloped laterals.

All of the variables except the rate of the pH decline, the rate of the pH increase and the final pH detected significant cultivar differences (Table 3.2a). Differential cultivar response to each treatment level was detected by average root weight (Table 3.3), and interaction effects were detected for the final pH and the rate of the pH increase (Table 3.4 and 3.5).

Table 3.2a Analyses of variance for average root and shoot weight and the pH variables for 8 wheat cultivars grown in nutrient solution at 2 Al^{3+} levels.

Source of Variation	df	Mean Squares						
		average root weight	average shoot weight	minimum pH	neg log mean H^+ conc	rate of pH decline	final pH	rate of pH increase
Replications	2	0.010	0.125	0.025	0.067	0.004	0.222	0.035
Al^{3+} levels	1	0.488 **	3.090 **	0.008 ns	1.519 **	0.025 ns	47.780 **	12.434 **
Cultivars	7	0.133 **	0.208 **	0.037 **	0.094 **	0.006 ns	1.331 ns	0.370 ns
Cultivars x Al^{3+} levels	7	0.022 *	0.072 ns	0.005 ns	0.050 ns	0.007 ns	1.537 **	0.617 **
Error	30	0.008	0.040	0.008	0.030	0.007	0.367	0.108
Total	47							
CV (%)		18.4	13.7	2.3	13.8	60.8	10.8	41.6

* Indicates statistical significance at the 5% level.
** Indicates statistical significance at the 1% level.

Table 3.2b Analyses of variance for root and shoot weight index values (RWI and SWI, respectively) for 8 wheat cultivars grown in nutrient solution.

		Mean Squares	
Source of variation	df	RWI	SWI
Replications	2	0.008	0.222
Cultivars	7	0.096 **	0.030 ns
Error	4	0.021	0.224
Total	13		
CV (%)		21.4	17.7

* Indicates statistical significance at the 5% level.
** Indicates statistical significance at the 1% level.

Table 3.3 Means for average root weight (averaged over replicates) for 8 wheat cultivars grown in nutrient solution at 2 Al levels.

	Average root weight $gpot^{-1}$	
Cultivar Al μM	0	74
Alondra 's'	0.844	0.534
Kenya Kongoni	0.784	0.576
Atlas 66	0.665	0.584
Thatcher	0.657	0.353
Scout	0.650	0.276
PF 7748	0.426	0.398
Garnet	0.455	0.257
Park	0.351	0.241
Mean	0.604	0.402
$LSD_{0.05}$	0.013	0.013

Table 3.4 Means for final plant-induced pH values (averaged over replicates) for 8 wheat cultivars grown in nutrient solution at 2 Al levels.

	pH values	
Cultivar Al µM	0	74
Kenya Kongoni	7.5	5.3
Atlas 66	6.9	4.9
Alondra 's'	6.5	4.9
Thatcher	7.1	4.3
Garnet	6.9	3.9
Scout	6.6	3.9
PF 7748	5.0	5.2
Park	6.0	[illegible].0
Mean	6.6	4.6
$LSD_{0.05}$	0.6	0.6

Table 3.5 Means for rate of pH change (averaged over replicates) for 8 wheat cultivars grown in nutrient solution at 2 Al levels.

	rate of pH change (pH units per 2 days)	
Cultivar Al µM	0	74
Kenya Kongoni	1.717	0.667
Atlas 66	1.493	0.503
Alondra 's'	1.320	0.513
Thatcher	1.600	0.003
Garnet	1.563	-0.017
Scout	1.423	-0.023
Park	1.013	-0.057
PF 7748	0.267	0.663
Mean	1.300	0.212
$LSD_{0.05}$	0.180	1.180

The analyses of variance for RWI and SWI are given in Table 3.2b, and the tolerance ratings are in general agreement with published literature reports (Taylor and Foy 1985 a,c,d) and from the results of Chapter II (experiment 1b). Alondra 's' did not perform as well as anticipated based on the results of root elongation Chapter II (experiment 1b). The performance of Atlas 66 and Scout confirmed literature reports of Al tolerance and sensitivity, respectively (Taylor and Foy 1985 a,c,d), and the general pH patterns observed among the cultivars confirmed literature reports (Taylor and Foy 1985 a,b,c,d; Foy and Fleming 1982). It is of interest to note that the rate of the pH increase, the rate of the pH

decrease and the final pH induced did not differ between spring and winter wheats, hence it was decided to examine both types of wheat together for these variables.

Although none of the cultivars exhibited increased root weight in the Al treatments, PF 7748 yielded 93% as well in the Al treatment as in the control treatments (Tables 3.3 and 3.6). PF 7748 did not induce high pH values in either treatment in comparison with the other cultivars however, the final pH induced in the Al treatment was 0.2 units higher than in the control treatment. Apparently PF 7748 has the ability to maintain a relatively low root microzone pH in the process of normal nutrition without Al. The rate of the pH increase was more rapid in the Al treatments than in the control treatments (Table 3.5). This latter phenomenon can be partially explained by anomalous data points for the control treatments in replicate 2, but analysis of the other replicates alone did not significantly alter the ranking of PF 7748. Kenya Kongoni and PF 7748 rapidly increased solution pH in comparison with Scout and Park. The coefficients of variation for the rate of the pH decline and the rate of the pH increase were high indicating that the reliability of the treatment means compared were subject to variation.

It is of interest to note that there were no treatment differences in the minimum pH induced by each cultivar (Table 3.2a). Apparently, 1/5 strength Steinberg solution (Foy *et al.* 1967) initially acidified to pH 4.5 with or without Al facilitated similar plant-induced minimum pH values. The result is consistent with published literature reports (Taylor and Foy 1985a; Foy and Fleming 1982). The negative log of the mean H^+ concentration (averaged over cultivars and replicates) was generally lower (pH=4.3) for the Al treatment than for the control treatment (pH=4.7). Taylor and Foy (1985b) reported a correlation ($r=0.436$, $\alpha=0.01$) between spring wheat cultivar tolerance and the negative log of the mean H^+ concentration.

The rates of the pH declines determined before pH inflection were similar for both treatments and all 8 cultivars. Differential genotypic response at either treatment could not be detected by this variable. Taylor and Foy (1985 a,b,c,d) reported cultivar differences in the rate of the pH decline in both winter and spring wheats. Foy and Fleming (1982) reported similar rates of pH decline for 2 cultivars of spring wheat (UC-44-111 and Anza). It is significant that in the present study, both the spring and winter cultivars did not statistically differ in rates of pH decline. Aluminum tolerant genotypes (PF 7748, Alondra 's', Kenya Kongoni and Atlas 66) only differed in pH changes after 10 days of growth. In the present experiment pH recordings were performed every other day instead of daily (Taylor and Foy a,b). Perhaps the sensitivity of experiment decreased with less frequent monitoring and this obscured differential rates of pH decline for cultivars. The nutrient solutions used in this experiment were not allowed to age for four days (Taylor

personal communication) and this factor may have increased Al hydrolysis and pH drift. Judging from the ANOVA tables (Table 3.2a) none of the pH variables were sensitive enough to detect both cultivar and cultivar x Al level interactions.

Table 3.6 Means for relative shoot and root weight index values, minimum pH, the negative log of the mean H^+ concentration, slope of the pH decline, slope of the pH increase, and final pH (average over replicates) for 8 wheat cultivars grown in nutrient solution.

Cultivar	RWI	SWI[2]	min. pH[3]	neg log mean H^+ conc[3]	slope of pH decline[2,3]	final pH[2,3]	slope of pH incr[2,3]
PF 7748	0.929 a[1]	0.804	4.1 a	4.5 b	-0.111	5.1	0.465
Atlas 66	0.913 a	0.701	3.9 abc	4.5 b	-0.208	5.9	0.998
Kenya Kongoni	0.745 ab	0.795	4.0 ab	4.8 a	-0.114	6.4	1.192
Park	0.683 abc	0.700	4.0 abc	4.4 b	-0.108	5.0	0.478
Alondra 's'	0.633 bc	0.834	3.9 abc	4.6 ab	-0.140	5.7	0.917
Garnet	0.563 bc	0.549	3.8 cd	4.5 b	-0.140	5.4	0.773
Thatcher	0.527 bc	0.599	3.9 abc	4.5 b	-0.135	5.7	0.802
Scout	0.425 bc	0.745	3.8 cd	4.4 b	-0.150	5.3	0.700
Mean	0.677	0.716	3.9	4.5	-0.138	5.6	0.791

1 Means followed by the same letter within a column are not significantly different at the 5% level of significance as determined by Duncan's Multiple Range Test.
2 Means not significantly different at 5% level of significance.
3 Variables are averaged over treatments.

The final pH induced by each cultivar in the Al treatments differed. Generally, the tolerant cultivars induced higher final pH values in Al (Table 3.4) (Taylor and Foy 1985 a,b,c,d; Mugwira and Elgawhary 1979; Mugwira and Patel 1977; Mugwira *et al.* 1978; Foy and Fleming 1982).

The results from regression analyses are presented in Table 3.7. Regression with SWI as the independent variable was not performed because SWI was incapable of distinguishing differential cultivar response, however, there was a significant but low correlation between RWI and SWI ($r=0.411$, $\alpha=0.01$; $n=24$). The observed variability accounted for by most of the pH variables was lower than previous literature reports (Taylor and Foy 1985a,b,c,d) with the exception of the rate of pH increase. The results indicated that the rate of the pH increase, the final plant-induced pH values and the negative log of the mean H^+ concentration were all significantly correlated with RWI. The rate of the pH decline was not significantly related to the RWI although visual examination of the

roots for the first 10 days of growth indicated Al toxicity. The rate of the pH increase explained a larger amount of the observed variance (46.5%).

Table 3.7 Regression analyses between root weight index values (RWI) and the pH variables (minimum pH, negative log of the mean H^+ concentration, rate of the pH decline, rate of pH increase, and final pH) for 8 wheat cultivars grown in nutrient solution.[1]

Independent Variable	Dependent Variable	b	a	r^2
RTI	min.pH	0.175 ns	3.843	0.107
	neg. log. mean H^+ conc.	0.297 **	4.165	0.194
	rate of pH decline	-0.042 ns	-0.087	0.051
	rate of pH increase	1.191 **	-0.525	0.465
	final pH	1.929 **	3.310	0.353

** Indicates statistical significance at the 1% level

1 Replicate values were combined and analyzed (n=24)

It is likely that some of the unexplained variance can be accounted for by different Al tolerance mechanisms operating in addition to plant-induced pH changes. The "pH effect" per se on root growth is thought to be minimal as long as pH values are not depressed below 4.0. The results in this experiment confirm this relationship since pH decreases in both treatments were similar. Tolerant plants in the Al treatments generally increased the pH of the solutions to neutrality albeit at a slower rate. The extended effects of the pH x Al toxicity resulted in decreased root biomass.

Acidity has been regarded as having an indirect effect on nutrient absorption (Webb and Loneragan 1985). Aluminum is directly toxic or Al coprecipitates with various anions and nutrient deficiencies are induced. Webb and Loneragan (1985) observed that increasing pH of solutions increased ^{32}Pi absorption in wheat seedlings in short term ^{32}Pi absorption studies for a wide array of pH values and nutrient compositions. The increase in ^{32}Pi absorption was not immediate, therefore the authors speculated that the pH induced metabolic changes which resulted in direct structural changes which affected ^{32}Pi absorption. It is possible that in the present long term experiment the extended effects of low pH altered P absorption. Wagatsuma and Ezoe (1985a) reported that root cation exchange capacity values in several crop species increased from pH 4.0 to 4.5, and that Al absorption, accumulation and translocation appears to be pH dependent. Changes in pH

result in Al speciation (Bohn *et al.* 1979). The toxicity effects associated with Al speciation appear to be species specific (Kim *et al.* 1986; Blamey *et al.* 1983; Wagatsuma and Ezoe 1985a). The additional variability in this experiment not accounted for is likely the result of nutrient x Al and/or nutrient x pH interactions operating in the root environment. This does not preclude the differential effect of Al tolerance mechanisms operating within the plant.

F. Conclusions

The results of this experiment indicate that the tolerant wheats characterized can alleviate Al toxicity by rapidly increasing pH in mixed N solutions in nutrient culture. This phenomenon was the result of differential cultivar tolerance which could be observed prior to pH inflection (Taylor and Foy 1985d). PF 7748, Kenya Kongoni and Alondra 's' rapidly increased solution pH at rates comparable to or better than Atlas 66. PF 7748 appears to be a superior source of Al tolerance as determined by relative root biomass values. There were no cultivar differences in the rate of the pH decline and cultivar tolerance was not related to the rate of the pH decline. Cultivar tolerance was correlated with the rate of the pH increase, the final pH and the negative log of the mean H^+ concentration.

CHAPTER IV
Soil Studies

A. Introduction

On Alberta soils in the pH range of 5.1-5.5, liming to pH 6.5 is expected to increase yields of barley by 10-15%, and increase the yields of red clover and canola by 5-10% (Penney *et al.* 1977). The yield response of wheat on acid and limed native soils is poorly documented, but it is less than that of barley (Agdex 834-2). Soil amelioration with lime and fertilizers (increased P) is not always feasible due to extensive subsoil acidity (as occurs in the Peace River region), and due to the economics of liming. Root penetration into the subsoil can be severely limited due to acidic subsoil conditions, even when the topsoil is not acidic (MacKenzie 1973). Additions of organic matter are known to decrease extractable Al in acid soils (Hoyt and Turner 1975; Hargrove and Thomas 1982; Bloom 1982) and to relieve Al toxicity symptoms in sorghum (Ahmal and Tan 1986). Applications of organic matter to ameliorate extensive soil acidity on a large scale is unlikely to improve marginal agricultural lands.

MacKenzie (1973) recognized the need for Al tolerant cr[illegible]types specifically adapted to native soil conditions. Selecting Al tolerant genoty[illegible]lified in nutrient solutions with controlled Al levels. Extraneous sources of [illegible]toxicity (as in soils) are eliminated and root handling is simplified with little or no [illegible]quantitative root data. The primary objective of this study was to determine whether the Al tolerance ratings of wheat genotypes grown in nutrient solution at controlled Al levels would be indicative of the relative tolerance ratings from a soil screening in Al toxic Silver Valley soil at two lime levels.

B. Materials and Methods

Twenty wheat cultivars (19 spring cultivars and 1 winter cultivar) were screened for Al tolerance in limed and unlimed soil in the greenhouse. The soil site characterization and legal location are given in Table 4.1. The soil sample was obtained from the surface 15 cm of topsoil collected from a farm field in the Silver Valley Region, and was adjacent to the site utilized for liming trials conducted by MacKenzie (1973).

Table 4.1 Soil site, legal location and soil characterization[1]

Site	Legal location	Soil Series	% Base Sat.	pH (1:2.5 soil:soln.) Water	pH (1:2.5 soil:soln.) $0.01MCaCl_2$
Silver Valley	NE-10-82-10-6	Boundary Complex	8.37	4.5	4.1

$0.01\ MCaCl_2$ $\mu g\ g^{-1}$		1.0N KCl $\mu g\ g^{-1}$		NH_4OAc meq $100g^{-1}$ soil			
sol Al^{3+}	sol Mn^{2+}	ex Al^{3+}	ex Mn^{2+}	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}
16.25	3.91	377.75	7.30	4.87	0.82	0.05	1.27

1 Results from the Alberta Soils and Feed Testing Laboratory.

The soil was bulk mixed and sieved through a 5 mm mesh screen. The lime requirement was determined by serial additions of $Ca(OH)_2$ to aliquots of 20 g of soil (University of Alberta Soil Science Laboratory). Bulk lots of soil were limed to pH 6.7 by adding $Ca(OH)_2$ at a rate of [illegible]. The soil was potted (800 g of air dry soil per pot), and the lime was equilibrated with the soil by watering to field capacity and drying for 1 week, for 4 consecutive weeks.

Planting was staggered over 3 days to accommodate 3 replicates which consisted of 2 treatments each (limed and unlimed). Before planting, each pot of soil was remixed and N was incorporated throughout the soil at a rate of 67.4 kg ha^{-1}. P was banded per pot at a rate of 39.3 kg ha^{-1} approximately 2 cm below the seed. The nutrients were solutions of $NH_4(NO_3)_2$ and $NH_4H_2PO_4$. Ten seeds of each cultivar were planted into separate pots, covered with the remaining 100 mls of air dry soil and watered to field capacity. Thereafter, the pots were watered to values between 85-100% of field capacity. At the emergent growth stage, some chemical drift of soap solution being used to control thrips in an adjacent greenhouse trial caused severe damage to the shoots of the emerging cultivars (particularly in replicate 3). Due to time and space constraints it was decided to continue the experiment. After 10 days of growth the seedlings were thinned to 7 seedlings per pot for most cultivars. Some cultivars in the control treatments emerged poorly due to soil compaction, and thinning to 6 seedlings per pot across treatments and replicates was necessary. An additional application of N at the rate of 60 kg ha^{-1} was applied after 2 weeks of growth.

The plants were grown in the greenhouse for the month of October, 1986. A 16-hour light cycle was used and the temperature averaged at 20±3°C. The replications were harvested on consecutive days commencing on November 3, 1986.

At harvest, the soil was washed away from the plant samples with tap water. The plant tissue was rinsed twice in distilled water. Plants were divided into roots and shoots and the plant tissues were dried to a constant weight at 60°C. Whole root and shoot samples from 5 selected cultivars (Laval 19, Garnet, Park, Kenya Kongoni and Alondra 's') were bulked over replicates and submitted to the Alberta Soil and Feed Testing Lab for Al assays ($HNO_3/HClO_4$ digestion and Inductively Coupled Plasma detection).

C. Plant Measurements and Calculated Variables

The root and shoot dry matter weights for each cultivar were determined and used to construct root and shoot weight indices (RWI and SWI, respectively) by dividing the biomass in the unlimed treatment by the biomass in the limed treatment (Foy *et al.* 1974; Mugwira *et al.* 1981) for each replicate.

D. Statistical Design and Analyses

The treatments were 2 lime levels (limed and unlimed) and 20 wheat cultivars. The experimental design was a 20 x 2 factorial arranged with treatments in randomized complete blocks. Due to the spray damage in replicate 3 only 2 replicates were analyzed. The analyses of variance were performed for fixed effects models from the mean of 7 plants per plot for most cultivars. The plots of Concorde, Opal, Park and Prelude consisted of 6 plants per plot across treatments and replicates. The root and shoot weight index values were used in one way analyses of variance. Mean comparisons were performed as outlined in Chapter 2 (experiment 1a). Simple Pearson correlations were determined for the root and shoot weight variables, and Spearman rank correlations were determined to compare Al tolerance ratings in soil with the results from Chapter 2 (experiment 1b). The relationship between root weight in the unlimed treatment was examined by regressing root weight on relative root length values calculated from Chapter 2 (experiment 1b).

E. Results and Discussion

The seedlings in replicate 3 were the latest to emerge and were most severely affected by the drift from insecticidal soap solution used on nearby plants. Shoots displayed tip chlorosis and epinasty. The limed treatments exhibited soil compaction, salt accumulation and soil crusting, and these factors appeared to escalate the spray damage (particularly in replicate 3). As a consequence, replicate 3 was eliminated from the

analyses. It should be noted that this soil is high in sulfate and the Ca^{2+} from the lime probably increased $CaSO_4$ precipitation in the soil.

Seedlings in the unlimed treatments emerged 4-5 days before their counterparts in the compacted lime treatments. This early growth advantage was negated by the stress of Al and soil acidity after 2 weeks of growth. BH 1146 Cinquentenario, Atlas 66, Pitic 62, Kenya Kongoni and Alondra 's' were the first seedlings to emerge in the limed treatments. This growth advantage in the limed and compacted soil reflected the more vigorous root systems of these cultivars.

The shoots of the seedlings in the unlimed treatments were tall with narrow leaves and displayed a range of acid and Al induced symptoms (N deficiency, tip necrosis and chlorosis). The seedlings in the limed treatment were generally more vigorous with wider leaves and tended to recover from the spray induced damage at a later growth stage. Roots of most cultivars from unlimed treatments were finely branched but distinctively thinner than roots in limed treatments. The roots of the most sensitive cultivars in the unlimed treatment exhibited undeveloped laterals and stubby root protrusions. The root system in the limed treatments tended to grown around the compacted soil.

Significant cultivar differences were detected for both root and shoot weight (Table 4.2a) however only shoot weight indicated a significant cultivar x Al level interaction. Average root weight was not a reliable indication of differential cultivar response because the variability in root growth was high between limed treatments ($r=0.169$) in comparison with the unlimed treatments ($r=0.44$, $\alpha=0.05$). Penney (1973) did not detect significant barley cultivar x Al level interactions in field plots at the same soil site. Additional soil factors other than Al have contributed to the erratic plant responses. The coefficient of variation was high but this was not unexpected in cereal root research (Noordavijk 1985) and this factor may have further obscured detection of differential cultivar response.

Table 4.2a Analyses of variance for root weight and shoot weight for 20 wheat cultivars grown in soil at 2 Al levels.

		Mean Squares	
Source of variation	df	root weight	shoot weight
Replications	1	1.584	3.703
Al levels	1	2.628 **	53.324 **
Cultivars	19	0.192 **	0.965 *
Cultivars x Al levels	19	0.072 ns	0.431 **
Error	39	0.083	0.101
Total	79		
CV(%)		29.2	12.6

* Statistical significance at $\alpha = 0.05$

** Statistical significance at $\alpha = 0.01$

There was no association between root and shoot weight in the limed treatments (r=-0.221) or in the unlimed treatments (r=0.102). Differences in biomass for the treatments (Table 4.3) indicated that additional factors other than Al affect crop growth. Spray damage, soil compaction of the limed treatment (this likely decreased aerobic conditions), and an indication of N deficiency due to the acid and/or Al stress in the unlimed treatment may have contributed to the lack of correlation. It is unlikely that Mn^{2+} was toxic (Foy 1976). MacKenzie (1973) found no correlation between soluble soil Mn^{2+} and barley response at 10 different Alberta field sites. In the present study, other micronutrients may have been limiting, although there were not clear-cut deficiency symptoms. Acidity effects in the unlimed treatment due to excessive H^+ may have been pronounced. In contrast to these results Reid *et al.* (1969) reported a high correlation (r=0.93, α=0.01) for barley roots and shoots grown in the Al toxic Tatum soil for 7 weeks in the greenhouse. Reid (1969) concluded that for Tatum soil, barley could be effectively screened by measuring shoot yield in the unlimed treatment only.

Table 4.3 Means for root and shoot weight at each Al level, average root and shoot weight, and RWI and SWI (averaged over replicates) for 20 wheat cultivars grown in soil at 2 Al levels.

	Root weight (g/pot)		Shoot weight (g/pot)		(g/pot)	(g/pot)		
Cultivar	No Lime	Lime	No Lime	Lime	Average root weight	Average shoot weight	RWI[1]	SWI
1. Cinquentenario	0.91	1.86	1.73	3.30	1.38 a[2]	2.52 bcd	0.48	0.53 cde
2. BH 1146	1.15	1.31	2.18	4.34	1.23 ab	3.26 ab	0.87	0.50 cde
3. Pitic 62	1.00	1.44	1.57	3.15	1.22 ab	2.36 bcd	0.68	0.50 cde
4. Atlas 66	0.94	1.49	1.58	3.32	1.22 abe	2.45 bcd	0.72	0.48 cde
5. Katepwa	0.99	1.35	1.22	3.66	1.17 a-d	2.44 bcd	0.69	0.34 cde
6. PF 7748	0.79	1.47	1.59	3.09	1.13 a-e	2.34 bcd	0.54	0.52 cde
7. Kenya Kongoni	1.07	1.18	1.74	2.88	1.13 a-e	2.31 bcd	0.89	0.61 bc
8. Alondra 's'	1.17	1.04	1.60	3.08	1.11 a-e	2.34 bcd	1.08	0.53 cde
9. Concorde[3]	0.93	1.26	1.20	2.48	1.09 a-f	1.84 d	0.84	0.48 cde
10. Canuck	0.74	1.37	1.42	2.73	1.06 a-f	2.08 cd	0.56	0.52 cde
11. Chinook	0.94	1.09	1.95	2.66	1.02 a-f	2.31 bcd	0.95	0.74 ab
12. Sinton	0.67	1.20	1.20	2.82	0.93 a-f	2.01 d	0.55	0.42 cde
13. Norquay	0.82	1.02	1.70	2.78	0.92 a-f	2.24 bcd	0.80	0.61 abc
14. Pioneer	0.86	0.88	2.26	2.80	0.87 b-f	2.53 bcd	0.99	0.81 ac
15. Opal[3]	0.54	1.09	1.96	4.40	0.82 b-f	3.18 abc	0.51	0.45 cde
16. Laval 19	0.77	0.78	1.96	3.24	0.77 b-f	2.60 bcd	0.99	0.60 bc
17. Sonora 64	0.52	0.93	1.79	3.58	0.72 c-f	2.69 bcd	0.63	0.50 bcd
18. Park[3]	0.42	1.02	1.20	3.01	0.72 d-f	2.10 cd	0.41	0.40 de
19. Prelude[3]	0.53	0.80	2.11	3.76	0.66 ef	2.94 a-d	0.65	0.55 cd
20. Garnet	0.40	0.82	2.18	5.70	0.61 f	3.94 a	0.48	0.38 de
$LSD_{0.05}$	0.60	0.60	1.37	1.37	-----	-----	-----	-----

1. RWI not significant at $\alpha = 0.05$ (Table 4.2b)
2. Means followed by the same letter are not significantly different at the 5% level of significance as determined by Duncan's Multiple Range Test.
3. The mean of 6 plants per plot.

Analysis of variance for the calculated variable RWI (Table 4.2b) did not detect cultivar differences. One-way ANOVA partitions out less of the total variance attributed to model specifications in comparison to the two-way ANOVA with interaction. For the calculated variable RWI, the coefficient of variation was high (34.5% Table 2.2) and the model specification R^2 value indicated that only 60% of the total variation observed could be explained by this model.

Table 4.2b Analyses of variance for RWI and SWI for 20 wheat cultivars grown in soil at 2 Al levels.

		Mean Squares	
Source of variation	df	RWI	SWI
Replications	1	0.219	0.027
Cultivars	19	0.081 ns	0.477 **
Error	19	0.061	0.006
Total	39		
CV(%)		34.5	15.3

* Statistical significance at $\alpha = 0.05$.

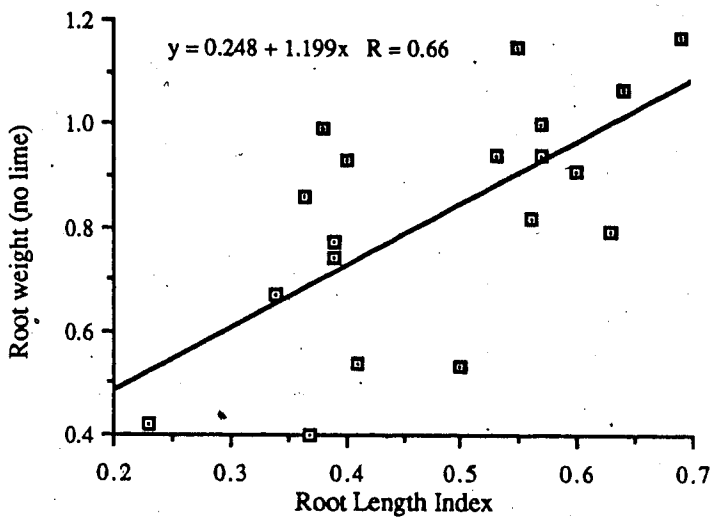
** Statistical significance at $\alpha = 0.01$

Analysis of variance for the calculated variable SWI indicated that the shoot weight index was capable of detecting differential cultivar response. However, the lack of correlation for root and shoot weight in either of the limed and unlimed treatments lends doubt to the ability to detect Al tolerance per se. In addition, there was a significant positive relationship between RWI and SWI variables ($r=0.675$, $\alpha=0.01$), suggesting that these index values were more stable than the raw values at each treatment level. The results from Chapter 2 (experiment 1b) indicated that shoot length and the shoot length index did not reflect differential Al tolerance in nutrient solution after 10 days of growth. Mugwira *et al.* (1981) reported no consistent relationship between relative top growth of wheat and triticale grown on Bladen soil and Al tolerance ratings determined from root elongation in nutrient solution.

For this experiment, root weight in the unlimed Silver Valley soil is the best measure of Al tolerance eliminating the confounding effects of spray damage and soil compaction. This is illustrated by the regression of root weight from the unlimed treatment on to the relative root length index (RLI) (Figure 4.1) values obtained from Chapter 2 (experiment 1b). The Spearman rank correlation coefficient for these two variables was significant ($r=0.567$, $\alpha=0.01$). In general, the two tolerance indices were comparable for the most tolerant cultivars (Alondra 's', Kenya Kongoni, BH 1146, Pitic 62, Atlas 66, Chinook and Norquay) and for the most sensitive cultivars (Park, Garnet and Sinton). The most sensitive cultivars, as measured by root biomass in the soil (Opal, Sonora 64, Park, Prelude and Garnet) exhibited a lower range of scores than in nutrient solution. This phenomenon is likely the result of the extended exposure to Al stress. Sonora 64 did not perform as well as expected from previous reports (Konzak *et al.* 1976; Polle *et al.* 1978) of Al tolerance determined by nutrient culture methods. Katepwa, Prelude and Opal also

deviated from the expected results of Chapter 2 (experiment 1b). The scatter of the data points around the regression line reflect differential cultivar responses to soil factors other than Al toxicity and the high variance associated with index measurements.

Figure 4.1 Regression of root weight from unlimed soil on relative root length (+Al/-Al) for 20 wheat cultivars.[1]



1 Data points represent cultivar values averaged over replicates

Al determinations for whole root and shoot samples from the limed and unlimed treatments are given in Table 4.4. In all cultivars, more Al accumulated in the root tissue than in the shoot tissue at both Al levels. The tolerant cultivars appeared to accumulate more Al in root tissue from the unlimed treatments in comparison with the sensitive cultivars. This observation needs to be retested and replicated, but the results indicated that tolerant cultivars have more Al accumulating power than the sensitive cultivars (Wagatsuma and Ezoe 1985a).

Table 4.4 Al content (μg g-) of five wheat cultivars grown in Silver Valley soil at 2 Al levels.[1]

Cultivars	Roots		Shoot	
	Lime	No Lime	Lime	No Lime
Laval 19	8706	9519	144	398
Garnet	6823	5567	392	268
Park	10237	4844	371	224
Kenya Kongoni	8798	11643	391	437
Alondra 's'	6985	13660	412	391
Mean	8309.8	9046.6	342.0	343.6

1 Samples were bulked over replicates.

F. Conclusions

The results from the present study suggest that screening wheat cultivars in Al toxic Silver Valley soil in the greenhouse and measuring root biomass gives an indication of the differential genotypic tolerance as verified by root elongation measurements in controlled nutrient solution studies, but additional soil factors are likely involved in crop response to the Silver Valley soil pot tests. The cultivars tested were from a diverse parentage and deviations in crop response predicted by root elongation studies in which Al was controlled can also indicate that regional soil differences can indirectly induce selection pressure for adaptation to a particular soil, (Foy *et al.* 1974; Lafever *et al.* 1977; Mugwira *et al.* 1981). Lafever *et al.* (1977) also concluded that relative lengths in nutrient solutions with Al were correlated to cultivar response on acid Wooster soil. Aluminum tolerant cultivars appear to have more Al accumulating power than sensitive cultivars.

CHAPTER V
Genetic Studies

A. Introduction

There have been various literature reports on the genetic control of Al tolerance in wheat. Kerridge and Kronstad (1968) reported that a single dominant gene (as measured by root length in Al treatments) was responsible for the tolerance exhibited in a cross between a susceptible and a moderately tolerant cultivar of wheat. The authors speculated that additional genes could be involved in cultivars with greater tolerance. Lafever and Campbell (1978) found that Al sensitivity in 4 crosses of soft red winter wheat was conditioned by a single recessive gene, but selection for tolerant plants based upon F_3 family means was not effective indicating tolerance was more complex. Campbell and Lafever (1978) also derived 6 populations from each of 8 different crosses of winter wheat and determined that dominance effects were slightly larger than additive gene effects. F_2 and backcross data implicated a single gene for the large dominance effect, but additional genes were likely involved to account for the abundance of intermediate plant types.

Aniol (1984) found that different segregation ratios occurred when the same hybrid populations (tolerant x sensitive classes) were tested at 2 Al levels by the root regrowth technique. Heterogeneity within the populations was thought to be indicative of complex genetic control. Camargo (1984 b) demonstrated that a moderately tolerant Brazilian cultivar "C-3" differed from sensitive "Siete Cerros" by 1 pair of dominant genes when tested with the root regrowth method at 3 mg l^{-1} of Al . There was a gradual decrease in dominance (increase in susceptibility) of this gene pair when tested at 10 mg l^{-1} Al.

Slootmaker (1974) indicated that the D genome in reconstituted hexaploid Canthatch derivatives was responsible for much of the Al tolerance, but that the A genome carried minor genes involved in tolerance. Polle *et al.* (1978) demonstrated that the substitution of chromosome 4D from Thatcher into Chinese Spring reduced Al tolerance in Chinese Spring close to the level of Thatcher. Aniol and Gustafson (1984) employed the root regrowth method on ditelosomic wheat lines to determine the chromosomal locations for the Al tolerance factors and concluded that genes on chromosome arms of 6AL, 7AS, 2DL, 3DL, 4DL and 4BL were responsible for most of the tolerance. Nullisomic-tetrasomic Chinese Spring wheat lines implicated chromosome 4B as contributing to tolerance. Rye additions to Chinese Spring indicated that chromosomes 3R, 4R and 6R were mainly responsible for tolerance in rye. Wheat chromosomes of homeologous group 6A and 6D appeared to suppress the Al tolerance genes located on chromosome 6R. The root regrowth technique

aptly demonstrates the polygenic nature of Al tolerance however, polygenic segregation values do not preclude the existence of major gene(s) controlling tolerance.

The objectives to the present research were to:

1. Investigate the inheritance of Al tolerance in Canadian wheats combined with elite imported cultivars in order to facilitate breeding strategies for Al tolerance.
2. Investigate the gene effects and numbers of genes segregating for Al tolerance in specific crosses.
3. Compare methodologies currently used to evaluate segregants (root length and root regrowth) and to determine whether the results are indicative of the same phenomena.

B. Materials and Methods

Six parents used in the study were selected on the basis of the hematoxylin staining results (Chapter 2; experiment 1a). Garnet, PF 7748, and Kenya Kongoni were chosen to represent tolerant genotypes. Park and Thatcher consistently scored as sensitive; Alondra 's' was chosen for its intermediate tolerance apparently acquired from Weique rye (Rajaram *et al.* 1981).

Table 5.1 Diallel crossing scheme of parental cultivars

Male	Garnet	Thatcher	Park	PF 7748	K.Kongoni	Alondra 's'
Female						
Garnet		X	X	X	X	X
Thatcher			X	X	X	X
Park				X	X	X
PF 7748					X	X
K.Kongoni						X

Fifteen F_1's among the 6 inbred lines were obtained by controlled pollinations in a half diallel crossing design. The 15 lines were allowed to self pollinate to form an F_2 population. Backcrosses of the F_1 to the maternal parent (the sensitive cultivar in most crosses) were also obtained. Reciprocal crosses were not included in this design. From previous results (Chapter 2), Park (parent 1) and PF 7748 (parent 2) appeared to possess substantial amounts of contrasting tolerance and had diverse pedigrees. These parents were reciprocally crossed to obtain F_1, F_2, BCP_1 and BCP_2 populations. All of the derived progenies were examined in 3 separate experiments.

Experiment 1: Park x PF 7748 Generation Mean Analysis

The discriminating sensitive x tolerant cross of Park x PF 7748 was chosen to employ a generation mean analysis and estimate gene effects using root length as the independent variable. Previous results (Chapter II; experiment 1b) indicated a significant correlation (r=0.71, α=0.01) between seminal root length in aluminum and the relative root length index.

The 6 populations (Park(P_1), PF 7748 (P_2), F_1, F_2, BCP_1 and BCP_2 were grown in nutrient solution (cells were drilled in the planting trays to accommodate single seeds) with 300 µM Al to obtain an idea of segregation patterns for the Al tolerance trait. Reciprocal crosses did not differ, therefore, they were combined for presentation. The growth conditions were similar to those described (Chapter 2; experiment 1b) except that on the fifth day Al (supplied as $AlCl_3$) was added. The seedlings were grown for a total of 10 days and preserved in 95% ethanol until the lengths of the seminal roots were determined.

Experiment 2: Root Regrowth Evaluation for the F_2 Segregants

All of the 15 derived F_2 populations were examined by a modified root regrowth technique (Moore *et al.* 1976; Aniol 1984). Lethal concentrations of Al (supplied as $AlCl_3$ in distilled water) were determined for each cultivar. A lethal dose of Al was considered to cause the complete inhibition of root growth (10 seedlings per sample; 2 replicates) after 24 hours of Al treatment and a 48 hour recovery period in full strength nutrient solution (Polle *et al.* 1978). Cultivars which reinitiated root growth from the primary seminal root apexes were considered tolerant (Moore *et al.* 1976).

Regrowth in the tolerant segregants was normal in appearance with no demarcation or injury of the tissue. In contrast, root tips of the sensitive cultivars were knobby and discolored. Some sensitive cultivars initiated lateral root profusions approximately 4 mm from the root apex, but no reinitiation occurred from the primary meristem. The following lethal concentrations were determined for each cultivar:

	Al ($mg\,l^{-1}$)
Park	2.5 *
Thatcher	2.5
Alondra 's'	5.0
PF 7748	10.0
Kenya Kongoni	10.0
Garnet	15.0

* Lethal concentrations determined in 2 replicate sample of 10 plants per sample.

Pregerminated seeds were planted in polystyrene planting trays (3 seeds per cell) and seedlings were grown for 4 days in full strength nutrient solution (Polle *et al.* 1978). Plant growth conditions (temperature, light cycle, irradience, humidity) was as previously described (Chapter 2; experiment 1b). Aluminum concentrations for screening F_2 segregants were determined by using the midpoint between 2 lethal parental doses for each cross. After the recovery period, the seedlings were preserved in 95% ethanol until assessments for tolerance or sensitivity could be conducted.

Experiment 3: Root Length Distributions of Populations Derived From 12 Crosses

In order to confirm if the results from the root regrowth experiment were indicative of segregation for Al tolerance per se, root length distributions were constructed using root length as the independent variable. Five populations (P_1, P_2, F_1, F_2, BC-maternal parent) from 12 different crosses were examined for root length distributions in 300 μM Al (supplied as $AlCl_3$ and assessments were the same as previously described (Chapter 5; experiment 1).

The following crosses were evaluated:

Cross no.	Parents (female x male)
1	Garnet x Thatcher
2	Garnet x PF 7748
3	Garnet x Kenya Kongoni
4	Garnet x Alondra 's'
5	Thatcher x Park
6	Thatcher x PF 7748
7	Thatcher x Kenya Kongoni
8	Thatcher x Alondra 's'
9	Park x Kenya Kongoni
10	Park x Alondra 's'
11	PF 7748 x Alondra 's'
12	Kenya Kongoni x Alondra 's'

C. Statistical Design and Analyses

Experiment 1: Park x PF 7748 Generation Mean Analysis

Six generations (P_1, P_2, F_1, F_2, BCP_1 and BCP_2) were grown concurrently in 300 µM Al in randomized complete blocks with 2 replications. F_2 sample size in the generation mean analysis consisted of 2 bulked F_2 families (n=40). It should be noted that in a biometrical analysis F_2 families are bulked and considered on a population basis. Generation means were calculated on the mean seminal root length per replication. The number of plants per generation varied and is reported in Table 5.2. The generation means per replicate for each cross were used in a two-way analysis of variance to determine the least significant difference between generation mean root length. Within generation variances were calculated for each replicate. In addition, 5 different F_2 families were grown concurrently in replicated randomized blocks, and familial data was examined on root distributions. The ABC Scaling Tests of Mather and Jinks (1971) were used to test for the additivity of the scale in estimating gene effects.

$$A = 2\overline{BCP}_1 - \overline{P}_1 - \overline{F}_1$$
$$B = 2\overline{BCP}_2 - \overline{P}_2 - \overline{F}_1$$
$$C = 4\overline{F}_2 - 2\overline{F}_1 - \overline{P}_1 - \overline{P}_2$$

where:

$\overline{P}_1$, $\overline{P}_2$, etc. are the generation means of P_1, P_2, etc.

If gene effects are additive then each parameter should equal 0. The significance ($P \leq 0.05$) of the scaling tests were determined by using standard errors calculated from the within generation variances and deriving sampling variances according to the following formulae (Mather and Jinks 1971):

$$V_A = 4V_{BCP1} + V_{P1} + V_{F1}$$
$$V_B = 4V_{BCP2} + V_{P2} + V_{F1}$$
$$V_C = 16V_{F2} + 4V_{F1} + V_{P1} + V_{P2}$$

where:

V_{P1}, V_{F1}, etc. equal the square of the within generation standard error of P_1, F_1, etc.

If gene effects were not additive a logarithmic transformation was employed to shorten the upper end of the scale. Gene effects were determined by the 6 parameter generation mean model outlined by Gamble (1962). This model uses the following notation to parameterize gene effects:

	Parameter
mean	m
additive	a
dominance	d
additive x additive	aa
additive x dominance	ad
dominance x dominance	dd

The means of the 6 populations were used to obtain estimates of the six parameters based on the formulae:

$$
\begin{aligned}
m &= \bar{F}_2 \\
a &= \overline{BCP}_1 - \overline{BCP}_2 \\
d &= -1/2\bar{P}_1 - 1/2\bar{P}_2 + \bar{F}_1 - 4\bar{F}_2 + 2\overline{BCP}_1 + 2\overline{BCP}_2 \\
aa &= -4\bar{F}_2 + 2\overline{BCP}_1 + 2\overline{BCP}_2 \\
ad &= -1/2\bar{P}_1 + 1/2\bar{P}_2 \\
dd &= \bar{P}_1 + \bar{P}_2 + 2\bar{F}_1 - 4\bar{F}_2
\end{aligned}
$$

The sampling variances of these estimates are obtained by squaring the corresponding standard errors of the within generation variances. Significance of gene effects ($P \leq 0.05$) were obtained from a table of normal standard deviates.

The potence ratio (a net measure of phenotypic dominance (Mather and Jinks 1971)) was calculated according to the method of Petr and Frey (1966).

$$h = (\bar{X}F_1 - \bar{X}MP)/\bar{X}HP - \bar{X}MP$$

where:

$\bar{X}F_1$, $\bar{X}MP$ and $\bar{X}HP$ are the means of the F_1, the 2 parents, and the high parent, respectively.

Broad sense heritability was calculated by the method of Petr and Frey (1966):

$$H = VF_2 - \sqrt{(VP_1 \cdot VP_2)/VF_2} \times 100$$

where:

VF_2, VP_1 and VP_2 are the variances of the F_2 and the parents.

To facilitate calculation of narrow sense heritability it was necessary to perform the calculations on the log transformation of the original data (to equalize the variances). Narrow sense heritability was calculated by the method proposed by Warner (1952)

$$h = 1/2D / VF_2$$

$$1/2D = 2(VF_2) - (VB_1 + VB_2)$$

where:

VB_1 and VB_2 are the sum of the within generation variances for the backcrosses.

Experiment 2: Root Regrowth Evaluation of F_2 Segregants

Approximately 150 randomly selected seeds representative of 1-5 F_2 families per cross (an F_2 family is derived from 1 F_1 plant) were used to study F_2 segregation ratios. Parents and the F_2 families were randomized within seedlings trays, and the 15 crosses were grown concurrently. Familial data of the F_2 segregants was tested for homogeneity by χ^2 and if the classes were homogeneous they were pooled and tested for the χ^2 fixed ratio at the appropriate degrees of freedom. In some crosses only 1 family was represented in which case the χ^2 fixed ratio test was applied at 1 degree of freedom. Yates' correction was employed to correct for continuity when individual classes could not be pooled.

Experiment 3: Root Length Distributions of Populations Derived From 12 Crosses

Five populations (parents, F_1, F_2 and BC-maternal parent) for each of 12 crosses were grown concurrently in a completely randomized design at 300 µM Al (supplied as $AlCl_3$). Parental cultivars were also grown concurrently at 0 µM Al. Only one F_2 family of adequate sample size could be accommodated in this study, therefore 1F_2 family was randomly selected for examination from the populations of F_2 families in every cross. Each cross was analyzed on a separate basis and root length means, ranges and distributions were used to describe the data. The degree of dominance in the F_1 was calculated according

to the method outlined in experiment 1 (Chapter 5). χ^2 for a fixed ratio was performed on backcross and F_2 populations for crosses in which parental types were recovered and root length limits were specified for parental types. The null hypothesis for a fixed ratio was accepted unless $P \leq 0.05$.

D. Results and Discussion

Experiment 1: Park x PF 7748 Generation Mean Analysis

There were significant differences in mean root lengths of the parents (Table 5.2). Roots of PF 7748 grew approximately 5 times longer than those of Park. The mean root length of the F_1 population mean was significantly greater than Park but did not statistically differ from PF 7748. The potence ratio (0.81) indicated partial dominance of the trait as measured by root length. The mean of the root length backcross to Park was not significantly different than the F_2 mean. There were no indications of transgressive segregants in the F_2 population examined in the generation mean analysis. Parental types were recovered in both of the backcross and F_2 generations.

Table 5.2 Generation means, standard errors of the within generation variances and number of plants tested in Park x PF 7748 cross at 1 level of Al. (experiment 1).

Generation	Means Seminal Root length (cm plant^{-1})	Std. Err. of Mean	number of plants
P_1	6.625	0.396	20
P_2	37.935	2.081	20
F_1	35.031	3.058	16
F_2	29.575	2.232	40
BCP_1	30.090	4.099	20
BCP_2	39.140	2.080	20
$LSD_{0.05}$	8.530		

Table 5.3 Within generation variances (experiment 1).

Generation	s^2 (Original Scale)	s^2 (Log Transformation)
P_1	3.14	0.069
P_2	86.60	0.080
F_1	149.62	0.143
F_2	199.27	0.486
BCP_1	36.04	0.651
BCP_2	86.53	0.079

Within generation variances (Table 5.3) for the 2 parents and the F_1 population exhibited considerable variability. These distributions should be similar and the variability within them should be entirely nonheritable. PF 7748 appeared to be a heterogeneous population based upon the within generation variance of the original data. The variance of the backcross to Park was greater than the F_2 distribution because the deviations from the mean were substantially larger and because the sampling variances were inversely related to sample size. The F_2 sample size (n=40) was greater so that precision was gained in representing the F_2 segregants. The F_2 distribution (Figure 5.1) was shown to be wider than the distribution of the backcross to Park. An attempt was made to stabilize the variances but reciprocal values distorted the variances (Table 5.3).

Table 5.4 Significance of the ABC scaling tests based upon the generation means of Park x PF 7748 grown in nutrient solution at 300 µM Al (experiment 1).

TEST	A	B	C
	-18.57 ± 8.76 *	5.31 ± 5.57	3.68 ± 11.03

*Indicates statistical significance at the 5% level.

The A scaling test (Table 5.4) was significant at the 5% level but not at the 1% level, therefore nonadditivity of the scale and consequent gene effects were implicated. The original scale of measurement was cm plant^{-1} and resulted in a distribution with parental values at either extreme. A logarithmic transformation shortened the upper end, but the fit of the A scaling test was not improved. It was decided that the original scale of measurement was suitable to estimate gene effects.

Table 5.5 Gene effects estimates and standard errors (experiment 1).

Parameter	Estimate	Std. Err. of Mean
m	29.57**	±2.23
a	-9.05**	±3.53
d	32.91**	±13.22
aa	12.68	±12.82
dd	6.61	±4.72
ad	-44.00	±21.44

**Indicates statistical significance at the 1% level.

Figure 5.1 Root length distributions of Park x PF 7748

	Root length (cm)																																						
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60								
P_1			4	8	5	3																																		
P_2										1			1	2		1	1	1	2	2	1	1	1	3		1	1	1												
F_1						1						1				1		2	4	2	2	2	1																
BCP_1[1]				2		2	3		1				1					1		1	1	1	1	2		2	2												
					1	1	1	2			3	1	1	1		2	1	1	3	1	1																		
			2	2		2		2		3	1	3	1	1		2	4	1	1	5	2		1	1	3	1	1												

1. [illegible] as 1:1. $\chi^2 = 0.450$, p = 0.50 - 0.25
2. [illegible] was 3:1. $\chi^2 = 0.033$, p = 0.90 - 0.75

Figure 5.2 Segregation patterns of 5 F_2 families derived from Park x PF 7748

	Root length (cm)																																						
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60								
family 1[1]					1	1	1		1	1		1		2			1	3		2	2		1		1	1		1												
family 2[2]					2	1		1		1		2						2	1		1	1	2	2		3		1											
family 3								1		2			1		2		2	2	1		1				2	2	1		1	2									
family 4		1								2	1	1	1	2	1	1	2		2	1					1		1	1		2									
family 5[3]			1	1	1		1	2				1	1		1		1	2	1	1	1		1	1		1	1		1										

1-3 Ratios tested were 3:1. $\chi^2 = 0.067$, p = 0.90 - 0.75

The gene effect estimates (Table 5.5) indicated that dominance plays a large role in the genetics of Al tolerance. This result is consistent with those of Campbell and Lafever (1978) using similar methods of screening and gene effect estimation in 8 crosses of winter wheat. Root length distributions of the populations are presented in Figure 5.1. The backcross to Park indicated a 1:1 segregation ratio and there was no phenotypic overlap between the parents to obscure the segregation values. This segregation indicated that a single gene accounts for most of the tolerance within the studied population. The F_2 distribution (Figure 5.1) of the bulked families indicated that 1 gene was responsible for the Al tolerance observed. The significant additive gene effect (Table 5.5) can be accounted for by the predominance of intermediate root lengths. Significant additive gene effects and high heritabilities have been reported for various winter wheat crosses (Campbell and Lafever 1981, 1978). In the generation mean analysis, epistasis was not implicated in inheritance.

The root length distributions for the 5 F_2 families examined individually are presented in figure 5.2. Family sizes were small (n=20) but χ^2 for a fixed ratio was still applied to determine segregation ratios. Root length limits between parental types were delineated (Figure 5.2). Homogeneous families were not pooled because the graphical depiction of pooled families obscured the discrete segregation classes presumably due to the sensitivity of the root length variable to environmental influences. The results indicated that 3 of the families (families 1, 2 and 5) were segregating in a 3:1 (t:s) ratio with recovery of both parental types. Heterozygotes and Al tolerant homozygotes were dominant. Families 3 and 4 did not segregate in a 3:1 fashion, and there was no significant recovery of the sensitive parental type. Discrete distributions in approximate 1:3 (t:s) ratios were observed on either side of the 44 cm limit. It is possible that these families were segregating for genetic factors other than Al tolerance. Another plausible explanation could be that the parental stocks used in these families were not homogeneous for the Al tolerance gene. F_1 hybrids with 2 tolerant alleles would produce similar offspring in this backcross generation. The discrete distributions within families 3 and 4 likely indicate heterozygote (< 44 cm) and homozygote (> 44 cm) expression due to genotypic differences in dose response. Transgressive segregants with roots longer than PF 7748 were apparent, based upon root length distributions (Figure 5.2). The recovery of 1 extremely sensitive plant in family 4 (Figure 5.2) was associated with slow germination.

The broad sense heritability (85%) indicated high heritability for the Al tolerance trait. To facilitate narrow sense heritability estimates, log transformations were used on the original data to adjust for unequal variances (particularly the F_1 and the 2 parents). Narrow sense heritability (50%) indicated that selection for the Al tolerance trait, as measured by root length, would be effective in this F_2 population. Campbell and Lafever (1978)

reported that selection for Al tolerance was effective in tolerant x sensitive crosses based on the regression of F_3 family root length of the F_2. Tolerant x tolerant crosses occasionally produced F_3 progeny with short roots (Campbell and Lafever 1978). This phenomenon was likely the result of selection of the heterozygotes. If 1 gene was segregating for Al tolerance, only 50% of the F_2 heterozygotes would achieve homozygosis in the F_3, however, this percentage would be lower if the parents were not true breeding.

Experiment 2: Root Regrowth Evaluation of F_2 Segregants

The results of the root regrowth evaluation of the F_2 segregants and parents for the 15 different crosses examined separately are summarized in Table 5.6. Although the amounts of root regrowth varied within and between crosses, no attempt was made to subjectively classify the segregants into another phenotypic class. The null hypothesis of a fixed ratio is accepted unless statistically significant at $P \leq 0.05$. Scoring of PF 7748 and K. Kongoni was not as clear cut as in the preliminary determinations because root tip reinitiation frequently occurred within 1 mm from the root tip apex.

The data indicated changing segregation ratios within and between crosses when tested at 1 level of Al. These results support Aniol's (1984) data indicating segregation for Al tolerance factors within crosses. Aniol (1984) attributed the differing segregation ratios as being due to the heterozygosity for Al tolerance in the parent. In the present study, the parental data does indicate substantial heterogeneity particularly in the more tolerant cultivars (PF 7748, Kenya Kongoni and Alondra 's'). The heterogeneous response could be due to variable penetrance depending on the Al concentration used.

The Canadian cultivar "Garnet" exhibits much less heterogeneity and this may be attributed to the substantial inbreeding used in the Canadian wheat breeding system to achieve crop uniformity. Aniol (1984) and Aniol and Gustafson (1984) determined that each segregation ratio can be explained by a different gene (or genes) and that genes for Al tolerance are located in different genomes. The predominance of simple segregation ratios (3:1 and 9:7 (tolerant:sensitive)) may imply that 1 or 2 genes, respectively, are segregating within families of any hybrid population. With one gene involved, dominance of the tolerant homozygote and the heterozygote is expressed and the homozygous recessive frequently scores as sensitive. With 2 independently assorting loci (2 alleles per locus and allelic differences among the parents) the 9:7 ratios are indicative of epistasis (interloci interaction) with dominance for the tolerance alleles at the 2 loci. If there are no dominant tolerance alleles at either loci the phenotype scores are sensitive. Perhaps some threshold concentration is exceeded and only the dominant tolerant phenotype is expressed. It is interesting to consider that the 3:1 ratios could be representative of 2 genes segregating in a

12:4 ratio, and the presence of homozygous sensitivity alleles at either locus would produce phenotypically indistinguishable progeny. Aniol (1984) obtained different F_3 segregation ratios for the same hybrid population when tested at 2 Al levels, and polygenes were assumed to be involved since segregation ratios was not the same at varying levels of Al. However, these different segregation ratios can be accounted for if the Al concentrations used influenced the penetrance and expression of tolerance. Camargo (1984b) demonstrated that expression of dominance in a heterozygote segregating in a 3:1 ratio can be manipulated by changing Al concentrations. Aniol's interpretation of polygenes involved in Al tolerance due to changing F_3 segregation ratios at different Al concentrations, is questionable since the reverse and deviant ratios (Aniol 1984) could also be due to variable Al concentrations or different alleles for the same gene.

The predominance of ratios indicating the dominant nature of Al tolerance was apparent. However, the 1:3 and 7:9 ratios indicated that dominance was variable, and could be explained by an abundance of sensitive phenotypes (notably in crosses involving Park and Alondra 's'). Park showed distinct segregation at 1.25 mgl^{-1} of Al and appeared to be a heterogeneous population. The Al sensitivity factor at 1.25 mgl^{-1} behaved as if it were dominant at the Al concentration employed. Camargo (1984b) and MacNair (1981) reported that variable dominance can be displayed if the dose-response curves of the homozygote and heterozygote are different. That is, the 1:3 ratio can likely be reversed and the dominant nature of Al tolerance expressed at an Al level below the 1.25 mgl^{-1} threshold. Park was found to be tolerant at 0.5 mgl^{-1} (data not shown). It is conceivable that the 1:3 and 3:1 ratios represent different genes, or allelic variants of the same locus since both ratios are present together in some crosses. MacNair (1981) reported that directional selection for both copper tolerant and intolerant populations of *Mimulus guttatus* were important in the evolution and adaption to contaminated and uncontaminated soils, respectively. Park and Alondra 's', therefore, appear to possess genes and/or alleles which confer adaptability to soils with nontoxic levels of Al .

Families within the Thatcher x Park cross (sensitive x sensitive) indicated 1 and 2 gene segregation ratios with the sensitive phenotype fully dominant at 1.25 mgl^{-1} of Al. Park and Thatcher share a common parentage, but the heterogeneous response of Park was apparent at this Al concentration. The cross of PF 7748 x Kenya Kongoni (tolerant x tolerant) satisfied the χ^2 criterion for a 3:1 ratio, but there were no sensitive segregants found in any of the families, and both parents had exhibited considerable heterogeneity in other cross combinations at 6.25 mgl^{-1}. If quantitative inheritance was involved there should have been a few individuals sensitive to 5 mgl^{-1} unless the genes were only operational above 5 mgl^{-1}. It may be that the Al tolerance genes in PF 7448 and K.

Kongoni are the same. The segregation of Garnet x K. Kongoni could not be explained by Mendelian ratios.

The different segregation ratios within families of a cross may suggest that each genotype carries 3 loci (2 alleles per locus) but that only 2 loci are activated at a specific Al concentration. Al tolerance may be a branched biochemical pathway with 1 gene per branch. Activation of the genes and the expression of tolerance would be dependent on the Al concentration applied and the alleles present.

An alternate explanation for the different segregation ratios within a cross would implicate the heterogeneity of the parental lines. The root regrowth technique appeared to be sensitive enough to detect differences in the dose-response of the heterozygote and the homozygote as illustrated by parental segregation ratios (Table 5.6). In crosses with substantial parental heterogeneity (particularly Garnet x Thatcher and PF 7748 x Alondra 's') the 9:7 and 7:9 ratios may be deviant 3:1 or 1:3 ratios, respectively, if the parental lines were heterogeneous in different proportions. The only way to prove this would be to screen parental lines for homogeneity before use in crossing, but the segregation ratios from the present study (Table 5.6) indicated that the dose response of the heterozygote can not always be separated from the homozygote. Heterogeneity in the parents may also explain the reverse ratios with ambidirectional dominance, and may explain why there were different segregation ratios in different crosses using the same parent at 1 Al level.

Root regrowth results have been compared with a few standard reference cultivars (Moore *et al.* 1976; Aniol 1983) and it appears that Al tolerance is in some way related to regrowth. However, it has not previously been demonstrated that the genetic system controlling root regrowth is the primary determinant in Al tolerance and that the segregation ratios observed are prima-facie evidence for genetic control of Al tolerance. Many physiological mechanisms have been postulated whereby a plant can tolerate Al and it is conceivable that root regrowth reflects one or more mechanisms operating. The regrowth technique is somewhat analogous to the genetic versus somatic analysis described by Mather and Jinks (1971). When the trait of interest cannot be measured directly it can be partitioned and studied in subunits. Root regrowth from root apexes may be more related to genotypic differences in the plant's ability to exclude or accumulate Al in the root tips and still initiate root regrowth and it is questionable whether this mechanism is the sole determinant in Al tolerance. Garnet scored highly tolerant in the hematoxylin test, but in subsequent tests (as measured by the relative root length index, plant-induced pH changes and soil studies) scored as sensitive. In the present study, 15 mgl^{-1} of Al was required to inhibit root regrowth. Garnet does not accumulate much Al in the root tip (Chapters 2 and 4). Root regrowth may, therefore, depend on the critical concentration required to break the

threshold level at which Al accumulates in the root tip. Aniol and Gustafson (1984) determined that genes for Al tolerance in Chinese Spring ditelosomic and nullisomic-tetrasomic lines resided on 7 different chromosomes in the D and A genomes. The present data does not confirm polygenic control of Al tolerance in any one cross, but indicates that at the maximum no more than 3 genes are responsible for tolerance in most crosses. Aniol and Gustafson (1984) reported that the ditelosomic lines did not regrow consistently in the root regrowth method. It is also conceivable that ditelosomy altered the processes involved in root regrowth and consequently altered the expression of Al tolerance.

Experiment 3: Root Length Distributions of Populations Derived From 12 Different Crosses.

Population root length means, ranges, degrees of dominance, sample sizes, and the population root length distributions are summarized in Tables 5.7 and Figures 5.3 - 5.14, respectively. Root length means of the sensitive parent were low and the kurtosis was high in comparison with the tolerant cultivars. The root length distributions of Thatcher x Park (sensitive x sensitive (cross 5)) did not show F_1 or F_2 root length means significantly different from either parent. The degree of dominance was judged insignificant.

The tolerant x tolerant crosses (#2,3,11, and 12) all displayed complete or overdominance for Al tolerance in the F_1. It should be noted that the potence ratio tends to overestimate dominance if the parents are similar (Petr and Frey 1966), therefore in these cases judging root length means may be more appropriate. In crosses 2 and 11 the roots of the backcross and F_2 were significantly higher and/or similar to the high parent. The range of the F_2 distribution of K. Kongoni x Alondra 's' (cross 12) was narrow in comparison to the backcross population and the root length distribution of the F_2 showed no segregation of plant types. The F_2 root length distribution of PF 7748 x Alondra 's' (Cross 11) suggested a single dominant gene may have been responsible for the Al tolerance in this family but this could not be determined with certainty because of the overlap between heterogeneous parental types. The F_2 and backcross population of Garnet x PF 7748 and Garnet x K. Kongoni (crosses 2 and 3, respectively) did not segregate into distinct classes because root length differences between the parents were negligible. Positive trangressive segregants in the F_2 and the backcross distributions of Garnet x K. Kongoni were apparent. The F_1 root length of distribution of Garnet x Alondra 's' (cross 4) appeared to segregate in a 3:1 ratio highlighting the apparent heterogeneous genotypes (particularly Alondra 's'), but the F_2 and backcross distributions did not illustrate any segregation of phenotypes.

Some of the sensitive x tolerant crosses (9 and 10) exhibited incomplete to overdominance for Al tolerance in the F_1. F_2 and backcross distributions for crosses 7 and 9 indicated that much of the observed tolerance was conditioned by a single gene. The χ^2 ratio for Thatcher x K. Kongoni (cross 7) supported the 1 gene hypothesis but the poor fit may be due to heterogeneous parental lines. Parental root length distributions in crosses 8 and 10 overlapped, therefore F_2 and backcross segregation ratios were obscured. The backcross distribution of Thatcher x PF 7748 (cross 6) also indicated a 1:1 ratio, but there was no segregation in the F_2 probably due to inadequate family size. The F_2 distribution of Garnet x Thatcher (cross 1) segregated in a 3:1 ratio which supported the 1 gene hypothesis.

Examinations of the parental lines used in the various F_1 hybrid combinations revealed different degrees of dominance for Al tolerance in the parents. Parental contributions to dominance appear to be as follows: PF 7748 > Garnet ≡ Alondra 's' ≡ K. Kongoni > Thatcher ≡ Park. Varying degrees of dominance for Al tolerance within cultivars of diverse origin indicated different magnitudes of tolerance, and the segregation of some of the diallel crosses in 3:1 ratios provided evidence for a multiple allelic series at a specific locus. If the independent variable had been more sensitive to allelic differences in parents of comparable root length, additional variants may have been revealed.

E. Conclusions:

Experiment 1: Park x PF 7748 Generation Mean Analysis

The results of this experiment indicated that dominance was important in the inheritance of Al tolerance. F_2 familial data suggested that 1 gene was responsible for the Al tolerance observed. Significant additive gene effects were thought to be due to the use of heterogeneous parental lines (particularly PF 7748). Narrow sense heritability was intermediate (50%) and therefore selection for Al should be effective but slower if heterogeneous parental lines were used.

Experiment 2: Root Regrowth Evaluation of F_2 Segregants

Parental lines and the derived hybrids were heterogeneous for Al tolerance genes. In many cases, familial segregation ratios were different, but all were characterized by simple Mendelian inheritance with 1 or 2 genes segregating for Al tolerance. It was proposed that not more than 2 loci may be activated at one time depending on the Al level employed. Alternatively, it was proposed that heterogeneous parental sources produced progeny which segregated in deviant 1 gene ratios. Heterogeneous parental lines may also

produce the reverse segregation ratios with ambidirectional dominance within crosses. Ambidirectional tolerance classes within crosses involving Albondra 's' and Park suggest and abundance of sensitivity alleles selected for on soils with nontoxic levels of Al.

Experiment 3: Root Length Distributions of Populations Derived From 12 Crosses

F_2 root length distributions for crosses (1, 7 and 9) indicated that Al tolerance was conditioned by a single gene. Backcross data for crosses 7 and 9 supported the 1 gene hypothesis. Backcross data for cross 6 also supported this hypothesis, but the F_2 distribution of this cross did not segregate possibly due to inadequate family size. Root length distributions did not discern fine differences between parents of comparable tolerance. Existence of varying degrees of dominance and the segregation of some diallel crosses into 3:1 ratios suggested that multiple alleles for the same locus were responsible for much of the phenotypic expression of Al tolerance.

F. Comparisons Among Methodologies

Rigorous comparisons between the 3 genetic experiments are not possible because of differences in protocol, however general comparisons may elucidate whether the assessments are measuring the same phenomenon (Al tolerance). In the 2 experiments utilizing the root length variable, monogenic inheritance with domiance of the tolerant phenotype was postulated for the crosses in which segregation ratios could be determined, however, the root regrowth method revealed different segregation ratios within families. These ratios were monogenic and digenic with ambidirectional tolerance classes. The idea of different segregation ratios within a cross is difficult to conceptualize unless 2 loci are activated at any one time depending on the Al level x allelic interaction (Chapter 5; experiment 2). Alternatively, the regrowth technique may be a sensitive measure of heterogeneity, but may not accurately reflect inheritance unless the parents had previously been screened for homogeneity.

Root length and root regrowth may not measure the same phenomenon. Root length determinations are made after continual growth in an Al treatment and Al tolerance genes could be assumed to be active during the stress. Root regrowth is a measure of recovery in Al free solution and this process may be regulated by different genes.

Familial F_2 segregation ratios for the Park x PF 7748 crosses were well characterized in experiments 1 and 2, but the results were not similar. The 1:3 segregation ratio for the cross detected in regrowth technique may indicate differentially detected

heterozygotes (in comparison to the root length assessments), residual heterogeneity in the parents, or the Al treatment in the regrowth technique may have had a severe effect on the direction of dominance. Similar discrepancies were revealed between the root regrowth and root length data for F_2 progeny derived from Garnet x Thatcher, Thatcher x PF 7748, Thatcher x K. Kongoni and Park x K. Kongoni. More rigorous research is required to determine the similarity of these methodologies, and caution is warranted in interpreting segregation results until these discrepancies are clarified.

Table 5.6 Segregation of 15 F_2 populations and 6 parents evaluated by root regrowth (experiment 2).

Al level (mg1-1)	Cross & parents	# Families	Ratio	Observed:Expected t:s[1]	t:s	χ^2	p
8.75	Garnet x Thatcher	3	9:7	90:73	92:71	0.142	0.95-0.90
	Garnet			29:2			
	Thatcher			10:18			
8.75	Garnet x Park	1	1:3	15:37	13:39	0.228	0.75-0.50
		2	9:7	65:43	61:47	0.132	0.99-0.95
	Garnet			23:5			
	Park			2:21			
12.50	Garnet x PF 7748	5	3:1	125:39	123:41	0.446	0.95-0.90
	Garnet			23:4			
	PF 7748			10:21			
12.50	Garnet x K.Kongoni	1	3:1	106:59	124:41	9.940**	0.00
	Garnet			22:8			
	K. Kongoni			11:21			
10:00	Garnet x Alondra 's'	1	3:1	110:40	112:38	0.079	0.90-0.75
	Garnet			25:5			
	Alondra 's'			7:23			
1.25	Thatcher x Park	3	1:3	19:75	23:71	0.800	0.75-0.50
		2	7:9	27:35	27:35	0.000	1.00
	Thatcher			29:1			
	Park			20:10			
6.25	Thatcher x PF 7748	1	9:7	24:14	21:17	0.[illegible]	0.50-0.25
		2	3:1	88:26	85:29	0.302	0.75-0.50
	Thatcher			2:27			
	PF 7748			28:4			
6.25	Thatcher x K. Kongoni	1	9:7	97:63	90:70	1.072	0.50-0.25
	Thatcher			0:30			
	K. Kongoni			26:5			
3.75	Thatcher x Alondra 's'	1	7:9	12:21	14:19	0.279	0.75-0.50
		1	3:1	92:25	95:32	0.261	0.75-0.50
	Thatcher			0:28			
	Alondra 's'			25:3			
6.25	Park x PF 7748	1	7:9	49:59	45:58	0.276	0.75-0.50
		1	1:3	15:32	12:35	0.700	0.50-0.25
	Park			2:26			
	PF 7748			29:5			
6.25	Park x K. Kongoni	1	7:9	69:86	68:87	0.006	0.95-0.90
	Park			1:26			
	K. Kongoni			26:6			

Al level (mg1-1)	Cross & parents	# Families	Ratio	Observed:Expected t:s[1]	t:s	χ^2	p
3.75	Park x Alondra 's'	2	9:7	44:29	39:31	0.002	0.95-0.90
		1	3:1	22:9	23:8	0.0421	0.90-0.75
		2	1:3	7:22	7:22	0.000	1.00
	Park			0:27			
	Alondra 's'			18:9			
5.00	PF 7748 x K. Kongoni	5	3:1	150:0	115:35	0.000	1.00
	PF 7748			30:0			
	K. Kongoni			30:0			
7.50	PF 7748 x Alondra 's'	1	3:1	24:9	25:8	0.041	0.90-0.75
		1	1:3	8:23	8:24	0.041	0.90-0.75
		1	7:9	14:19	14:18	0.0317	0.90-0.75
		1	9:7	20:15	20:15	0.000	1.00
	PF 7748			18:14			
	Alondra 's'			0:33			
7.50	K. Kongoni x Alondra 's'	2	1:3	20:46	16:50	0.000	1.00
		3	9:7	55:41	54:42	0.584	0.75-0.50
	K. Kongoni			31:3			
	Alondra 's'			4:25			

** Indicates statistical significance at the 1% level.
1 t = tolerant; s = susceptible

Table 5.7 Population root length means, ranges, degrees of dominance and number of plants grown in nutrient solution (experiment 3).

Al level (μM)	Cross no.	Cross or population	root length (cm) mean	range	dominance	no.plants
0		Garnet	57.71	41.2-82.9	-	30
		Thatcher	48.22	36.4-72.1	-	30
		Park	43.56	14.7-70.9	-	30
		PF 7748	58.94	34.3-78.4	-	27
		K. Kongoni	56.71	32.3-84.1	-	30
		Alondra 's'	58.60	19.0-89.7	-	30
300	1	Garnet x Thatcher				
		Garnet	36.59 c*	19.6-45.3	-	30
		Thatcher	10.77 a	4.7-20.8	-	27
		F_1	34.97 c	7.7-44.5	(0.87)[1]	30
		BCP_1	38.58 c	6.2-49.3	-	24
		F_2	26.62 b	11.1-47.0	-	33
	2	Garnet x PF 7748				
		Garnet	27.87 a	12.5-35.4	-	30
		PF 7748	34.61 b	13.7-41.5	-	30
		F_1	42.39 c	27.5-51.4	3.31	30
		BCP_1	39.74 c	31.7-49.4	-	27
		F_2	30.00 a	11.4-54.0	-	33
	3	Garnet x K. Kongoni				
		Garnet	29.00 a	12.0-44.3	-	30
		K. Kongoni	28.29 a	1.7-40.2	-	30
		F_1	41.68 b	20.4-54.6	36.22	24
		BCP_1	38.20 b	17.5-47.8	-	15
		F_2	40.29 b	25.2-57.8	-	30
	4	Garnet x Alondra 's'				
		Garnet	29.39 a	7.6-44.3	-	30
		Alondra 's'	25.53 a	8.6-44.4	-	27
		F_1	29.24 ab	8.7-46.6	(0.92)[1]	29
		BCP_1	31.54 b	9.3-44.3	-	30
		F_2	33.98 b	26.3-48.0	-	33
	5	Thatcher x Park				
		Thatcher	11.11 ab	2.8-29.2	-	24
		Park	9.84 a	2.0-17.7	-	23
		F_1	10.28 ab	7.8-14.8	(-0.32)[1]	18
		BCP_1	12.58 b	6.9-17.4	-	24
		F_2	9.90 a	5.0-17.1	-	30
	6	Thatcher x PF 7748				
		Thatcher	10.71 a	6.0-16.9	-	18
		PF 7748	41.65 d	27.6-51.5	-	30
		F_1	34.85 c	9.1-52.3	0.56	24
		BCP_1	28.22 b	10.1-40.9	-	21
		F_2	40.28 d	26.3-55.2	-	30

Al level (μM)	Cross no.	Cross or population	root length (cm) mean	range	dominance	no.plants
	7	Thatcher x K. Kongoni				
		Thatcher	10.54 a	4.4-18.5	-	21
		K. Kongoni	36.23 c	18.3-50.2	-	30
		F_1	37.00 c	25.3-45.9	(1.06)[1]	24
		BCP_1	26.45 b	7.4-39.2	-	30
		F_2	29.64 b	4.8-48.6	-	33
	8	Thatcher x Alondra 's'				
		Thatcher	10.40 a	3.9-17.1	-	24
		Alondra 's'	37.69 c	6.7-55.1	-	30
		F_1	33.91 c	18.1-44.7	(0.72)[1]	15
		BCP_1	21.46 b	7.5-43.3	-	32
		F_2	32.44 c	9.2-46.1	-	30
	9	Park x K. Kongoni				
		Park	9.42 a	3.1-18.2	-	24
		K. Kongoni	33.14 c	20.2-44.5	-	30
		F_1	38.87 d	11.6-51.3	1.48	30
		BCP_1	26.13 b	8.6-44.4	-	24
		F_2	33.74 c	7.8-55.0	-	33
	10	Park x Alondra 's'				
		Park	8.40 a	5.4-11.9	-	27
		Alondra 's'	27.27 c	3.3-46.2	-	27
		F_1	34.97 d	22.8-43.1	1.82	15
		BCP_1	19.70 b	5.2-46.7	-	33
		F_2	32.85 cd	11.1-49.2	-	33
	11	PF 7748 x Alondra 's'				
		PF 7748	35.90 b	11.5-51.5	-	30
		Alondra 's'	26.19 a	6.7-41.9	-	33
		F_1	36.27 b	10.8-47.4	1.08	30
		BCP_1	40.07 b	22.2-49.8	-	18
		F_2	35.27 b	27.5-45.5	-	33
	12	K. Kongoni x Alondra 's'				
		K. Kongoni	31.94 ab	14.6-43.7	-	31
		Alondra 's'	29.27 ab	3.9-41.1	-	24
		F_1	33.76 c	9.6-43.4	2.37	18
		BCP_1	35.61 c	7.7-48.6	-	27
		F_2	26.95 a	20.4-36.3	-	33

* Within a cross, means followed by the same letter are not significantly different at the 5% level as determined by Duncan's Multiple Range Test.

1 Degree of dominance is not significant.

Figure 5.3 Root length distributions of Garnet x Thatcher (Cross 1)

	Root length (cm)																																		
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	65	58	60				
P_1											1		1		1		3	3	6	6	2	4	2	1												
P_2			1	2	5	7	9	1		1	1																								
F_1					1				2					1	1	2		2	6	4	4	5	2												
BCP_1														2		2		3	1	3	5	2		5		1									
F_2[1]						1	3	1	5	1		2	1	1	2	2	1	6	2	2	1	1		1												

1. Ratio tested was 3:1. $\chi^2 = 1.031$, $p = 0.50\text{-}0.25$.

Figure 5.4 Root length distributions of Garnet x PF 7748 (Cross 2)

	Root length (cm)																																		
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60				
P_1							1			2	1	1	2	1	7	6	7	1	1																
P_2								1			1			1	1	1	1	5	8	6	3	1	1												
F_1								1							1			1	2	1	3	4	3	8	4		2								
BCP_1																	3	2	2	5	6	2	2	3		1								
F_2							4		2	5			1	1	2	1		3	3	1	4	2	1	1				1							

Figure 5.5 Root length distributions of Garnet x K. Kongoni (Cross 3)

	Root length (cm)																																						
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60								
P_1							1		1		3		3	1	6			2	2	1	1		1																	
P_2	1							1		1			3	1	2	3	5	4	3	1	1																			
F_1											1					2	3		2			4	1	4	1	4	1	1											
BCP_1									1	1		1						1	1		1	3		2	4														
F_2														1	2	1	1	2	3	1	4	4	4	3	2		1			1									

Figure 5.6 Root length distributions of Garnet x Alondra 's' (Cross 4)

	Root length (cm)																																						
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60								
P_1					1				1	2	1	1	1	5	3	3	3	4	3		1			1															
P_2					3	2	2		1	1		1	1	2	1	2	3		3	3	1		1																
F_1					2	1	2		3	1				1	1		2	5	2	3	2	2	1	1															
BCP_1						1	1				1		1	1	6	4	1	3	1	1	4	2	2																
$F_2{}^1$												1		3	3	3	3	9	3	2	2	2	1	1															

Figure 5.7 Root length distributions of Thatcher x Park (Cross 5)

	Root length (cm)																																
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60
P_1		1	2	4	4	3	2	3	3				1			1																
P_2		1	1	1	6	8	4		1	1																							
F_1				1	6	6	1	4																									
BCP_1				1	2	4	7	6	3	1																							
F_2			1	4	8	4	11		1	1																							

Figure 5.8 Root length distributions of Thatcher x PF 7748 (Cross 6)

	Root length (cm)																																
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60
P_1				2	5	4	4		2	1																							
P_2															1		1	3	4	2	5	3	4	1	4	1	1						
F_1						1								3	1	1	1	2	1	5	7	2											
BCP_1[1]						1		3	1	2			1		1	2	1	1	3	1	3		1										
F_2														1	1	1	3	1	3	5	3	2	1	3	2	1	2		1				

1. Ratio tested was 1:1. $\chi^2 = 0.430$, $p = 0.50\text{-}0.25$

Figure 5.9 Root length distributions of Thatcher x K. Kongoni (Cross 7)

	Root length (cm)																																		
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60				
P_1			1	1	3	7	5	3		1																										
P_2										1			1	1	2	4	2	3		4	1	4	5		1		1								
F_1														1		2	3	1	6	1	5	4		1											
BCP_1[1]						1	4	2	3				1	1	1	4	1	1	4	3	4															
F_2[2]			1		1	3	1	2					1	1	2	3		1	3	1	7	3	2												

1. Ratio tested 1:1. $\chi^2 = 2.700$, p = 0.25-0.10
2. Ratio tested 3:1. $\chi^2 = 0.000$, p = 0.99

Figure 5.10 Root length distributions of Thatcher x Alondra 's' (Cross 8)

	Root length (cm)																																		
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60				
P_1			1	3	2	9	5	2	1	1																										
P_2				1	1	1	1	2						1			1		1		1	5	4	4	3	1		2	1						
F_1										1				2	2		2		3	1		1	3												
BCP_1					2	5	6	4	3					1	1		1	4		2	1	1	1												
F_2					4		3								1		2	4	2	3	3	2	2	4											

Figure 5.11 Root length distributions of Park x K. Kongoni (Cross 9)

	Root length (cm)																																				
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60						
P_1		1	1	5		8	4	1		1																												
P_2											2	2	1	4	2		2	1	4	5	4		3															
F_1							1								1	1	1	2	4	4	2	7	3	1	2		1										
BCP_1[1]					1	2	2	4	2	1					1			2	1	1	2	3	2															
F_2[2]					1	3	2		1					1				1	5	6	4	4	2	1	1	1		1									

1. Ratio tested was 1:1. $\chi^2 = 0.000$, p = 0.99
2. Ratio testred was 3:1. $\chi^2 = 0.041$, p = 0.90-0.75

Figure 5.12 Root length distributions of Park x Alondra 's' (Cross 10)

	Root length (cm)																																				
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60						
P_1				9	7	7	4																																
P_2			1	1	4			2					2	2	2	2		1	1		3	3	2	1														
F_1												1		1		1	3	1	1	5		1	1														
BCP_1				1	2	3	7	5	4	1				1		2		1	3		1		1	1														
F_2							3	2	4	1					2		1		1	1	7	3	1	6		1												

Figure 5.13 Root length distributions of PF 7748 x Alondra 's' (Cross 11)

	Root length (cm)																																	
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60
P_1							1	1					1				2	4	6	6	4	2	2				1				
P_2				1	1	2	2	1	1	1	1	2		2	2	4	4		5	2		2									
F_1						1	1		1	1						1	3		3	2	9	2	2	3	1						
BCP_1												1			1		1		2	2	1	5		2	2	1					
F_2														1	5	2		8	5	4	4	2	1	1							

Figure 5.14 Root length distributions of K. Kongoni x Alondra 's' (Cross 12)

	Root length (cm)																																	
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60
P_1								1				2	3	1	5		2	6	2	6	1	1	1								
P_2			1		2			1				1	2		3	3	1		6	1	2	1									
F_1						1								1		2	2	4	2	2	2	1	1									
BCP_1					1				1			1				1	3	4	5	3	2		1	4	1							
F_2											2	4	5	6	6	5	4		1													

CHAPTER VI
Summary

The hematoxylin procedure detected cultivar differences in Al tolerance after 18 hours of Al pretreatment. Seedling age did not affect the tolerance ratings, but the assessment was simplified and less subjective for young seedlings (4 days). Quantitative root length determinations were used to construct a tolerance index and the index was found to be a better measure of Al tolerance in comparison with the visual hematoxylin assessments in terms of precision and accuracy. The two screening methods were not highly correlated ($r = 0.595$, $\alpha = 0.01$).

Most of the economically important Canadian spring wheat cultivars were moderately sensitive to sensitive in reaction to Al. On the basis of the root length index, the recently released cultivars, HY 320 and Vernon, ranked as very tolerant, and Norquay and Fielder ranked as tolerant. Historical Canadian cultivars used in early Canadian cultivar development (Kitchener, Renown, Marquis, Prelude and Lake) scored moderately tolerant in reaction to Al but this trait was subsequently lost in derived lines suggesting that it was not an important adaptive character. The results emphasized the necessity of breeding for Al tolerant Canadian wheats, particularly hard red spring cultivars.

Regression of root dry weights for 20 cultivars grown in Al toxic Silver Valley soil indicated a positive relationship ($R = 0.66$, $\alpha = 0.01$) between root dry weights and the root length index determined by root elongation in nutrient solution. Cultivar testing in the field is required to substantiate this result. Preliminary whole root Al assays for 5 cultivars suggested that Al tolerant genotypes trap more Al in root tissue than sensitive cultivars.

In a physiological study, eight wheat cultivars were grown in mixed N solutions with and without Al to characterize genotypic differences in plant-induced pH changes. Aluminum tolerant genotypes (Atlas 66, PF 7748, Kenya Kongoni and Alondra 's') rapidly increased the pH in Al treatments but sensitive cultivars maintained lower root microzone pH values. PF 7748 maintained a low root microzone pH in control treatments in the process of normal nutrition. Cultivar tolerance was not related to the rate of the pH decline or the minimum pH induced, but cultivar tolerance was correlated with the rate of the pH increase ($r^2 = 0.465$, $\alpha = 0.01$), the final pH ($r^2 = 0.353$, $\alpha = 0.01$) and the negative log of the mean H^+ concentration ($r^2 = 0.194$, $\alpha = 0.01$). Statistical detection of cultivar tolerance based on root weights [illegible] be less sensitive than previous studies utilizing root length. On [illegible] root [illegible] PF 7748 was a superior source of Al tolerance comparable with Atlas 66.

The genetic studies utilizing the variable root length detected monogenic inheritance with dominance of the tolerant phenotypes in the following crosses: Garnet x Thatcher,

Thatcher x PF 7748, Thatcher x Kenya Kongoni, Park x Kenya Kongoni and Park x PF 7748. Significant additive gene effects in Park x PF 7748 crosses were thought to be due to an abundance of heterozygous segments with intermediate root lengths. Varying degrees of dominance in the F_1, and multigenic ratios for some the diallel crosses suggested that multiple alleles for the same locus may explain much of the tolerance observed. Evaluation of all of the 15 F_2 diallel at 1 Al level may substantiate this hypothesis. Future investigations should concentrate on characterizing allelic variants. The root regrowth technique appeared to be somewhat effective in detecting heterogeneity within the progeny. Families within some crosses exhibited both monogenic and digenic segregation with ambidirectional tolerance classes. Discrepancies between the results of the root regrowth and root length determinations warrant caution in interpretation of inheritance data utilizing the regrowth technique. Rigorous comparisons between these two methodologies may reveal their relationship.

Simple Mendelian inheritance for Al tolerance was implicated in all of the genetic experiments. Chromosomal location of the Al tolerance gene should be verified and genic mapping could facilitate gene cloning. Physiological studies may differentiate between major gene control and minor adaptability genes conferring Al tolerance.

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Appendix

Appendix Table 1 List of wheat cultivars screened for Al tolerance together with their pedigree and origin. (Year of cultivar release is given for Canadian cultivars).[1]

Cultivar/Selection	Pedigree & Origin	Year Released
Acadia	Selection from Canus x RL.729 E. Canada	1952
Alondra's'	D6301-Nainari 60 x Weique- Red Mace x Ciano2-Chris (CM11683) Mexico	NA[2]
Apex	(H.44-24 x Double Cross) x Marquis W. Canada	1937
Atlas 66	US winter wheat	NA
Bananaquit	CIMMYT cultivar	NA
Benito	Neepawa/3/RL4255*4//Manitou/C.I.7090 W. Canada	1979
BH 1146	Brazilian cultivar	NA
Bishop	Ladoga x Gehun E. Canada	*[3]
Canthatch	Thatcher 6 x Kenya Farmer W. Canada	1959
Canuck	Canthatch and a selection from Mida/Cadet/Rescue W. Canada	1974
Canus	Marquis x Kanred W. Canada	1941
Cascade	[(Quality A x Pacific Bluestem) x C26-59-2D] x Onas E. Canada	1947
Ceres	Kota x Marquis USA	1928
Chester	Mida/Cadet//(Renown/S-615// Rescue)/Kendee W. Canada	1976
Chinook	Thatcher x S-615-11 E. Canada	1952

Chuckar's	CIMMYT cultivar	NA
Cinquentenario	CIMMYT cultivar	NA
Columbus	Neepawa *6/RL 4137 W. Canada	1980
Concorde	C.J. 13931 (Penjamo-Yaqui 54) E. Canada	1976
Coronation II	Pentad x Marquis E. Canada	1937
Cypress	Rescue x Chinook W. Canada	1962
Dundas	Opal x Inia 66 E. Canada	1979
Fielder	Introduction from Idaho, USA	1976
Garnet	Preston A x Riga M E. Canada	1925
Glenlea	(Pembina² Bage) x CB100 W. Canada	1972
Huron	White Fife x Ladoga E. Canada	*
HY 320	Tobari 66 x Romany W. Canada	1984
Katepwa	Neepawa *6/RL 2938/3/Neepawa *6// C.I. 8154 /2* Frocor W. Canada	1981
Kenhi	Kenya 338 AC²E² x Lemhi² W. Canada	1958
Kitchener	Head slection from Marquis W. Canada	not released
Kenya Kongoni	C.I. 8154/2 *Fr/2/3* Rom/3/ W.S.245-II-50-A/C.I. 8154/2 12* Fr Kenya	NA
Kota	Selected from Russian durum USA	*
Ladoga	Introduction from Russia	not released
Lake	Regent x Canus	1954

	W. Canada	
Laval 19	(F.W. 606-A x Opal) x Opal E. Canada	1979
Leader	Fortuna x Chris W. Canada	1981
Lee	Hope x Timstein USA	1950
Lemhi 53	Kenya x Lemhi[5] USA	1956
Lemhi 62	Kenya x Lemhi[6] USA	1968
Manitou	(Thatcher[7]-Frontana x Thatcher[6]-Kenya Farmer) x Thatcher[6]-P.I. 170925 W. Canada	1965
Maringa	Brazilian cultivar	NA
Marquis	Hard Red Calcutta x Red Fife E. Canada	*
Milton	Kentville Selection *6/Pompe E. Canada	1981
Napayo	Manitou[2] x R.L.4124.1 W. Canada	1972
Neepawa	R.L. 4125 x R.L.4008 W. Canada	1969
Norquay	(Lerma Rojo x Sonora 64) x Justin W. Canada	1974
Opal	Triesdorf Stamm 21/40 x Von Römke Erli Introduction from Netherlands Grown in E. Canada	1969
Park	(Mida x Cadet) x Thatcher W. Canada	1963
Pembina	Thatcher x R.L. 2564 W. Canada	1959
PF 7748	(North Dakota 81 x IAS59) x IAS58 Brazilian cultivar	NA

Pioneer	Riga xPreston E. Canada	*
Pitic 62	Yaktana 54 x (Norin 10 x Brevor) Mexican introduction Grown in W. Canada	1969
Prelude	Downy Gehun x Fraser W. Canada	*
Preston	Ladoga x Red Fife E. Canada	*
Quality A	Selection of Florence Introduction from USA	*
Red Bobs 222	Reselection of Early Triumph W. Canada	1926
Red Fife	Reselection from a Polish introduction E. Canada	*
Redman	Regent x Canus W. Canada	1946
Regent	H-44 x Reward W. Canada	1939
Reliance	Kanred x Marquis USA	1932
Renfrew	Selection from Marquis W. Canada	1924
Renown	H-44 xReward W. Canada	1937
Rescue	Apex x S-615 E. Canada	1946
Reward	Marquis x Prelude E. Canada	*
Romany	Kenyan cultivar	NA
Ruby	Downy Riga x Red Fife E. Canada	*
Saunders	C.26-44.7 x Thatcher E. Canada	1947
Scout66	US cultivar	NA

Selkirk	(McMurachy x Exchange) x Redman[3] W. Canada	1953
Sinton	Manitou x CT262 W. Canada	1975
Sonora 64	Mexican cultivar	NA
Springfield	Norin10/Brevor//3 *Lemhi 53/3/ Lemhi 62/4/Lemhi 53*5/3/Lee*7// Chinese/(*Ae. umbellulata*) USA	19
Stanley	Ladoga x Red Fife E. Canada	*
Thatcher	[Marquis x Iumillo] x [Marquis x Kanred] USA	1935
Vernon	Opal *4/Pompe E. Canada	1979
Chinese Spring	Collection from Dr. J. Kuspira	N
Early Red Fife	Selection from Red Fife E. Canada	*
Hard Red Calcutta	Introduction from India	not re

1. Canadian pedigrees obtained from Agriculture Canada - Handbook of Canadian Varieties, 1976. Recent pedigree information obtained directly from Agriculture C Ottawa.
2. Not applicable.
3. * Released prior to 1923.

Appendix Table 2 Analyses of variance for root length (RL), shoot length (SL) and longest seminal root (LSR) for 76 young wheat seedlings grown in nutrient solution at 5 Al levels (experiment 1a).

Source of Variation	df	Mean Squares		
		RL	SL	LSR
Replications	2	307.1	96.9	15.3
Al levels	4	864.6 **	12.8 **	61.5 **
Error a	8	7.3	2.3	0.6
Cultivars	75	21.6 **	5.7 **	1.6 **
Cultivars x Al level	300	1.9 ns	0.7 ns	0.2 ns
Error b	750	1.9	1.0	0.2
Total	1139			
CV (%)		16.5	22.1	15.0

* Statistical significance at α=0.05

** Statistical significance at α=0.01

Appendix Table 3 Treatment means for root length (RL), shoot length (SL) and longest seminal root (LSR) for 76 wheat cultivars grown in nutrient solution at 5 Al^{3+} levels (experiment 1a).

	RL (cm/plant)					SL (cm/plant)					LSR (cm/plant)				
Cultivar Al^{3+} µm	0	180	360	720	1400	0	180	360	720	1400	0	180	360	720	1400
1. Acadia	10.80	8.30	7.80	5.20	5.77	5.97	5.93	4.97	4.03	4.53	3.47	3.13	3.03	2.20	2.73
2. Alondra 's'	13.43	11.67	11.83	9.90	8.27	4.63	4.33	5.27	5.10	3.87	4.80	4.00	4.27	3.43	3.13
3. Apex	12.73	7.40	5.97	5.90	6.33	5.70	4.47	5.10	4.90	4.40	4.00	2.87	2.50	2.37	2.60
4. Bananaquit	11.90	11.13	8.00	8.03	6.77	3.97	4.67	4.00	4.97	4.47	3.60	3.77	2.60	2.80	2.57
5. Benito	10.27	8.23	6.63	5.53	4.80	4.90	4.57	3.83	3.37	3.27	3.67	3.00	2.73	2.53	2.33
6. Bishop	13.93	8.70	7.73	7.43	6.80	5.47	4.63	4.57	4.67	4.93	4.72	2.83	2.77	2.77	2.83
7. Canthatch	9.17	6.67	6.97	6.77	5.87	3.73	3.47	4.03	3.73	4.33	3.30	2.70	2.77	2.73	2.30
8. Canuck	9.23	8.03	7.23	5.50	5.70	4.43	4.70	3.87	3.93	3.87	3.57	2.87	2.97	2.20	2.37
9. Canus	9.47	6.40	5.57	4.77	4.47	4.33	4.30	4.30	3.83	3.57	3.83	2.67	2.50	2.37	1.83
10. Cascade	13.70	12.10	9.37	9.03	8.07	5.23	4.87	4.10	5.03	4.57	4.77	4.17	3.47	3.13	2.97
11. Ceres	13.17	9.87	9.00	7.60	6.73	5.60	5.93	5.93	4.73	5.20	4.27	3.53	3.17	3.03	2.70
12. Chinook	8.07	6.37	6.33	5.93	4.07	3.97	3.90	4.87	4.80	2.70	3.07	2.47	2.30	2.13	1.63
13. Chuckar 's'	9.63	7.60	8.87	8.07	7.77	3.70	3.57	4.27	4.07	3.53	3.37	2.83	3.40	3.07	2.93
14. Cinquentenario	10.97	9.60	9.43	7.87	6.10	4.33	4.63	4.43	4.33	4.40	4.20	3.23	3.17	2.87	2.37
15. Columbus	12.33	8.03	8.20	8.20	6.80	4.70	4.37	4.63	4.63	4.10	4.57	3.23	3.50	3.50	2.90
16. Concorde	10.77	8.87	8.80	7.70	6.77	4.10	4.77	4.40	4.43	4.30	3.70	3.30	3.17	2.97	2.70
17. Coronation II	13.30	9.37	7.83	7.00	7.63	5.67	6.30	4.50	4.07	5.13	4.73	3.63	3.37	3.20	3.40
18. Cypress	10.67	6.67	5.90	5.93	9.60	4.27	4.10	4.03	3.67	4.67	3.80	2.90	2.60	2.73	3.00
19. Dundas	9.60	8.43	6.93	5.47	4.53	4.67	5.33	4.70	4.23	4.10	3.00	2.87	2.37	2.13	1.70
20. Fielder	8.97	7.63	6.43	6.03	5.20	3.73	3.60	2.90	2.80	2.87	3.37	2.83	2.40	2.43	2.07
21. Garnet	16.27	12.03	11.37	9.00	8.67	7.03	6.43	5.63	5.83	6.10	4.73	3.90	3.67	3.00	3.03
22. Glenlea	12.30	10.53	8.53	7.67	6.40	4.47	6.07	5.17	3.77	3.93	3.90	3.23	3.10	2.99	2.43
23. Huron	11.93	9.87	9.10	9.03	7.17	5.27	5.03	4.70	5.37	4.77	3.90	3.57	2.97	3.00	2.37
24. Katepwa	11.07	8.13	7.10	6.97	6.03	5.00	5.37	3.57	4.53	4.47	4.27	3.07	2.90	2.77	2.67
25. Kenhi	9.77	8.03	7.03	5.50	5.20	4.00	4.00	4.50	3.97	3.27	3.57	2.97	2.80	2.33	2.03
26. Kitchener	13.33	9.50	7.73	7.60	7.80	4.20	5.23	5.03	4.40	4.57	4.00	3.67	2.87	3.00	2.90
27. Kenya Kongoni	11.13	9.70	8.50	7.13	6.77	3.80	4.13	4.03	3.33	3.97	3.97	3.67	3.23	2.67	2.53
28. Kota	10.50	8.43	6.87	7.13	6.60	5.03	6.23	5.63	5.13	4.77	4.00	2.93	3.07	2.87	2.60
29. Ladoga	15.37	10.73	9.70	8.93	6.83	5.33	6.23	4.97	5.63	4.90	4.53	3.30	3.33	3.43	3.00
30. Lake	10.17	9.33	7.53	7.03	6.10	4.90	5.70	5.70	4.73	4.17	3.00	3.53	3.20	2.53	2.33
31. Laval 19	10.83	7.73	8.07	7.20	6.90	5.50	4.87	5.53	4.53	5.27	3.43	2.67	2.90	2.47	2.57

32. Leader	10.03	8.03	6.90	6.77	6.57	4.17	4.30	4.57	4.50	4.20	3.93	3.13	3.00	2.70	2.77
33. Lee	9.93	7.87	7.37	6.70	5.97	4.43	4.30	4.57	4.03	3.27	3.80	3.10	3.00	2.63	2.43
34. Lemhi 53	12.83	10.30	8.30	6.60	7.17	4.67	5.37	4.33	4.23	4.07	4.33	3.63	2.80	2.57	2.70
35. Lemhi 62	12.87	9.97	7.43	7.73	6.80	4.93	5.33	4.30	4.13	4.50	4.27	3.50	2.70	2.80	2.70
36. Manitou	11.10	8.07	7.90	6.77	7.37	3.93	3.83	3.77	4.60	4.13	3.97	3.20	2.90	2.53	2.83
37. Maringa	14.37	10.57	8.10	7.23	4.57	4.27	2.90	2.03	2.50	2.43	4.70	3.40	3.17	2.93	2.13
38. Marquis	13.37	8.73	6.80	5.93	[illegible]	5.23	4.53	5.13	3.90	4.50	4.17	3.07	3.00	2.57	2.63
39. Milton	9.57	7.80	7.13	6.77	5.80	5.75	4.60	5.73	5.10	4.17	3.27	2.70	2.67	2.37	2.23
40. Napayo	9.90	7.97	5.90	6.10	5.30	4.40	4.30	3.87	3.57	2.97	3.80	2.80	2.37	2.[illegible]	2.63
41. Neepawo	10.90	8.33	7.20	6.27	5.47	4.20	5.83	4.73	4.63	3.57	4.07	3.13	2.57	2.50	2.33
42. Norquay	12.57	9.57	9.37	8.63	7.13	4.20	4.20	5.07	4.80	3.90	4.03	4.03	3.23	2.83	2.73
43. Opal	10.13	8.70	8.40	7.87	6.13	4.37	5.63	4.67	5.47	4.87	3.33	3.23	2.87	2.73	2.40
44. Park	11.70	8.23	6.00	7.40	7.73	5.00	5.67	4.30	4.70	4.97	4.47	3.50	2.73	3.07	3.07
45. Pembina	13.50	7.07	7.17	6.57	6.47	5.07	4.97	4.97	4.67	5.27	4.17	3.00	3.07	2.73	2.67
46. Pioneer	9.07	8.57	6.73	6.27	5.63	5.13	5.87	5.40	5.37	5.33	3.40	3.00	2.37	2.53	1.97
47. Pitic 62	12.13	10.07	7.93	8.47	8.43	4.97	5.23	4.13	4.07	4.70	4.23	3.53	2.93	3.13	3.20
48. Prelude	13.33	10.07	9.13	9.50	7.40	6.57	6.50	6.63	6.80	5.27	3.97	3.37	3.27	3.13	2.93
49. Preston	11.57	9.73	9.90	7.60	7.77	4.40	5.70	5.53	4.83	4.07	4.10	3.40	3.43	2.70	3.00
50. Quality A	12.23	8.80	8.03	6.80	5.97	4.67	5.30	5.03	4.00	4.37	4.07	3.10	3.33	2.70	2.63
51 Red Bobs	10.67	8.63	6.63	6.83	6.20	4.33	4.47	4.27	4.43	4.40	3.83	3.50	2.73	2.67	2.53
52. Red Fife	9.27	7.93	7.37	7.70	7.57	4.73	5.70	5.50	5.17	4.67	3.60	2.97	2.77	3.07	3.07
53. Redman	11.70	8.23	7.13	6.57	5.23	4.23	5.23	4.40	5.13	4.13	3.80	2.93	2.93	2.40	2.30
54. Regent	11.33	8.90	8.33	7.27	6.73	4.33	5.57	5.00	5.37	4.53	3.93	3.53	3.17	2.97	2.67
55. Reliance	9.67	6.27	5.93	6.57	6.27	4.20	3.97	3.90	4.20	3.90	3.70	2.60	2.40	2.53	2.30
56. Renfrew	14.60	12.10	10.83	9.43	8.90	4.27	5.77	5.60	5.10	5.03	4.17	3.90	3.43	3.30	3.30
57. Renown	11.83	9.60	8.60	8.67	8.60	4.13	4.60	5.07	4.40	4.83	4.23	3.47	3.50	3.53	3.27
58. Rescue	10.80	8.30	7.10	6.43	6.43	4.33	5.07	5.03	4.20	4.27	4.10	2.83	3.10	2.70	2.77
59. Reward	10.50	8.07	7.43	6.83	6.63	4.63	5.00	4.53	4.47	4.53	3.27	2.77	2.90	2.57	2.47
60. Romany	12.53	12.37	10.47	9.10	8.40	3.70	4.23	4.23	3.87	3.40	4.00	3.70	3.37	3.10	3.07
61. Ruby	11.17	9.50	7.47	6.83	6.80	4.30	5.40	4.80	3.93	4.70	3.93	3.30	2.90	2.50	2.60
62. Saunders	11.73	8.70	7.33	6.80	6.17	4.17	4.67	3.17	3.37	4.53	3.97	3.33	3.03	2.50	2.70
63. Selkirk	10.93	7.77	5.90	6.20	5.33	4.10	4.83	4.03	3.50	3.67	3.50	3.00	2.50	2.60	2.23
64. Sinton	11.53	6.00	5.57	5.87	5.53	5.00	3.97	2.67	3.73	3.70	4.10	2.47	2.30	2.43	2.33
66. Springfield	10.00	8.93	6.93	6.30	5.63	4.13	3.27	3.67	3.37	2.70	3.50	3.67	2.73	2.53	2.30
67. Stanley	11.67	9.53	8.13	7.30	5.60	3.77	5.13	6.27	4.43	3.77	4.03	3.13	2.97	3.00	2.63
68. Thatcher	11.83	9.80	7.90	7.87	6.77	4.43	3.97	5.03	4.53	4.87	4.17	3.63	2.93	2.97	2.77
69. Vernon	9.70	9.20	8.03	7.10	5.63	5.13	5.87	4.33	5.47	4.03	3.47	3.37	2.47	2.80	2.47
70. BH 1146	13.80	10.93	9.60	7.47	6.17	4.70	4.20	3.83	3.77	4.33	4.10	3.50	3.10	2.80	2.60
71. Early Red Fife	10.50	10.27	7.57	6.27	6.23	4.90	4.23	3.90	4.33	3.77	3.30	3.20	2.80	2.40	2.53

72. Hard Red Calcutta	10.43	6.97	7.30	6.37	6.73	4.30	3.83	4.67	3.53	4.30	3.37	2.50	2.57	2.20	2.23
73. HY 320	16.30	12.83	10.03	11.43	8.50	4.27	4.47	3.50	4.63	4.30	5.07	4.03	3.23	3.43	3.10
74. PF 7748	13.10	10.37	9.70	8.73	7.63	4.70	4.27	4.63	3.90	4.10	4.10	3.37	3.50	2.97	2.67
75. Red Bobs 222	12.77	10.07	7.80	7.90	7.83	4.50	5.10	4.73	4.23	5.43	4.43	3.50	2.93	3.07	3.17
76. Chester	9.47	8.47	7.63	7.23	5.83	4.43	5.17	3.57	4.73	3.73	3.60	3.13	2.90	2.77	2.27
Mean	11.49	8.96	7.82	7.21	6.52	4.61	4.85	4.53	4.38	4.22	3.92	3.22	2.95	2.75	2.60

Appendix Table 4 Means for longest seminal root index (LSRI), longest seminal root (LSR), shoot length index (SLI) and shoot length (SL) for 76 wheat cultivars (experiment 1a).

Cultivar	LSRI	LSR	SLI	SL
Chuckar 's'	0.906 a[1]	3.12 g-v	1.039 c-m	3.83 p-r
Kitchener	0.860 ab	3.29 d-n	1.306 a-c	4.69 b-p
Romany	0.854 abc	3.45 b-i	1.063 b-m	3.89 n-r
Opal	0.851 abc	2.91 l-y	1.174 a-f	5.00 b-h
Renfrew	0.849 abc	3.62 a-e	1.345 a-b	5.15 b-e
Early Red Fife	0.839 a-d	3.09 h-v	0.841 i-o	4.23 f-q
Renown	0.828 a-e	3.60 a-f	1.146 a-i	4.61 b-p
Red Fife	0.826 a-e	2.85 z-a_2	1.105 a-k	5.15 b-e
Reward	0.825 a-e	2.79 z-a_2	1.009 c-n	4.63 b-p
Concorde	0.824 a-e	3.17 g-r	1.093 b-l	4.40 e-p
Bananaquit	0.816 a-f	3.67 i-w	1.185 a-f	4.41 e-p
Regent	0.812 a-f	3.25 e-o	1.181 a-f	4.96 b-j
Acadia	0.808 a-f	2.91 e-y	0.813 j-o	5.09 b-g
Prélude	0.808 a-f	3.33 c-l	0.980 d-n	6.35 a
Canthatch	0.806 a-f	2.76 z-a_2	1.078 b-l	3.96 m-r
Vernon	0.801 a-g	2.91 l-y	1.007 c-n	4.97 b-j
Preston	0.793 a-h	3.33 c-l	1.254 a-e	4.91 b-l
Springfield	0.792 a-h	2.87 m-z	0.793 l-o	3.43 q-s
Fielder	0.775 a-i	2.70 z-b_2	0.797 k-o	3.18 r-s
Alondra 's'	0.774 a-i	3.93 a	1.003 c-n	4.64 b-p
Sonora 64	0.774 a-i	2.49 z-b_2	0.887 f-r	2.87 s
Laval 19	0.771 a-i	2.81 z-a_2	.916 f-n	5.10 b-f
Chester	0.770 a-i	2.93 k-y	0.998 d-n	4.33 e-q
Milton	0.768 a-i	2.65 z-b_2	0.920 f-n	5.03 b-h
Dundas	0.767 a-i	2.45 a_2-b_2	1.012 c-n	4.61 b-p
Norquay	0.766 a-i	3.28 d-n	1.072 b-l	4.43 e-p
Huron	0.766 a-j	3.16 g-s	0.957 e-n	5.03 b-h
PF 7748	0.762 b-j	3.32 c-l	0.906 f-n	4.32 e-q
Kenya Kongoni	0.761 b-j	3.31 e-q	1.036 c-m	3.85 o-r
Pitic 62	0.759 b-j	3.40 b-j	0.913 f-n	4.62 b-p
Lee	0.752 b-k	3.00 j-x	0.971 e-n	4.12 h-q
Lake	0.751 b-k	3.09 h-v	1.031 c-m	5.04 b-h
Glenlea	0.748 b-k	3.11 g-v	1.064 b-l	4.68 b-p
Red Bobs	0.747 b-k	3.05 i-x	1.047 b-m	4.38 e-p
Selkirk	0.747 b-k	2.77 z-a_2	1.005 c-n	4.03 k-r
Thatcher	0.741 b-k	3.29 c-m	1.062 b-m	4.57 b-p
Leader	0.740 b-k	3.11 g-v	1.063 b-m	4.35 e-p
Canuck	0.734 b-l	2.79 z-a_2	0.937 f-n	4.16 h-q
Coronation II	0.733 b-l	3.67 a-d	0.888 f-n	5.13 b-f
Stanley	0.733 b-l	3.15 g-s	1.388 a	4.67 b-p
Pioneer	0.730 b-l	2.65 z-b_2	1.071 b-l	5.42 b-c
Saunders	0.728 b-l	3.11 g-v	0.951 e-n	3.98 m-r
Cascade	0.728 b-l	3.70 a-c	0.893 f-n	4.76 b-o
Ceres	0.727 b-l	3.34 c-k	0.973 e-n	5.48 b
BH 1146	0.727 b-l	3.22 e-p	0.887 f-n	4.17 g-q
Quality A	0.727 b-l	3.17 g-r	1.002 c-n	4.67 b-p
Benito	0.726 b-l	2.85 z-a_2	0.755 m-o	3.99 l-r

Kota	0.724 b-l	3.09	h-v	1.119 a-j	5.36	b-d
Columbus	0.722 b-l	3.51	b-h	0.948 e-n	4.50	d-p
Manitou	0.722 b-l	3.08	i-v	1.047 b-m	4.05	j-r
Ruby	0.722 b-l	3.05	i-x	1.151 a-h	4.63	b-p
Ladoga	0.722 b-l	3.52	b-g	1.025 c-m	5.41	b-d
Garnet	0.721 b-l	3.67	a-d	0.852 h-n	6.21	a
HY 320	0.718 c-l	3.77	ab	1.003 c-n	4.23	e-q
Red Bobs 222	0.714 c-l	3.42	b-i	1.093 b-l	4.80	b-n
Cinquentenario	0.713 c-l	3.17	g-r	1.044 b-m	4.43	e-p
Hard Red Calcutta	0.712 c-l	2.57	z-b_2	1.003 c-n	4.13	h-q
Kenhi	0.710 c-l	2.74	z-a_2	1.163 a-g	3.95	m-r
Cypress	0.703 d-l	2.87	n-z	0.937 f-n	3.94	m-r
Park	0.702 d-l	3.37	c-j	0.982 d-m	4.93	b-k
Redman	0.700 d-l	2.87	m-z	1.281 a-d	4.63	b-p
Chinook	0.699 d-l	2.32	bz	1.080 b-l	4.05	j-r
Pembina	0.697 d-l	3.13	g-r	1.018 c-m	4.99	b-i
Rescue	0.695 d-l	3.10	g-v	1.084 b-l	4.58	b-p
Lemhi 62	0.694 d-l	3.19	f-q	0.925 f-n	4.64	b-p
Marquis	0.686 f-l	3.09	i-v	0.861 g-o	4.66	b-p
Reliance	0.685 f-l	2.71	z-b_2	1.112 a-j	4.03	k-r
Lemhi 53	0.677 f-l	3.21	f-q	0.961 e-n	4.53	c-p
Katepwa	0.673 f-l	3.13	g-t	0.895 f-n	4.59	b-p
Napayo	0.672 f-l	2.79	z-a_2	0.855 h-o	3.82	p-r
Bishop	0.660 g-l	3.09	i-v	0.860 g-o	4.85	b-m
Apex	0.654 h-l	2.87	n-z	0.828 j-o	4.91	b-k
Neepawa	0.647 k-l	2.92	k-y	1.113 a-j	4.59	b-p
Maringa	0.621 k-l	3.27	d-o	0.575 o	2.83	s
Canus	0.611 k-l	2.64	z-b_2	0.935 f-n	4.07	i-q
Sinton	0.592 l	2.73	z-a_2	0.710 n-o	3.81	q-r
Mean	0.744	3.10		1.003	4.52	

1 Means followed by the same letter are not significantly different at the 5% level of significance as determined by Duncan's Multiple Range Test.