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UNIVERSITY OF ALBERTA

Studies on the origin, screening methodologies, and inheritance of manganese tolerance in spring wheat (*Triticum aestivum* L.)

BY

Juan Sergio Moroni

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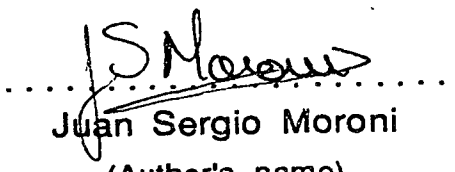

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"In the temple of science are many mansions . . . and various indeed are they that dwell therein and the motives that have led them there.

Many take science out of joyful sense of superior intellectual power; science is their own special sport to which they look for vivid experience and the satisfaction of ambition; many others are to be found in the temple who have offered the products of their brain on this altar for purely utilitarian purposes. Were an angel of the Lord to come and drive all the people belonging to these two categories out of the temple, it would be noticeably emptier but there would still be some men of both present and past times left inside . . . If the types we have just expelled were the only types there were, the temple would never have existed any more than one can have a wood consisting of nothing but creepers . . . those who have found favour with the angel . . . are somewhat odd, uncommunicative, solitary fellows, really less like each other than the host of the rejected.

What has brought them to the temple . . . no single answer will cover . . . escape from everyday life, with its painful crudity and hopeless dreariness, from the fetters of one's own shifting desires. A finely tempered nature longs to escape from his noisy cramped surroundings into the silence of the high mountains where the eye ranges freely through the still pure air and fondly traces out the restful contours apparently built for eternity".

Albert Einstein
Berlin, May 1918.

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studie and Research for acceptance, a thesis entitled **Studies on the origin, screening methodologies, and inheritance of manganese tolerance in spring wheat (*Triticum aestivum* L.)**, submitted by **Juan Sergio Moroni** in partial fulfillment of the requirements for the degree of **Master of Science in Plant Breeding.**

K. G. Briggs
(Supervisor)

G. J. Taylor

M. Nyborg

Date: July 26, 1991

To my mother,
Jacoba.
Single mother, independent woman.

Abstract

After aluminum (Al) toxicity, manganese (Mn) toxicity is probably the world's second most important growth limiting factor in acid soils. Breeding wheat (*Triticum aestivum* L.) for tolerance to Mn might be in some cases more feasible and economical than use of soil amendments. This study was conducted (1) to determine the level of Mn tolerance in Canadian wheat cultivars and its probable origin, by relative root weight (RRW) estimates in solution culture, and by analysis of cultivar pedigrees and drawing of phylogenetic maps to discern filial relationships, (2) to develop a rapid, seedling based, screening bioassay for Mn tolerance by testing several physiological parameters, and (3) to determine the inheritance and estimate genetic effects on Mn tolerance using the progeny generation mean analysis method of progeny generations from five cultivars (Norquay, Laura, Oslo, Columbus, and Katepwa) crossed in all combinations, excluding reciprocals.

A range of tolerance to Mn among Canadian cultivars was observed. Manganese tolerance appears to have originated from the Brazilian land races Polyssu (= Ponta Grossa 142) and/or Alfredo Chavez 6.21. The differential response in chlorophyll concentration of Mn-stressed seedlings and leaf elongation rate (LER) of seedling regrowth of cultivars differing in Mn tolerance, as well as the significant correlation of these parameters with Mn tolerance assayed by the relative root weight methodology (RRW) indicates the suitability of chlorophyll content or LER for screening seedlings tolerant to Mn toxicity. The continuous frequency distribution of segregating generations which indicated differential tolerance to Mn toxicity, the similarity of the F_1 and F_2 means, and high levels of additive gene action indicated quantitative inheritance of Mn tolerance. Furthermore, heritability and gene effects estimates indicated that the genetic control of Mn tolerance in cv Norquay and cv Laura may be different. A preponderance of additive effects coupled with high heritability and small dominance (potence ratio) estimates indicate that selection for Mn tolerance should be effective in early generations, particularly where cv Norquay is used as the Mn tolerant parent. This information will help breeders to develop plant breeding systems, and may also help in the study of the mechanisms for Mn tolerance in wheat.

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Chapter I

I.1. Introduction

Of the worlds non-irrigated arable lands, nearly half are acid (Clark, 1982), and manganese (Mn) toxicity is probably the second most important growth-limiting factor after aluminum toxicity in acid soils (Foy, 1984a). In some Canadian soils, soil acidity is becoming a major factor limiting crop growth. Of the cultivated land of Alberta and the Peace River region of British Columbia there are an estimated 328,000 ha of strongly acid soils (pH < 5.5) and about 1,600,000 ha of moderate acid soils (pH range of 5.6 to 6.0) (Penny *et al.*, 1977). Furthermore, the use of fertilizers, particularly ammonia-based nitrogen which has a strong acidifying action, is rapidly increasing and is the major cause of acidification in Western Canada (Hoyt *et al.*, 1981). It has been projected that 25% of the soils in Alberta could be acid by 1985 (Hoyt *et al.*, 1981). While technology is readily available to detect and correct nutrient toxicity problems where they exist, economics become a significant factor with the rising cost of amendments (Miller, 1983). In the past, an approach to soil fertility problems has emphasized "changing the soil to fit the plant", but a more economical approach to soil stress problems emphasizes "tailoring the plant to fit the soil" (Foy, 1983a).

Although incorporation of Mn tolerance into Canadian wheat cultivars has not yet been consciously implemented, selection of Mn tolerant Canadian cultivars has been recently reported (Macfie *et al.*, 1989). The possibility of exploiting genotypic differences within cultivated wheat in a breeding program may improve the advantage to producers wishing to maximize efficiency of resource utilization. The objectives of this study were (1) to determine the extent and origin of Mn tolerance in selected Canadian spring wheat cultivars, (2) to develop a rapid seedling based screening bioassay for Mn tolerance which is suitable for use in segregating populations of wheat, and (3) to determine the mode of inheritance of tolerance to Mn toxicity in wheat. The lack of knowledge in these three areas has hampered development of a suitable breeding program for selection of wheat cultivars tolerant to Mn toxicity. This has been further hampered by the complex and diverse role and effects of Mn in plants.

No attempt is made in this chapter to review all the available literature of Mn toxicity on plants, nor of wheat in particular; rather the complexity of Mn toxicity in plants is demonstrated. Brief reviews of the literature concerning the origin and range of Mn tolerance in wheat, the screening methodologies used for selecting Mn tolerance of field crops, and the inheritance of Mn tolerance of plants are presented in Chapter II,

Chapter III, and Chapter IV, respectively. For more detailed and recent information on the role and effects of Mn in plants see Foy *et al.* (1978), Foy (1984a; 1983a, b), Kamprath and Foy (1985), and Graham *et al.* (1988).

I.2. Literature review

I.2.a. Manganese soil toxicity

The first report of Mn toxicity in crop plants was published in 1909 by Kelly, who described Mn toxicity symptoms for pineapple in manganiferous soils of the drier regions of the Hawaiian islands (cited in Mulder and Gerretsen, 1952). Since then, several reports of Mn toxicity have been reported from the tropics, sub-tropics and temperate regions of the world (Schilchting and Sparrow, 1988). In global terms, Dudal (1976) related Mn toxicity mainly to Acri- and Ferralsols (especially on ultrabasic rocks), and acid Nito- and thionic Fluvisols (FAO-Unesco, 1974). Wambeke (1976), on the other hand, emphasized Oxi(=Ferral-) more than Ultisols (=Acri- and Nitosols), and also mentioned Alfisols (=Luvisols).

In North America Mn toxicity problems have been reported for several crop species in several different soils. Bortner (1935) reported Mn toxicity for tobacco in poorly drained soils and soils on limestone. Adams and Pearson (1967), and Adams (1984), reported Mn toxicity in tobacco and cotton in soils on limestone in Ultisols of the older land surfaces. Foy and Campbell (1984b) described Mn toxicity for several species in Fragiudalfs. Snider (1943) reported problems for several species in Illinois in soils characterized by low pH and exchangeable bases and high exchangeable Mn in the profiles. Hati *et al.* (1979) reported Mn toxicity problems for several species, especially cotton, in Missouri, and Mclean and Brown (1984) in an acid Hapludoll of Kansas. Moraghan (1979) described a case where flax suffered from Mn toxicity in Calcia quolls of North Dakota low in available Fe. Evidence in field experiments was given by Lee and McDonald (1977) for potatoes in a loamy Ultisol (Acri- or Nitosol) by Gupta *et al.* (1973), and by Prausse *et al.* (1972) for potatoes and barley in loamy Brown-earths and Stagnogleys soils. In Canada, Mn toxicity problems have been reported for barley on a sandy-loam Podzol in New Brunswick, for apple orchards in the Okanagan Valley of British Columbia (Fisher *et al.*, 1977), and for crops being rotated with potatoes, in which the potatoe fields associated with strongly acidic pH received low lime application as a measure of disease control (France, 1986, cited by Stokes, *et al.*, 1988).

Although reports of Mn toxicity have come from diverse areas of the world (Schlichting and Sparrow, 1988), the information indicates that these problem soils have unique physical and chemical characteristics of specific ecology and are not wide spread in term of land area. On the other hand, the availability and the potential toxicity of Mn to a given crop depends on many soils properties, including total Mn content, pH, organic matter level, aeration, and microbial activity (Foy, 1973; Stahlberg *et al.*, 1976). Manganese toxicity generally occurs in soils with pH values of 5.5 or below if the soil contains sufficient total Mn. It can also occur at higher soil pH values in poorly drained or compacted soils where reducing conditions favor the production of divalent Mn (Foy, 1984). Manganese toxicity is frequently induced or intensified by ammonia-based nitrogen fertilization, which lowers the pH, or by fumigation with steam or methyl bromide, air -drying, or flooding (Nelson, 1977; Kluthcouski and Nelson, 1979). The addition of organic matter can reduce Mn toxicity, probably by chelating excess divalent Mn that the plant could otherwise absorb (Masui and Ishida, 1975). The availability of Mn in soils is closely related to the activities of microorganisms that can oxidize the soluble and toxic divalent Mn to the tetravalent, nontoxic form (Kamura and Nishitaani, 1977; Bromfield, 1979). Sidorenko *et al.* (1979) reported that *Metallogenium* Perf. oxidized Fe and Mn on rice roots. These reports indicate that Mn toxicity not only depends on the Mn soil content but also on the different factors which would affect its availability to plants. Thus, theoretically, all soils can be changed into Mn toxic ones by increasing Mn reserves and/or their availability (Schlichting and Sparrow, 1988).

I.2.b. Uptake and functional role of manganese

Manganese is readily taken up and transported from the roots to the shoots (Nable and Loneragan, 1984). There is general agreement that divalent Mn (II) is the prevailing source of Mn at the root surface and is the one species taken up by the plant (Clarkson, 1988). The mobility of Mn in plants is not yet understood (Loneragan, 1988). Mobility of Mn in the phloem appears to be species-dependent, while in the xylem of all species, primarily as a divalent ion, Mn is readily mobile (El-Baz *et al.*, 1990).

Manganese has a role in many biochemical processes in plants, but unlike other essential trace elements, Mn usually acts as an activator of enzymes and is often able to be replaced by other metal ions. Manganese resembles Mg in its biochemical function and is involved in activating enzyme catalyzed reactions including phosphorylation, decarboxylation, reduction and hydrolysis reactions and therefore affects processes such

as respiration, amino acid synthesis, lignin biosynthesis and the level of hormones in plants (Burnell, 1988).

The average concentration of Mn in the cytoplasm is about 100 µM (Burnell, 1988). A significant proportion of total cell Mn is involved in dissociable metal-activated systems requiring relative high (0.1-1.0 mM) concentrations of ionic metal (Burnell, 1988). A pool of tightly bound Mn occurs in the chloroplast where amounts of 6 atoms per 400 molecules of chlorophyll is found (Chanie, 1970). Assuming a chlorophyll concentration of 1.8 mM, the bound Mn is equivalent to about 30 µM (Burnell, 1988). The Mn-containing superoxide dismutase may contribute about 1% of the bound Mn (Sevilla *et al.*, 1980).

Although a relatively large number of enzymes are activated by Mn ions, to date only three Mn-containing enzymes have been reported. These include the photosynthetic Mn-containing complex, the Mn-containing superoxide dismutase and the Mn-containing acid phosphatases (Burnell, 1988). There has been a report of a metalloprotein dependent on Mn in peanuts, named manganin (MW 56,000-58,000). This protein has been isolated and contains one atom of Mn, but its function is unknown (Diekert and Rozacky, 1969).

Physiological functions of Mn in plants are affected adversely by either deficient or toxic levels of Mn. It is use of these extremes of Mn nutrition that have been largely responsible for elucidating its physiology (Campbell and Nable, 1988). The physiology of Mn in plants is governed by the chemistry of Mn, particularly the configuration of the electrons in the 'd' shell (Hughes and Williiams, 1988). When Mn is in the Mn(II) oxidation state there is one unpaired electron in each of the five 'd' orbitals *i.e.* the stable, energetically favoured 'd^5' configuration is attained (Bartlett, 1988). Two consequences of this configuration are (i) Mn is a relatively weak ligand and (ii) Mn has the potential to form compounds in several oxidation states (Campbell and Nable, 1988). These factors have contributed to the difficulties in unraveling the physiology of Mn. Thus, many functions have been derived by inference (Campbell and Nable, 1988).

According to Campbell and Nable (1988), Mn has a profound influence on three particular physiological (metabolic) functions: (i) photosynthesis, particularly electron transport in photosystem II, photodestruction of chlorophyll and chloroplast structure, (ii) N metabolism, especially the sequential reduction of nitrate, and (iii) aromatic ring compounds as precursors for aromatic amino acids, hormones (auxins), phenols and lignins.

I.2.c. Manganese toxicity symptoms and effects

Because Mn is readily taken up and transported from the roots to the shoots (Nable and Loneragan, 1984), Mn is generally less rhizotoxic than other metals. Symptoms of Mn toxicity occur first on the shoot (Brown and Devine, 1980), however, when high concentrations of manganese are applied to roots in order to accelerate plant response, root growth may be affected directly (Wong and Bradshaw, 1982; Macfie *et al.*, 1989) and alterations in shoot metabolism may then be a secondary effect (Horst, 1988). Inhibition of root growth is accompanied by brown discoloration indicative of accumulation of oxidized Mn (MnO_2) on the root surface and oxidized phenolics in root cortical cells (Keil *et al.*, 1986; Macfie and Taylor, 1989).

The extent of injury from Mn toxicity is generally proportionately to the concentration of excess Mn accumulated (Fales and Ohki, 1982). Although there are no typical Mn toxicity symptoms in shoots, three major groups of symptoms may be distinguished (Horst, 1988). The first group includes symptoms of physiologically old, non-growing plant tissues (stem, petioles, leaves). These symptoms appear as small distinct dark-brown speckles (Elamin and Wilcox, 1986a; Riedell and Schmid, 1986; Bussler, 1958; Horst and Marschener, 1978), necrotic lesions ('stem streak necrosis of potato' (Berger and Gerloff, 1947), 'internal bark necrosis of apple' (Eggert and Hayden, 1970)), chlorotic spots (Elamin and Wilcox, 1986b), leaf-margin and leaf-tip chlorosis (Blatt and van Diest, 1981). Leaf shedding is also known from other plant species (*e.g.* abnormal defoliation of Satsuma mandarin (Aoba, 1986) and potato (Andrees, 1971)). The second group of symptoms are growth inhibition and distortion of young expanding leaves. These symptoms are commonly known as 'crinkle leaf'. They have been described for cotton (Neal, 1937; Adams and Wear, 1957), soybean (Parker *et al.*, 1969; Heena and Carter, 1975) and bush bean (Horst and Marschener, 1978b). The third group of symptoms is chlorosis of young expanding leaves. This symptom has been interpreted as Mn-induced Fe deficiency (Amberger *et al.*, 1982; Clark *et al.*, 1981). All symptoms may occur on the same plant simultaneously (Kohno *et al.*, 1984).

The relative importance of these symptoms is dependent on the genotype, both inter- and intra-specific (Moris and Pierre, 1949; Foy, 1984a), and growing conditions affecting Mn tolerance of the plant tissue. While a wide range of Mn toxicity symptoms have been reported, which depend on plant genotypes, soil/soil factors interactions and other ecological factors, Mn toxicity does not necessarily inhibit production of biomass (Schlichting and Sparrow, 1988).

2.d. Manganese toxicity and environmental interactions

Climatic factors such as photon flux intensity and temperature modulate the severity of expression of injury from Mn toxicity. Plant tolerance to high levels of applied Mn increased with increasing temperature, despite significantly greater tissue concentrations of Mn during plant growth (Rufty *et al.*, 1979; Nelson, 1982). It has been proposed that the increased tolerance is associated with more rapid rates of leaf expansion accompanied by increased vacuolar capacity for disposal of accumulated Mn (Rufty *et al.*, 1979). The temperature effect on Mn toxicity, however, is further complicated by the interaction with photon flux intensity. McCool (1935) reported that as light intensity decreased, visible injury to plants grown in Mn-treated soil decreased. He ascribed the effect of light on the plants to stimulation of Mn absorption because the percentage of Mn in the leaves decreased with the decrease in light intensity. Horiguchi (1988b) working with bush bean and corn, also reported that an increase in light intensity increased the Mn uptake by the plant at high levels of Mn in nutrient solution, and resulted in a decrease in the chlorophyll content of the leaves. Even at similar levels of Mn within the leaves, high light intensity increased the severity of Mn-induced chlorosis. Horiguchi (1988b) suggested that high light intensity stimulates not only Mn uptake by the plant but also the destruction of chlorophyll when Mn is in excess.

The effects of temperature and photon flux intensity on Mn toxicity indicate that the 'critical concentration' for expression of toxicity symptoms is not constant and exhibits large variability when the temperature and photon flux intensity environment is altered. It follows that the use of 'critical concentration' in prediction models of Mn toxicity must be limited to plants with a similar genetic background, grown in similar temperature and photon flux intensity environments. More practically, critical concentrations should be characterized at specific temperature and photon flux intensities.

Along with the effect of temperature and light intensity on Mn toxicity in plants, a third major interaction which has been reported is the amelioration of Mn toxicity by silicon (Lewin and Reimann, 1969; Okuda and Takahashi, 1962a,b; Williams and Vlamis, 1957; Vlamis and Williams, 1967). Silicon supply alleviates the Mn toxicity of rice plants not only by decreasing the Mn uptake by plant but also by increasing the internal tolerance to an excessive amount of Mn in the tissues (Horiguchi, 1988a). Whether this is a wide spread phenomenon in nature has not been reported since studies have been conducted under laboratory conditions.

I.2.e. Hypotheses concerning manganese toxicity mechanisms

Two general hypotheses have been proposed to explain the physiological disorders caused by Mn toxicity. First, numerous workers have postulated that the symptoms of Mn toxicity reflect a Mn/Fe interaction thereby leading to physiological disorders as a consequence of limitation of uptake/utilization of Fe (Foy *et al.*, 1978; Foy, 1984a). Second, several workers have postulated that excess Mn accumulation results in increased peroxidative destruction of IAA, increased synthesis of ethylene (Fowler and Morgan, 1972; Morgan *et al.*, 1966; Morgan *et al.*, 1976; Sirkar and Amin, 1974) and subsequent acceleration of senescence processes. Recently, Houtz *et al.*, (1988) presented evidence that the inhibiting effects of Mn excess on photosynthesis result from a Mn-induced modification of the kinetic properties of Rubisco activity.

I.2.f. Hypotheses concerning manganese tolerance mechanisms

Three mechanisms for the tolerance of plants to Mn toxicity have been proposed. (1) Evidence exists for the exclusion of Mn uptake operating as a Mn tolerance mechanism in certain species and some cultivars (Helyar, 1978). (2) Evidence also exists for the restriction of Mn transport from roots to shoots (Oulette and Dessureaux, 1958; Andrew and Hegarty, 1969; Robson and Loneragan, 1970; Edwards and Asher, 1982). (3) Tolerance of shoots to high internal Mn concentrations (Foy, 1984a) is the most widely discussed mechanism in the literature and has been suggested as a mechanism for tolerance in wheat (Foy *et al.*,) as well as for several other crop species (Brown and Devine, 1980; Heenan and Campbell, 1981; Foy *et al.*, 1973; Foy *et al.*, 1981; Horst, 1983). Internal tissue tolerance to Mn may be due to: (a) the formation of metabolically inactive, organic manganese complexes (Foy, 1984a); (b) binding to cellwalls and/or deposition in vacuoles (Helyar, 1978; Pfeffer *et al.*, 1986); and (c) tolerance by some vital enzyme systems of high concentrations of ionic manganese (Scott *et al.*, 1987).

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Chapter II

Pedigree analysis of the origin of manganese tolerance in Canadian spring wheat (*Triticum aestivum* L.) cultivars.[1]

II.1. Introduction

Nearly half of the world's non-irrigated arable soils are acidic (Clark, 1982). After aluminium toxicity, manganese (Mn) toxicity is probably the second most important growth limiting factor in acid soils (Foy, 1984). Fortunately, interspecific and intraspecific differences in tolerance to Mn have been identified among crop plants, providing potential to develop cultivars adapted to acid soils (Foy *et al.*, 1988). Breeding for tolerance to Mn might be in some cases more feasible and economical than the use of soil amendments. For example, Mn toxicity can be a problem in subsoils (Bromfield *et al.*, 1983), thus making soil amendment difficult and expensive. In addition, extreme climatic conditions (*eg.* water logging; dry, hot conditions) can lead to Mn toxicity on limed soils (Siman *et al.*, 1974) and near-neutral soils (Grasmanis and Leeper, 1966).

Demonstration of genotypic variability and suitable screening techniques are needed prior to formulation of a breeding program (Devine, 1982). In wheat (*Triticum aestivum* L.), relatively few reports have documented a range of tolerance to Mn (Neenan, 1960; Foy *et al.*, 1973; Brauner and Sarruge, 1980; Camargo and Oliveira, 1983; Scott *et al.*, 1987; Macfie *et al.*, 1989). Most of these reports speculate that Mn tolerance has originated from germplasm developed in Latin America or Brazil, but no specific data have been presented to support this suggestion. More detailed knowledge concerning the origin of Mn tolerance in wheat will help the plant breeder develop plant breeding systems. This knowledge may also help in the study of the mechanisms for Mn tolerance in wheat, especially for different sources of tolerance.

Macfie *et al.*, (1989) described the presence of Mn tolerance in some Canadian wheat cultivars. This study was undertaken to determine the extent of Mn tolerance and the origin of Mn tolerance in selected Canadian spring wheat cultivars. Screening of Canadian and foreign cultivars, analysis of cultivar pedigrees, and phylogenetic maps were used to discern the filial relationships of the cultivars tested and the origins of any Mn tolerances.

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II.2. Materials and methods

II.2.a. Germination

The experimental procedure used to screen for Mn tolerance in wheat (*Triticum aestivum* L.) was similar to that used by Macfie *et al.*, (1989) and by Briggs *et al.*, (1989). A total of 91 spring wheat cultivars, 76 of which were Canadian from several wheat classes (Canada Western Red Spring (CWRS), Canada Prairie Spring (CPS), Canada Utility (CU) and, Canada Western Soft White Spring (CWSWS)), were screened in this study. Seeds were surface sterilized in 1.2% sodium hypochlorite for 20 minutes and germinated overnight at room temperature in an aerated solution of the systemic fungicide Vitavax (*Carbathiin + Thiram* ; 0.005 gL^{-1}). Germinated seeds were laid on a plastic mesh, crease down, suspended over 10 L of a nutrient solution composed of (µM): Ca, 1000; Mg, 300; NO_3, 2900; and NH_4, 300; and were grown for three days at room temperature. For the first two days of germination the containers were covered with black plastic.

Three days after germination, fifty seedlings of similar size were selected and grown for an additional five days in a complete nutrient solution containing (µM): Ca, 1000; Mg, 300; K, 800; NO_3, 3300; NH_4, 300; PO_4, 100; SO_4, 101; Cl 34; Na, 20; Fe, 10; B,6; Mn, 2; Zn, 0.5; Cu, 0.15; and Mo, 0.1. Iron was supplied as Fe-EDTA prepared from equimolar amounts of $FeCl_3$ and Na_2EDTA. All nutrient solutions were adjusted to pH 4.8 with KOH and HCl, and solutions were constantly aerated. Seedlings were grown under environmental conditions similar to those described below.

II.2.b. Growth conditions during assay

Sixteen uniform, nine-day-old seedlings of each cultivar were mounted with strips of polyurethane foam on black Plexiglas frames which covered each of 60 polyethylene containers of 10 L capacity. Each frame supported eight plants in four groups of two. Nutrient solutions were shielded from light to inhibit algal growth. Plants were grown in a temperature controlled growth room at a day/night temperature of 22/17 ± 1 °C and a relative humidity of 93/58 ± 5 %, with a 16 h photoperiod. Solution temperatures were maintained at 18.0 ± 0.5 °C by immersing all containers in a common water bath. The growth room was illuminated by 12 HID mercury (400W) and 4 HID high pressure sodium (400W) lamps located 1.3 m above the plant bases. The photosynthetic photon flux density (PPFD) was 319 ± 32 µmol m^{-2} s^{-1}.

The complete nutrient solution described above was used for all treatments, except that the concentrations of NH_4 and NO_3 were increased to 600 µM and 3600 µM, respectively. The control treatment consisted of unaltered nutrient solution. For the Mn treatment, 500 µM Mn (supplied as $MnCl_2 \cdot 4H_2O$) was superimposed over the basal nutrient solution. Control and Mn treatments for each cultivar within each replicate were blocked together to minimize variation. All nutrient solutions were adjusted to pH 4.8 with KOH and HCl, and solutions were constantly aerated. Nutrient solutions were adjusted periodically to 10 L with distilled water to compensate for water loss by evaporation and transpiration. After a 14-day treatment, the plants were harvested, divided into roots and shoots, oven-dried at 75 °C for three days, and weighed.

II.2.c. Analysis

To evaluate the Mn tolerance of selected Canadian and foreign cultivars a total of four experiments were performed (*Tables II.2* to *II.5*). In the first 3 experiments, 28 cultivars and 2 standards were screened. The standards used in this study were the Mn-tolerant Norquay and the Mn-sensitive Columbus (Macfie *et al.*, 1989). The experimental design consisted of a randomized block factorial design with 30 cultivars, two Mn treatments and three replicates (totalling 180 containers). Due to space constraints threefold replication was achieved in time. The last experiment (*Tables II.5*) consisted of 6 cultivars and the 2 standards (*ie.* Norquay and Columbus). For this experiment replication was achieved in space rather than in time. Because root weight and shoot weight of the various cultivars differed under normal and toxic Mn levels, cultivar tolerance of Mn was determined by the relative root weight (RRW), and relative shoot weight (RSW) method (RRW or RSW= root or shoot weight in the presence of 500 µM Mn divided by control root or shoot weight, respectively; Macfie *et al.*,1989). Both tolerance indices (RRW and RSW) were tabulated to evaluate cultivar differences in response to Mn toxicity (*Tables II.2* to *II.5*). Data were analyzed by analysis of variance (ANOVA) and Duncan's multiple range test using the arcsine transformation for RRW and RSW. To facilitate analysis of the transformed variables the RRW values for some of the replications of Frontana (1.05), Carazinho (1.02) and Lerma Rojo (1.01) were transformed to 1.00. Significance was defined at the 95% confidence level. Tolerance to Mn was determined by using RRW and was arbitrarily defined as follows: Mn-sensitive cultivar RRW $\leq$ 0.40; cultivar intermediate in tolerance to Mn 0.40 < RRW $\leq$ 0.70; Mn-tolerant cultivar RRW > 0.70.

II.2.d. Data tabulation

Results of the four experiments are presented in *Tables II.2* to *II.5*. In addition, *Table II.1* indicates the RRW and RSW of 30 wheat cultivars screened by Macfie *et al.* (1989) using the same experimental conditions. All cultivars screened by Macfie *et al.* (1989) were of spring habit except for Atlas 66, Monon, and Scout 66, which were of winter habit. In addition, each table indicates the pedigree of each cultivar, the country where the cultivar was developed, and the year in which the cultivar was released in the country where the cultivar was developed. The cultivar pedigrees were mainly drawn from references containing large wheat pedigree compendiums and, when possible, were expressed using the nomenclature system recently adopted by CIMMYT (Villareal and Rajaram, 1988). If information was compiled from more that one reference each source is cited.

II.2.e. Methodology of map drawing for the determination of origin of Mn tolerance

The RRW of the 91 cultivars screened in this study (*Tables II.2* to *II.5*), and the RRW of 28 of the 30 cultivars screened by Macfie *et al.* (1989) (*Table II.1*), were used to develop two filial pedigree maps (*Fig. II.1* and *Fig. II.2*) outlining the sources of Mn tolerance observed in the Canadian germplasm. For discussion purposes, the screened cultivars (119) were divided into two groups. The first group (93 cultivars) are referred to in the pedigree map analysis as *"Canadian"* material and consist of cultivars selected and/or developed in Canada (74) plus cultivars which have been developed and/or released in other countries prior to release in Canada (19). The second group (26) is composed of foreign cultivars which have not been released in Canada and are from Argentina (1), Australia (1), Brazil (11), Kenya (4), Mexico (6), and the USA (3).

The first pedigree map (*Fig. II.1*) was developed using the *"Canadian"* material to determine the source of Mn tolerance. For cultivars with a RRW $\geq$ 0.60 a special effort was made in tracing their pedigrees to determine if they have common ancestral parents. Most *"Canadian"* wheat cultivars were easily incorporated into the phylogenetic map (*Fig. II.1*), with the exception of the Mn-sensitive cultivars Max (0.24), Cassavant (0.17), Mondor (0.15) and the cultivar Belvedere (0.52) of intermediate Mn tolerance. Based on the genealogical analysis and interpretation of *Fig. II.1*, a pedigree map for two Mn-tolerant Canadian cultivars (*ie.* Norquay and Laura) was developed to trace the probable origin of the Mn tolerance trait (*Fig. II.2*). Seed for key cultivars in

the phylogenetic map (*Fig. II.2*) was obtained where possible, and was screened for Mn tolerance. The order of the crosses in each pedigree was not considered, and maternal effects were not taken into consideration in drawing either map. Country of origin, year released and/or developed, and RRW (where screened) are indicated on the maps. For further clarification, cultivars screened were surrounded by a thick-line margin. Some cultivars are repeated in *Fig. II.1* for purposes of simplification are indicated with an asterisk (*).

II.3. Results and discussion

II.3.a. Plant symptoms

A diverse range of Mn toxicity symptoms were observed among the cultivars, including leaf chlorosis, stunting, stiffness in leaf tissue, necrotic leaf spots, white flecking, leaf tip burn and, occasionally, leaf purpling. These symptoms were similar to those previously described by Keisling *et al.* (1984) and Ohki (1984). Symptoms were most apparent on the sensitive cultivars but, necrotic leaf spots were observed in some of the Mn-tolerant cultivars from Brazil. Obvious Mn toxicity symptoms were not observed in the roots except that some cultivars (*eg.* Hard Red Calcutta) showed brown discoloration. This root discoloration may reflect precipitation of Mn on the root surface, which varies with the plant-induced pH of the nutrient solution, and may not therefore be a direct symptom of Mn toxicity (Macfie and Taylor, 1989).

II.3.b. Differential response of Mn tolerance

The RRW and RSW of the Mn-tolerant and the Mn-sensitive standards (Norquay and Columbus, respectively) were not significantly different ($p \leq 0.01$) between experiments. This similarity across experiments indicated the consistency of the environmental conditions during the assay and the reliability of the standards themselves. It also allowed direct comparison of RRW and RSW values between experiments. Oneway ANOVAs for RRW and RSW indicated significant main effects due to cultivar. Because the RRW and RSW are expressed as growth with toxic level of Mn as a fraction of growth with a normal level of Mn, analysis of treatment effects were not appropriate.

A broad range of differential response to Mn stress was detected among cultivars. The range for RRW observed was 0.08 to 1.05 while for RSW it ranged from 0.27 to

0.98 (*Tables II.2* to *II.5*). The majority of the Canadian cultivars were sensitive or intermediate in tolerance to Mn. For RRW the range was 0.08 to 0.88, and for RSW it ranged from 0.27 to 0.89 (*Tables II.2* to *II.5*). Only 3 of the 76 Canadian cultivars screened in this study were Mn-tolerant (RRW > 0.70), namely Biggar (0.88), Laura (0.92), and Norquay (0.83). Among the remaining, 19 cultivars were intermediate in Mn tolerance (0.40 < RRW ≤ 0.70), while 54 cultivars were Mn-sensitive (RRW ≤ 0.40). The predominance of Mn sensitivity among Canadian cultivars is in agreement with the results reported by Macfie *et al.* (1989).

Of the 16 foreign cultivars screened in this study (*Tables II.2* to *II.5*), 8 were Mn-tolerant (RRW > 0.70), 3 were intermediate in Mn tolerance (0.40 < RRW ≤ 0.70), and 5 were Mn-sensitive (RRW < 0.40). Foreign entries determined to be Mn-tolerant were the Brazilian cultivars Frontana (1.05), Carazinho (1.02), Veranopolis (0.85), Polyssu (= Ponta Grossa 142) (0.83), Frondoso (0.77), and Cotipora (0.71), and the Mexican cultivars Lerma Rojo (1.01) and Yaktana 54 (0.93). The ranking of Mn-tolerant cultivars was comparable to results reported by several others. For example, among the Mn-tolerant cultivars screened in this, study Scott *et al.* (1987) reported Cotipora and Carazinho (Brazil) as among the "most Mn-tolerant cultivars", and Foy *et al.* (1988) reported Lerma Rojo (Mexico) as being Mn-tolerant. On the other hand, Brauner and Sarruge (1980) reported Frontana (Brazil) to be one of two Mn-sensitive cultivars among 30 cultivars screened, in contrast to the tolerance found in the present study. The results of Brauner and Sarruge (1980) are rather surprising considering that Frontana was widely grown in the southern states of Brazil (a region dominated by acid soils) for several years after its introduction in 1943 (Hettel, 1989).

The range of Mn-tolerance found in the Canadian cultivars was similar to that found in the Brazilian cultivars screened in this study. Three of the Canadian cultivars showed growth under conditions of Mn stress which was greater than 70% of control, a level similar to that reached by several of the Mn-tolerant Brazilian cultivars. It is likely that the mechanism (s) of Mn tolerance have been conserved in the Canadian cultivars. Although Mn tolerance in wheat has been reported to be controlled by a few major genes (Scott and Fisher, 1989), the wide range of response to Mn toxicity among the wheat cultivars screened in this study and those screened by Brauner and Sarruge (1980) and Macfie *et al.* (1989) indicate the possibility that many genes could play a role in determing tolerance to Mn and that Mn tolerance may be a quantitative trait (see Chapter IV).

The predominance of Mn sensitivity found in Canadian cultivars is not surprising. Although acid soils exist in Canada (Miller, 1983), Mn toxicity in cereals has not been reported, nor has a deliberate effort been made to select for Mn-tolerant cultivars. What is surprising, however, is that some of the Canadian cultivars were found to be Mn-tolerant even in the absence of an obvious and deliberate selection pressure for Mn tolerance. Of course, agronomic practices might have been conducive to a Mn toxic environment (*ie.* poorly drained, periodically flooded soils; compacted soils; nitrogen fertilization (see Foy, 1984)). However, it has not been established which of those factors could have provided a substantive selection pressure.

Scott and Fisher (1989) suggested that plants tolerant of acid soils (*ie.* aluminum- (Al) tolerant and/or Mn-tolerant) may be poorly adapted to non-acid soils. This hypothesis arose from the comparison of old acid-tolerant cultivars (which were low yielding) and more recent high yielding cultivars which have not been bred for acid tolerance. As demonstrated by the CIMMYT/Brazil wheat breeding program for acid soils, this is not the case in cultivated wheat (Hettel, 1989; Kohli and Rajaram, 1988). High yielding, Al-tolerant wheat cultivars have been already developed and released. Furthermore, should manganese tolerance be a disadvantage on a non-acidic soil, it would be expected that selection pressure would be towards Mn-sensitive cultivars in non-acid areas. The development and release of high yielding, Mn-tolerant, Canadian cultivars and the release of Sunstar, a Mn-tolerant cultivar from Australia, which has been selected in northern New South Wales in the absence of Mn toxicity (Scott and Fisher, 1989), suggests that this has not occurred. Similarly, a few high yielding, Al-tolerant, wheat cultivars from Canada (Briggs *et al.*, 1989) and Australia (Scott and Fisher, 1989) have been developed in the absence of direct selection for Al tolerance.

II.3.c. Source of Mn tolerance

Pedigree analysis of the *"Canadian"* cultivars (*Fig. II.1*) indicated that cultivars released and/or developed prior to the 1960's (45 cultivars) did not have Latin American germplasm in their background. Most of the parental germplasm of these cultivars is of European, Australian and or North American origin. Of these cultivars, 40 were found to be Mn-sensitive and 5 were found to be intermediate in Mn tolerance. None were found to be Mn-tolerant. On the other hand, most cultivars developed and/or released after the 1960's (*ie.* 47 cultivars) have some parental germplasm from Latin America. This parental germplasm is mainly of Mexican and/or Brazilian origin. Many of the Mexican cultivars are direct descendants of Brazilian germplasm, or have some

Brazilian germplasm in their lineage. Of these cultivars, 30 were found to be Mn-sensitive, 14 were found to be intermediate in Mn tolerance, and 3 were found to be Mn-tolerant.

The era of introduction and appearance of Mn tolerance in *"Canadian"* germplasm is distinct. It was not until the 1960's that cultivars intermediate in Mn tolerance appeared in the *"Canadian"* germplasm in relatively high frequency. Manganese-tolerant cultivars appeared in the 1970s and 1980s. This can be clearly seen in *Fig. II.1*, where most pre-1960s cultivars (found towards the bottom of *Fig. II.1*) are related to each other through European, Australian and/or North American germplasm. The post-1960s cultivars (found towards the upper part of *Fig. II.1*) are mainly related through the Latin American cultivars. The timing of the appearance of intermediate and high Mn tolerance coincides directly with the release of cultivars with parental germplasm of Brazilian and/or Mexican origin with known tolerance to acid soils. Amongst these are the Brazilian cultivars widely used by CIMMYT during the "green revolution' (*eg.* Frontana, Surpresa). These cultivars are from the wheat growing regions of southern Brazil which are dominated by acid soils.

II.3.d. Origin of Mn tolerance

Although Mn tolerance appear to be a quantitative trait (see Chapter IV), the inheritance of Mn tolerance has been reported to be relatively simple (Foy *et al.* 1988). Thus the origin of Mn tolerance can be demonstrated by pedigree analysis of the Mn-tolerant Canadian cultivars Norquay and Laura (*Fig. II.2*). Seed for most of the key cultivars in the pedigrees of these two cultivars was obtained and screened (with the exception of Supremo 211, Surpresa, and Alfredo Chavez 6.21). Norquay (0.83) most likely inherited tolerance from Lerma Rojo (1.01) through Supremo 211 and Surpresa (reported to be Mn-tolerant by Foy *et al.* (1988)). The parents of Surpresa are Polyssu (=Ponta Grossa 142) (0.83) and Alfredo Chavez 6.21. On one side of its lineage, Laura may have inherited tolerance from the experimental line BW 15, through Tobari 66 (0.64), Tezanos Pinto Precoz (0.60), and Frontana (1.05). From the other side of its lineage, Laura may have inherited tolerance from the experimental line BW 517, through Carazinho (1.02), Frontana (1.05), and Fronteira (0.60), whose parents are Polyssu (=Ponta Grossa 142) (0.83) and Alfredo Chavez 6.21. Thus, Frontana proves to be Laura's common ancestral linkage to Mn tolerance by either line of descent.

These results suggest that Polyssu (=Ponta Grossa 142) and/or Alfredo Chavez 6.21 are the progenitors of Mn tolerance in the Mn-tolerant Canadian cultivars Norquay

and Laura (*Fig. II.2*). Although developed and released almost 12 years apart, the common source for Mn tolerance appears to have been passed along without loss of expression. In addition, Biggar (0.88), the third Mn-tolerant Canadian cultivar identified in this study has Frontana as one of its ancestral parents. Similarly, the Mn-tolerant cultivars Siete Cerros (Mexico) (Camargo and Oliveira, 1983), Collafen, and Mexifen (Chile) (Scott *et al.*, 1987) also have Frontana as one of their parents. Frontana is also involved in the pedigree of most of the cultivars screened in this study with intermediate Mn tolerance. Despite these relationships, cultivars with Frontana or other Latin American germplasm in their ancestry do not necessarily retain tolerance of Mn, as exemplified by Columbus (0.16), Neepawa (0.15) and Napayo (0.31) (*Fig. II.1*), all of which have Frontana as one of their parents.

These results indicate that the progenitors of Mn tolerance may be the land races Polyssu and/or Alfredo Chavez 6.21, which passed tolerance on through Frontana and Surpresa. All of these cultivars are from Rio Grande do Sul, the southernmost state in Brazil, where one of the major constraints to production is soil acidity. According to Hettel (1989), wheat was first introduced to Brazil by European immigrants in the 16th century, and in Rio Grande do Sul, and native Indians grew wheat as early as 1627. The "Alfredo Chavez lines" were developed between 1920 and 1924 by making crosses with the best land race varieties used by the early Italian immigrants of Rio Grande do Sul. Thus, nearly 300 hundred years of undirected selection occurred before a major wheat breeding program began in Rio Grande do Sul in 1919. Frontana, one of the best of the improved varieties developed since 1925, is also found in the pedigree of most modern Brazilian varieties. Furthermore, Frontana and related varieties such as Surpresa were used widely by breeders during the 1940's and 1950's as a source of leaf rust resistance in USA, Canada, and Mexican programs (Hettel, 1989).

Whether Mn tolerance of wheat was brought to Brazil by early European immigrants or is the result of selection on the acid soils of the region is not known. Since the trait appears to be of a quantitative nature (see Chapter IV), point mutation could be safely discounted as a possible explanation for the appearance of Mn tolerance in Brazil. To my knowledge Mn tolerance of old and/or recent European cultivars has not been reported. In this study I found 2 old cultivars of European ancestry to be intermediate in Mn tolerance (Ruby (0.52) ~1917; White Fife (0.54) ~1908). Thus, it appears that 300 years of wheat cultivation on acid soils may have provided a selection pressure for germplasm with elevated tolerance to Mn toxicity.

II.4. Conclusion

This work confirms the availability of a range of Mn tolerance in wheat germplasm, and demonstrates that the probable origin of Mn tolerance in diverse cultivars is likely of common origin. This tolerance may have derived from land races from Rio Grande do Sul, the southernmost state of Brazil. This knowledge should assist the plant breeder in designing breeding programs for developing Mn-tolerant germplasm. Based on these results, I undertook a quantitative study investigating the inheritance of Mn tolerance in Canadian wheat germplasm (see Chapter IV) using a rapid, seedling based, screening bioassay for Mn tolerance (see Chapter III).

Table II.1. Pedigree and differential response to Mn (500 μM) by wheat cultivars as measured by relative root weight (RRW) and relative shoot weight (RSW) of Experiment Nº 1. (See Table 5 for references and footnotes).

Cultivar@	Pedigree	Origin	Released	RRW§#	RSW§#
Norquay[3]	Lerma Rojo/Sonora 64//Justin	Canada	1974	0.88*a*	0.89*a*
PT 742[17]	Tp//Cno/No66/3/Bb/Cno/4/Grajo'S'	Canada	¶	0.67*ab*	0.83*a-d*
PT 726[17]	Tp/Cno/No66/3/Bb/Cno/4/Grajo'S'	Canada	¶	0.66*ab*	0.88*ab*
Wildcat[4]	Bluebird/Glenlea	Canada	1987	0.61*bc*	0.77*a-f*
PT 741[17]	Tp//Cno/No66/3/Bb/Cno/4/Grajo'S'	Canada	¶	0.58*bc*	0.85*a-c*
PF 7748[22]	North Dakota 81/IAS59//IAS58 = Whydah (Zambia, 1989)	Brazil	?	0.58*bc*	0.88*a*
Monon[12]	Knox Sib/7/Kv/5/Fz/Hrn/2/IIINo1,W38/3/Wbs/4/Ff/6/Tb*3/2/H/Hr	USA	1959	0.55*b-d*	0.77*a-f*
Romany[20]	CLT262//51/YT54A	Kenya	1966	0.55*b-d*	0.78*a-f*
HY320[5]	Tobari 66/Romany	Canada	1985	0.53*b-d*	0.71*a-g*
Bluesky[4]	Potam/Glenlea	Canada	1987	0.53*b-d*	0.84*a-c*
Owens[4]	Fdr Sib/6/2*(Yta54A*4//No10/Bvr/3/Twin)/5/2*Fdw Sib /3/Twin Sib/PI227196//Twin Sib/4/Gns/Lmh53	USA†	1984	0.52*b-d*	0.81*a-e*
Kenya Tembo[19]	Wis245-II-50-17//CI8154/2*Fr/3/2*Tob66	Kenya	1975	0.50*b-d*	0.69*a-g*
Kenya Kongoni[19]	CI8154/2*Fr/2/3*Rom/3/Wis245-II-50-17/CI8154/2/2*Fr	Kenya	1981	0.46*b-e*	0.80*a-f*
Oslo[4]	Sn64/Y50E//Gte/3/Inia/4/Cno//Elgan/Sn64	USA†	1987	0.43*b-f*	0.60*e-j*
QT 8132[7]	Ciano/Siete Cerros//Kal/Bluebird/3/Pci 'S'	Australia	¶	0.39*c-g*	0.62*d-i*
Park[3]	Mida/Cadet//Thatcher	Canada	1963	0.39*c-g*	0.71*a-g*
Atlas 66[1]	Frondoso//Redhart 3/Noll 28	USA	1948	0.38*c-g*	0.70*a-g*
Kenya Nyumbu[19]	On/Tr207/3/Cno//Son 64/4/K.Nungu'S'	Kenya	1982	0.32*d-h*	0.62*d-i*
Roblin[5]	RL4302/RL4356//RL4359/RL4353	Canada	1986	0.24*e-h*	0.65*c-h*
Maringa[16]	Frontana/Kenya 58//P.G.1	Brazil	1966	0.24*e-h*	0.66*b-h*
Benito[5]	Neepawa/3/RL4255*4//Manitou/CI7090	Canada	1979	0.23*e-h*	0.69*a-g*
Marshall[21]	?	USA	?	0.21*f-h*	0.47*h-j*
Glenlea[3]	Pembina*2/Bage//CB100	Canada	1972	0.21*f-h*	0.64*d-h*
Scout 66[13]	Nebred/2/Hope/Turkey/4/Cheyenne/3/Ponca	USA	1967	0.19*f-h*	0.43*ij*
Kenyon[4]	Neepawa*5/Buck Manantial	Canada	1985	0.18*f-h*	0.60*f-j*
Conway[4]	Chris/Siete Cerros//Neepawa/Opal	Canada	1986	0.16*gh*	0.53*g-j*
Katepwa[5]	Nep*6/RL2938/3/Nep*6//CI8154/2*Fcr	Canada	1981	0.16*gh*	0.59*f-j*
Neepawa[3]	RL4125/RL4008	Canada	1969	0.15*gh*	0.56*g-j*
Columbus[5]	Neepawa*6/RL4137	Canada	1980	0.09*h*	0.41*j*
Lancer[5]	Fortuna/Chris	Canada	1985	0.08*h*	0.40*j*
SEM				0.06	0.07

Table II.2. Pedigree and differential response to Mn (500 μM) by wheat cultivars as measured by relative root weight (RRW) and relative shoot weight (RSW) of Experiment Nº 2. (See Table 5 for references and footnotes).

Cultivar@	Pedigree	Origin	Released	RRW§	RSW§
Laura[5]	Mitu/Tob/3/Czho/CT763//Atl66/CT262	Canada	1986	0.84*a*	0.92*a*
Norquay[3]	Lerma Rojo/Sonora 64//Justin	Canada	1974	0.72*b*	0.79*b*
Fielder[11,23]	Yt54A*4//No10/Bvr/3/2*Y50/4/No10/Bvr//Brt/Onas	USA†	1974	0.68*b*	0.79*b*
Pitic 62[20]	YT54/N10B126.1C	Mexico†	1962	0.53*c*	0.75*b-d*
Ruby[3]	Downy Riga/Red Fife D	Canada	1917$	0.52*c*	0.74*b-d*
Saunders[3]	C.26-44.7/Thatcher	Canada	1947	0.51*cd*	0.75*bc*
H. R. Calcutta[8,23]	Canadian introduction from India (Land race)	India†	1890$¶	0.45*c-e*	0.68*b-f*
Sinton[3]	Mitu/3/Th*6/KF//Lee*6/KF	Canada	1975	0.45*c-e*	0.69*b-f*
Kenhi[3]	Ken 338 AC_2E_3/Lemhi*2	Canada	1958	0.39*d-f*	0.61*c-h*
Prelude[3]	Downy Gehun/Fraser	Canada	1913$	0.39*d-f*	0.65*b-g*
Reward[3]	Marquis/Prelude	Canada	1928	0.37*e-g*	0.71*b-e*
Red Fife[3]	Reselection from a Polish Introduction	Canada	1845$	0.35*e-h*	0.58*d-h*
Stanley[22]	Ladoga/Red Fife	Canada	1895$	0.32*e-i*	0.61*c-h*
Chinook[3]	Thatcher/S-615-11	Canada	1952	0.31*f-j*	0.64*b-g*
Thatcher[3]	Marquis/Iumillo//Marquis/Kanred	USA†	1934	0.31*f-j*	0.67*b-f*
Manitou[3]	Th*7/Fn//Th*6/KF/3/Th*6/PI170925	Canada	1965	0.31*f-j*	0.75*b*
Ladoga[3]	Canadian Introduction from Russsia (Land race)	USSR†	1888$¶	0.31*f-j*	0.55*e-i*
Napayo[3]	Manitou*2/RL4124.1	Canada	1972	0.27*f-k*	0.61*c-h*
Reliance[3]	Kanred/Marquis	USA†	1932	0.25*g-k*	0.54*f-i*
Canthatch[3]	Thatcher*6/Kenya Farmer	Canada	1959	0.24*h-l*	0.56*e-i*
Marquis[3]	Hard Red Calcutta/Red Fife	Canada	1910$	0.21*i-l*	0.49*g-i*
Selkirk[3]	McMurachy/Exchange//Redman*3	Canada	1953	0.21*i-l*	0.47*hi*
Red Bobs 222[3]	Reselection of Early Triumph	Canada	1926	0.20*j-m*	0.46*hi*
Canuck[3]	Canthatch and a selection from Mida/Cadet/Rescue	Canada	1974	0.19*k-m*	0.59*d-h*
Rescue[3]	Apex/S-615	Canada	1946	0.17*k-m*	0.54*f-i*
Apex[3]	H.44-24/Double Cross//Marquis*2	Canada	1937	0.17*k-m*	0.48*g-i*
Cypress[3]	Rescue/Chinook	Canada	1962	0.17*k-m*	0.46*hi*
Garnet[3]	Preston A/Riga M	Canada	1925	0.16*k-m*	0.46*hi*
Columbus[5]	Neepawa*6/RL4137	Canada	1980	0.14*lm*	0.49*g-i*
Chester[3]	Renown*S-615*Rescue*Kendee*(Mida*Cadet)	Canada	1976	0.11*m*	0.40*i*
SEM				0.07	0.08

Table II.3. Pedigree and differential response to Mn (500 μM) by wheat cultivars as measured by relative root weight (RRW) and relative shoot weight (RSW) of Experiment Nº 3. (See Table 5 for references and footnotes).

Cultivar@	Pedigree	Origin	Released	RRW§	RSW§
Norquay[3]	Lerma Rojo/Sonora 64//Justin	Canada	1974	0.67*a*	0.78*a*
Belvedere[5]	Gamenya/Kolibri	Canada	1984	0.52*b*	0.73*ab*
Renfrew[3]	Selection from Marquis	Canada	1924	0.39*c*	0.71*ab*
Cascade[3]	Quality A/Pacific Blue Stem//C26-59-2D/3/Onas	Canada	1947	0.35*cd*	0.69*a-c*
Lemhi[2,23]	Federation/Dicklow	USA†	1939	0.33*c-e*	0.66*b-d*
Huron[3,8]	White Fife/Ladoga	Canada	1900$	0.32*c-f*	0.60*b-g*
Milton[5]	Kentville Selection*6/Pompe	Canada	1981	0.30*c-g*	0.64*b-e*
Dundas[5]	Opal/Inia 66	Canada	1979	0.30*d-h*	0.51*e-j*
Laval-19[5]	F. W. 606-A/Opal//Opal	Canada	1979	0.27*d-i*	0.62*b-f*
Vernon[5]	Opal*4/Pompe	Canada	1979	0.26*e-j*	0.57*c-h*
Concorde[22]	Penjamo/Yaqui 54	Canada	1976	0.25*e-j*	0.56*c-i*
Ankra[8,15]	Triesdorf St. 21-40/Erli	Netherland†	1979	0.24*f-k*	0.56*c-h*
Max[5]	Sappo/Kolibri	W. Germany†	1978	0.24*f-k*	0.55*d-i*
Pioneer[3]	Riga/Preston	Canada	1915$	0.22*g-l*	0.51*e-j*
Ceres[3,6]	Kota/Marquis	USA†	1926	0.22*h-l*	0.56*c-h*
Axminster[8,23]	Sel. Minister (ex Aus) or Marquis/Unknown cultivar	Australia†	1926	0.19*i-m*	0.48*f-k*
Lake[3]	Regent/Canus	Canada	1954	0.19*i-m*	0.54*d-j*
Bishop[3]	Ladoga/Gehun	Canada	1900$	0.19*i-m*	0.51*e-j*
Kota[3,8]	Found in a sample of Durum wheat obtained in USSR	Canada	1921$	0.18*j-n*	0.63*b-e*
Messier[15]	Opal/Concorde	Canada	1984	0.18*j-n*	0.48*g-k*
Acadia[3]	Canus//Marquis/Pentad	Canada	1951	0.18*j-n*	0.51*e-j*
Cassavant[8,16]	Peko/Ottawa 5381-9	Canada	1981	0.17*k-o*	0.47*g-k*
Redman[3]	Regent/Canus	Canada	1946	0.17*k-o*	0.53*d-j*
Mondor[15]	Peko/Ottawa 5381-9	Canada	1983	0.15*l-p*	0.47*g-k*
Coronation II[3]	Pentad/Marquis	Canada	1937	0.15*l-p*	0.42*i-k*
Garnet Ott[23]	Garnet (Ottawa)	Canada	?	0.14*m-p*	0.46*g-k*
Early Triumph[8,23]	Bobs/Early Red Fife	Canada	1918$	0.13*m-p*	0.40*jk*
Regent[3]	H-44/Reward	Canada	1939	0.11*n-p*	0.36*k*
Columbus[5]	Neepawa*6/RL4137	Canada	1980	0.11*op*	0.43*h-k*
Quality A[3]	Selected out of Florence, of Australian parentage	Canada	1922$	0.09*p*	0.36*k*
SEM				0.05	0.07

Table II.4. Pedigree and differential response to Mn (500 μM) by wheat cultivars as measured by relative root weight (RRW) and relative shoot weight (RSW) of Experiment Nº 4. (See Table 5 for references and footnotes).

Cultivar@	Pedigree	Origin	Released	RRW§	RSW§
Frontana[16]	Fronteira/Mentana	Brazil	1943	1.05*a*	0.95*a*
Carazinho[16]	Colonista/Frontana	Brazil	1957	1.02*ab*	0.83*bc*
Yaktana 54[23]	Yaqui 48/Kentana 48//Frontana	Mexico	1954	0.93*a-c*	0.92*ab*
Laura[5]	Mitu/Tob/3/Czho/CT763//Atl66/CT262	Canada	1986	0.92*a-c*	0.86*bc*
Biggar[17]	Tobari 66/Romany	Canada	1989	0.88*a-c*	0.79*b-f*
Veranopolis[16]	Trintecinco/Frontana	Brazil	1950	0.85*cd*	0.83*b-d*
Polyssu[16]	(=Ponta Grossa 142) Local Cultivar from Rio Grande Do Sul	Brazil	?	0.83*c-e*	0.74*c-h*
Norquay[3]	Lerma Rojo/Sonora 64//Justin	Canada	1974	0.83*b-d*	0.83*b-d*
Frondoso[16]	Polyssu/Alfredo Chavez 6.21	Brazil	?	0.77*c-f*	0.81*b-e*
Cotipora[16]	Veranopolis*2/Egypt NA101	Brazil	1965	0.71*d-f*	0.77*b-g*
Tobari 66[20]	TZPP/Son64A	Mexico	1966	0.64*e-g*	0.69*c-i*
Fronteira[16]	Polyssu/Alfredo Chaves 6.21	Brazil	1934	0.60*f-h*	0.76*b-g*
T. P. P.[16]	=Tezanos Pinto Precoz = Fn//Th/Svl	Argentina	¶	0.60*f-h*	0.77*b-g*
Leader[5]	Fortuna/Chris	Canada	1981	0.56*f-h*	0.72*c-h*
White Fife[8,23]	An Ottawa selection from Red Fife	Canada	1908$	0.54*f-i*	0.70*c-h*
Lemhi 53[3,2]	Kenya/Lemhi*6	USA†	1953	0.41*g-j*	0.46*j-n*
Supreme[8,23]	Selection from Red Bobs	Canada	1921$	0.38*h-k*	0.65*d-j*
Springfield[3,14]	No10/Bvr//3*Lmh53/3/Lmh62/4/ Lmh53*5/3/Lee*7//Chin/(*Ae.umbellulata*)	USA†	1970	0.36*h-k*	0.59*g-l*
Opal[15]	Triesdorf Stamm 21-40/Von Romke Erll	W. Germany†	1968	0.35*h-k*	0.61*f-k*
Colonista[16]	Roxo wheat selection	Brazil	?	0.35*h-k*	0.63*e-j*
Lemhi 62[23]	Lemhi 53*5/3/Lee*7/Chinese/*Ae.umbellulata*	USA†	1962	0.29*i-l*	0.60*f-k*
Kitchener[22,23]	Head selection from Marquis	Canada	1911$¶	0.28*j-l*	0.54*h-m*
Trintecinco[16]	Alfredo Chavez 3.21/Alfredo Chavez 4.21	Brazil	1936	0.24*j-l*	0.48*i-m*
Sonora 64[20]	YT54/N10B//2*Y54	Mexico	1964	0.18*j-l*	0.37*mn*
Early Red Fife[3]	Pure line selection from Red Fife	Canada	1903$	0.18*j-l*	0.41*k-n*
Pembina[3]	Thatcher/RL2564	Canada	1959	0.17*j-l*	0.47*j-n*
McMurrachy[23]	Found in Garnet	Canada	?	0.16*kl*	0.45*j-n*
Preston[3]	Ladoga/Red Fife	Canada	1895$	0.16*kl*	0.41*k-n*
Columbus[5]	Neepawa*6/RL4137	Canada	1980	0.16*kl*	0.37*l-n*
Lee[3]	Hope/Timstein	USA†	1947	0.11*l*	0.27*n*
SEM				0.10	0.09

Table II.5. Pedigree and differential response to Mn (500 μM) by wheat cultivars as measured by relative root weight (RRW) and relative shoot weight (RSW) of Experiment Nº 5.

Cultivar@	Pedigree	Origin	Released	RRW§	RSW§
Lerma Rojo[23]	Lerma 50/Yaqui 48//Maria Escobar*2/Supremo 211	Mexico	?	1.01*a*	0.98*a*
Norquay[3]	Lerma Rojo/Sonora 64//Justin	Canada	1974	0.71*b*	0.78*b*
Cutler[17]	Ciano'S'/Son64/Y50E5/GTO/4/Inia'S'	Canada	1991	0.56*b*	0.69*bc*
Kentana 48[19,23]	Kenya C9906/Mentana	Mexico	1948	0.26*c*	0.57*cd*
Yaqui 54[23]	Yaqui 48/Timstein//Kenya C9906	Mexico	1954	0.24*c*	0.60*cd*
Canus[3]	Marquis/Kanred	Canada	1935	0.20*c*	0.47*d*
Renown[3]	H-44/Reward	Canada	1937	0.16*c*	0.44*d*
Columbus[5]	Neepawa*6/RL4137	Canada	1980	0.14*c*	0.41*d*
SEM				0.06	0.09

§ Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

RRW and RSW from Macfie *et al.*, 1989.

† Released in Canada.

¶ Not released.

$ Aproximate year of released (Released prior to 1923).

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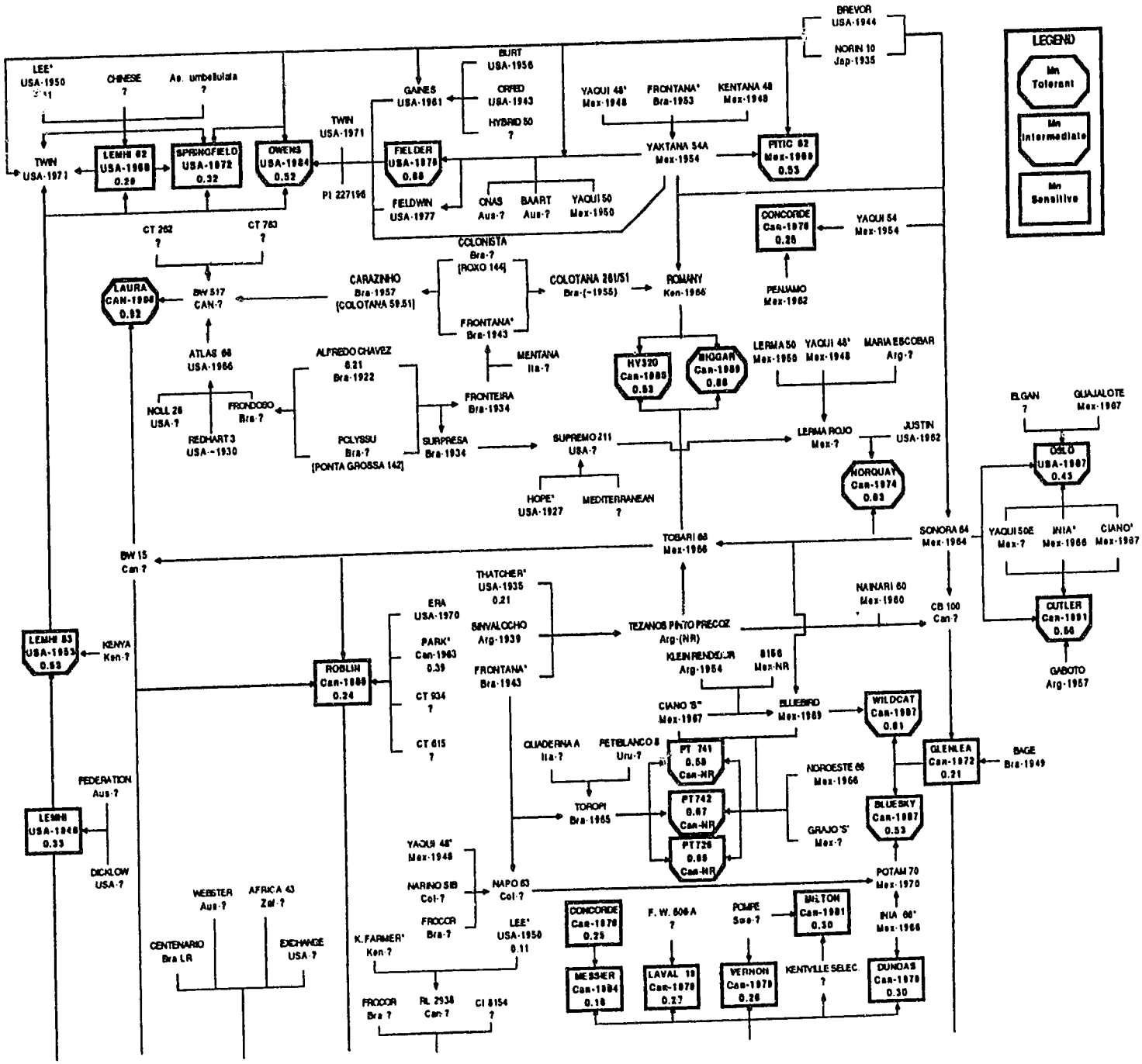
Fig. II.1. Phylogenetic map of filial pedigree relationships among 88 "*Canadian*" spring wheat cultivars. Included are the Canadian cultivars reported by Macfie *et al.*, (1989). Each cultivar is described by the country of origin, the year released and/or developed, and by the RRW value (where screened). To improve map clarity, cultivars denoted with an asterisk (*) are repeated more than once on the map.


LEGEND
Mn Tolerant
Mn Intermediate
Mn Sensitive
BREVOR USA-1944
NORIN 10 Jap-1935
LEE* USA-1950
CHINESE ?
Ae. umbellulata ?
BURT USA-1956
GAINES USA-1961
ORED USA-1943
HYBRID 50 ?
YAQUI 48* Mex-1948
FRONTANA* Bra-1953
KENTANA 48 Mex-1948
TWIN USA-1971
LEMHI 82 USA-1968 0.29
SPRINGFIELD USA-1972 0.32
OWENS USA-1984 0.52
FIELDER USA-1978 0.68
YAKTANA 54A Mex-1954
PITIC 62 Mex-1968 0.53
PI 227196
FIELDWIN USA-1977
ONAS Aus-?
BAART Aus-?
YAQUI 50 Mex-1950
CONCORDE Can-1978 0.26
YAQUI 54 Mex-1954
CT 262 ?
CT 763 ?
COLONISTA Bra-? [ROXO 144]
CARAZINHO Bra-1957 [COLOTANA 50.51]
COLOTANA 261/51 Bra-(~1955)
ROMANY Ken-1966
PENJAMO Mex-1962
LAURA CAN-1966 0.32
BW 517 CAN-?
FRONTANA* Bra-1943
MENTANA Ita-?
ATLAS 66 USA-1966
ALFREDO CHAVEZ 8.21 Bra-1922
HV320 Can-1985 0.83
BIOGAR Can-1989 0.88
LERMA 50 Mex-1950
YAQUI 48* Mex-1948
MARIA ESCOBAR Arg-?
NOLL 28 USA-?
FRONDOSO Bra-?
REDHART 3 USA-~1930
FRONTEIRA Bra-1934
POLYSSU Bra-? [PONTA GROSSA 142]
SURPRESA Bra-1934
SUPREMO 211 USA-?
LERMA ROJO Mex-?
JUSTIN USA-1962
HOPE* USA-1927
MEDITERRANEAN ?
NORQUAY Can-1974 0.83
ELGAN ?
GUAJALOTE Mex-1967
OSLO USA-1987 0.43
YAQUI 50E Mex-?
INIA* Mex-1966
CIANO* Mex-1967
OUTLER Can-1991 0.84
GABOTO Arg-1957
BW 15 Can-?
TOBARI 66 Mex-1966
SONORA 64 Mex-1964
THATCHER* USA-1935 0.21
NAINARI 60 Mex-1960
CB 100 Can-?
ERA USA-1970
SINVALOCHO Arg-1939
FRONTANA* Bra-1943
TEZANOS PINTO PRECOZ Arg-(NR)
KLEIN RENDIDOR Arg-1954
8156 Mex-NR
LEMHI 83 USA-1983 0.53
KENYA Ken-?
ROBLIN Can-1988 0.24
PARK* Can-1963 0.39
CT 934 ?
CT 615 ?
CIANO 'S' Mex-1967
BLUEBIRD Mex-1989
WILDCAT Can-1987 0.81
QUADERNA A Ita-?
PETIBLANCO 8 Uru-?
PT 741 0.58 Can-NR
NOROESTE 66 Mex-1966
GLENLEA Can-1972 0.21
SAGE Bra-1949
FEDERATION Aus-?
LEMHI USA-1946 0.33
DICKLOW USA-?
TOROPI Bra-1965
PT742 0.67 Can-NR
PT726 0.68 Can-NR
GRAJO 'S' Mex-?
BLUESKY Can-1987 0.53
YAQUI 48* Mex-1948
NARINO 5IB Col-?
FROCOR Bra-?
NAPO 63 Col-?
POTAM 70 Mex-1970
WEBSTER Aus-?
AFRICA 43 Zel-?
CONCORDE Can-1978 0.25
F.W. 606 A ?
POMPE Sue-?
MILTON Can-1981 0.30
INIA 66* Mex-1966
CENTENARIO Bra LR
EXCHANGE USA-?
K.FARMER* Ken-?
LEE* USA-1950 0.11
MESSNER Can-1984 0.16
LAVAL 19 Can-1978 0.27
VERNON Can-1979 0.28
KENTVILLE SELEC ?
DUNDAS Can-1978 0.30
FROCOR Bra-?
RL 2938 Can-?
CI 8154 ?

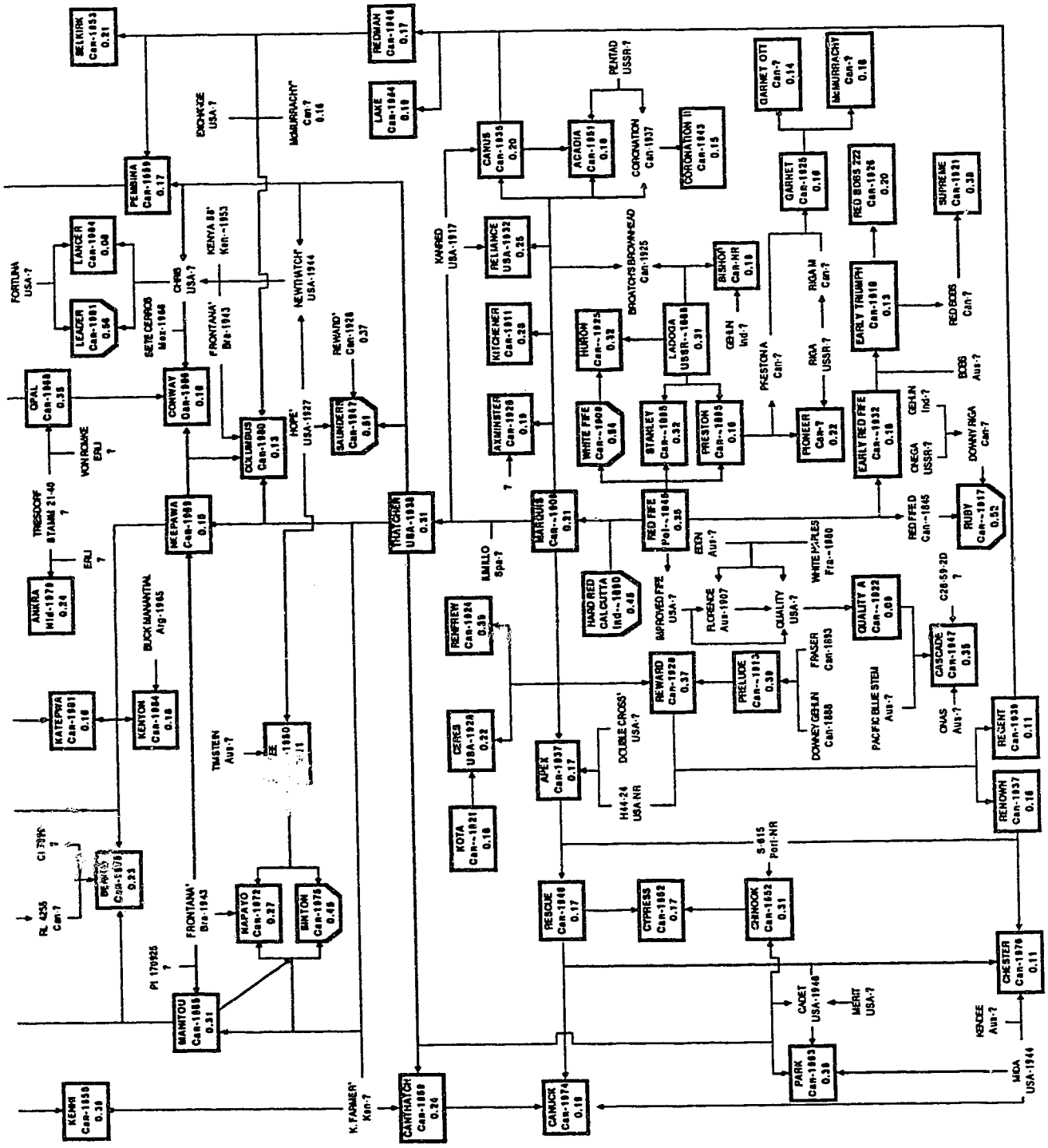
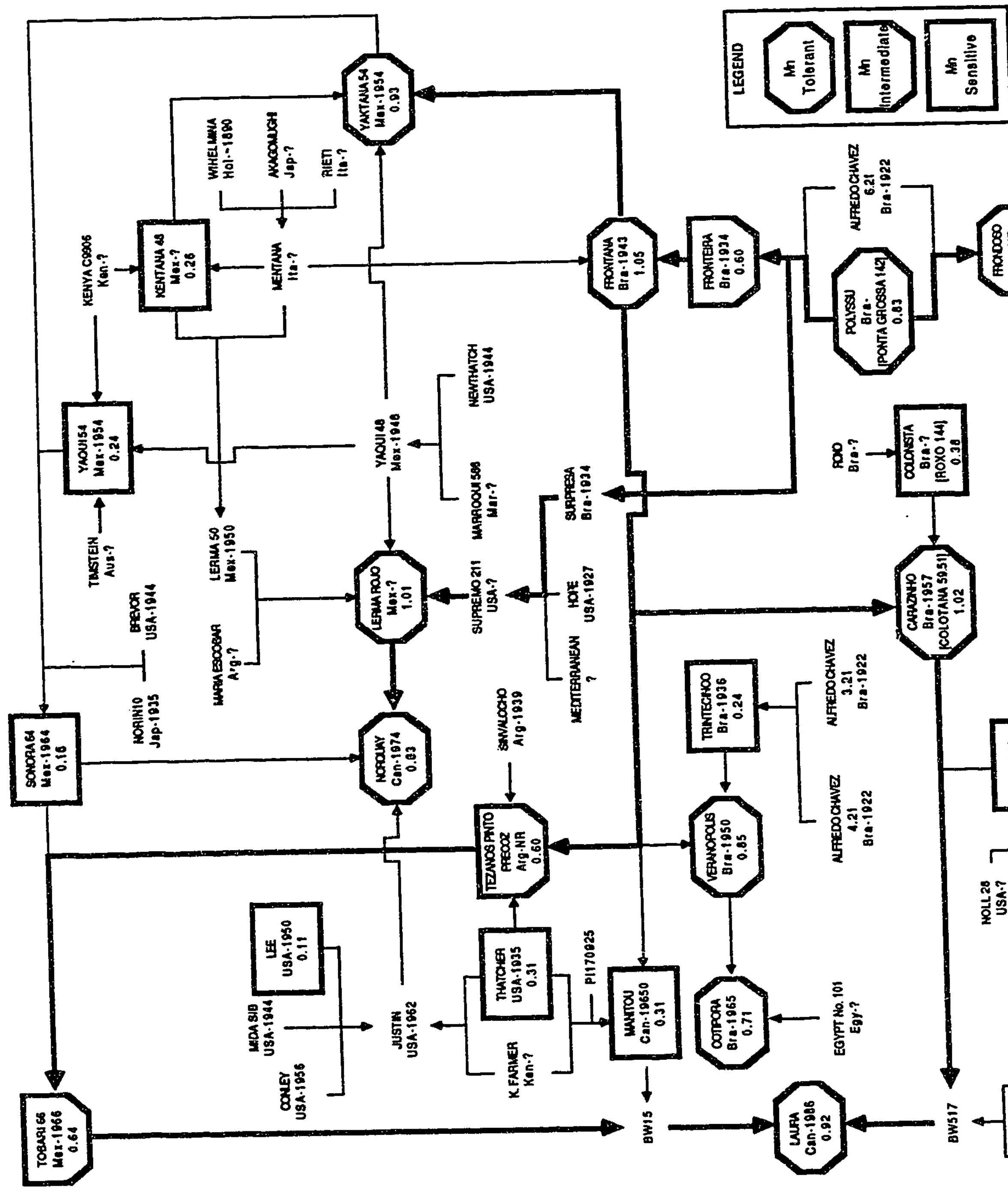

SELKIRK Can-1953 0.21
REDMAN Can-1946 0.17
LANE Can-1944 0.16
PEMBINA Can-1959 0.17
EXCHANGE USA-?
McMURRACHY' Can-? 0.16
CANUS Can-1935 0.20
ACADIA Can-1951 0.16
PENTAD USSR-?
CORONATION Can-1937
CORONATION II Can-1943 0.15
GARNET OTT Can-? 0.14
McMURRACHY Can-? 0.16
GARNET Can-1935 0.16
RED BOOKS 222 Can-1926 0.20
SUPREME Can-1931 0.38
LANCER Can-1964 0.08
LEADER Can-1961 0.06
FORTUNA USA-?
SIETE CERROS Mex-1946
CHRIS USA-?
KENYA 88' Ken--1953
FRONTANA' Bra-1943
NEWTHATCH' USA-1944
KARRED USA-1917
RELIANCE USA-1932 0.25
BRONTOTSBROWNHEAD Can-1925
BISHOP Can-NR 0.18
KITCHENER Can-1911 0.28
HURON Can--1925 0.32
LADOGA USSR--1968 0.31
GEHLN Ind-?
PRESTONA Can-?
RIGA Can-?
EARLY TRIUMPH Can-1918 0.13
REDBOOS Can-?
OPAL Can-1968 0.35
CONWAY Can-1980 0.18
COLUMBUS Can-1980 0.13
HOPE' USA-1927
REWARD' Can-1928 0.37
SAUNDERS Can-1967 0.81
AXMINSTER Can-1926 0.18
WHITE FFE Can--1909 0.84
STANLEY Can--1908 0.32
PRESTON Can--1885 0.18
PIONEER Can-? 0.22
RIGA USSR-?
EARLY RED FFE Can--1932 0.18
BOBS Aus-?
VON ROMKE ERLI ?
TRESDORF STAUNE 21-40 ?
ERLI ?
KEEPAWA Can-1969 0.18
THATCHER USA-1938 0.31
MARQUIS Can--1909 0.31
RED FFE Pol--1846 0.35
ONEGA USSR-?
GEHLN Ind-?
DONNY RIGA Can-?
RUBY Can--1917 0.52
RED FFE Can-1845
AMIRA Mid-1979 0.24
BUCK MANANTIAL Arg-1965
EMILIO Spa-?
HARD RED CALCUTTA Ind--1890 0.48
EDEN Aus-?
WHITE PEARLS Fra--1860
APPROVED FFE USA-?
FLORENCE Aus-1907
QUALITY USA-?
QUALITY A Can--1932 0.09
C28-59-2D ?
CASCADE Can-1947 0.38
ONAS Aus-?
PACIFIC BLUE STEM Aus-?
KATEPWA Can-1981 0.16
KENTON Can-1984 0.18
TASTEN Aus-?
REMFREW Can-1924 0.39
REWARD Can-1928 0.37
PRELUDE Can--1913 0.38
DONNEY GEHLN Can-1888
FRASER Can-1893
CERES USA-1928 0.22
DOUBLE CROSS' USA-?
APEX Can-1937 0.17
H44-24 USA-NR
RESCUE Can-1948 0.17
CYPRESS Can-1962 0.17
S-615 Pot-NR
CHINOOK Can-1952 0.31
RENOWN Can-1937 0.18
REGENT Can-1939 0.11
KOTA Can--1921 0.18
RL 4255 Can-?
CI 7996 ?
BERGEN Can-1978 0.23
MAPAYO Can-1972 0.27
BARTON Can-1976 0.48
FRONTANA' Bra-1943
PI 170925 ?
MANITOU Can-1985 0.31
CHESTER Can-1976 0.11
CADET USA-1946
MERIT USA-?
KENDEE Aus-?
MDA USA-1944
PARK Can-1983 0.38
KENN Can-1958 0.39
K. FARMER' Kan-?
CANTHATCH Can-1959 0.24
CANUCK Can-1974 0.18

Fig. II.2. Phylogenetic map of filial pedigree relationship of the Mn-tolerant wheat cultivars Laura and Norquay. Probable origins for Mn tolerance are shown by thick lines. Each cultivar is described by the country of origin, the year released and/or developed, and by the RRW value (where screened).


LEGEND
Mn Tolerant
Mn Intermediate
Mn Sensitive
YAKTANA 54 Mex-1954 0.93
KENTANA 48 Mex-? 0.26
KENYA C9906 Ken-?
WIHELMINA Hol-1890
AKAGOMUGHI Jap-?
RIETI Ita-?
MENTANA Ita-?
FRONTANA Bra-1943 1.05
FRONTEIRA Bra-1934 0.60
ALFREDO CHAVEZ 6.21 Bra-1922
POLYSSU Bra- (PONTA GROSSA 1) 0.83
FRONDOSO Bra-NR 0.77
YAQUI 54 Mex-1954 0.24
YAQUI 48 Mex-1948
NEWTHATCH USA-1944
MARROQUI 588 Mar-?
SURPRESA Bra-1934
RIO Bra-?
COLONISTA Bra-? (ROXO 144) 0.38
TIMSTEIN Aus-?
LERMA 50 Mex-1950
LERMA ROJO Mex-? 1.01
SUPREMO 211 USA-?
HOPE USA-1927
CARAZINHO Bra-1957 (COLOTANA 5951) 1.02
BREVOR USA-1944
MARIA ESCOBAR Arg-?
MEDITERRANEAN ?
NORIN 10 Jap-1935
SONORA 64 Mex-1964 0.16
NOROLWAY Can-1974 0.83
SINVALOCHO Arg-1939
TRINTECINCO Bra-1936 0.24
ALFREDO CHAVEZ 3.21 Bra-1922
ALFREDO CHAVEZ 4.21 Bra-1922
ATLAS 66 USA-1966 0.38
TEZANOS PINTO PRECOZ Arg-NR 0.60
VERANOPOLIS Bra-1950 0.85
NOLL 28 USA-?
REDHART 3 USA-1930
LEE USA-1950 0.11
THATCHER USA-1935 0.31
PI170925
MIDA SIB USA-1944
JUSTIN USA-1962
MANITOU Can-1965 0.31
COTIPORA Bra-1965 0.71
EGYPT No. 101 Egy-?
CONLEY USA-1956
K. FARMER Ken-?
TOBARI 66 Mex-1966 0.64
BW15
LAURA Can-1986 0.92
BW517
CT763
CT262

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Chapter III

Chlorophyll concentration and leaf elongation rate in wheat seedlings as a measure of manganese tolerance.[2]

III.1. Introduction

Nearly half of the world's non-irrigated arable lands are acid soils (Clark, 1982). After aluminium toxicity, manganese (Mn) toxicity is probably the second most important growth limiting factor in acid soils (Foy, 1984). Interspecific and intraspecific differences in tolerance to Mn have been identified among crop plants, providing breeding potential to develop cultivars adapted to acid soils (Foy *et al.*, 1988). To facilitate breeding programs a rapid, non-destructive, inexpensive, and repeatable seedling-based bioassay is required for selection of tolerant genotypes from early segregating generations (Devine, 1982). Physiological and biochemical responses to toxic levels of Mn have generally been used as screening techniques in determining Mn tolerance in a variety of crop plants. However, no test is available for the rapid evaluation of manganese tolerance in cereals (Scott and Fisher, 1989). In screening wheat (*Triticum aestivum* L.) cultivars for Mn tolerance, root and shoot weight (Brauner and Sarruge, 1980), relative root weight (Macfie *et al.*, 1989), and root length (Camargo and Ferreira Filho, 1990) have been used. Others have suggested use of seed Mn content (Guerrier, 1988), concentration of aconitic acid in seedlings (Burke, *et al.*, 1990) and whole leaf fluorescence (Homer *et al.*, 1980) as possible rapid screening procedures. All of these methodologies fail to fulfil at least one of the requirements for screening segregating populations described by Devine (1982).

In this report, two plant parameters which may provide a rapid seedling based bioassay for Mn tolerance in wheat are described, chlorophyll concentration and the leaf elongation rate for regrowth of Mn-stressed seedlings. These physiological parameters may provide suitable techniques for selecting tolerant seedlings in breeding programs.

2. A version of this chapter has been published.
J. S. Moroni, K. G. Briggs and G. J. Taylor. 1991. *Plant and Soil* 136:1-9.

III.2. Materials and methods

III.2.a. Germination

Five Canadian cultivars of spring wheat (*Triticum aestivum* L.) were used in this study. Columbus and Katepwa have been described as Mn-sensitive (tall genotypes), Oslo as Mn-intermediate (a semidwarf genotype) and Norquay as Mn-tolerant (semidwarf genotype) by Macfie *et al.* (1989). Laura, a Mn-tolerant cultivar (tall genotype), was also included in this study. These levels of tolerance were determined by the relative root weight method (RRW = root weight in the presence of 500 μM Mn, divided by control root weight; Macfie *et al.* 1989). Seeds were surfaced sterilized in 1.2% sodium hypochlorite for 20 minutes and germinated overnight at room temperature in an aerated solution of the systemic fungicide Vitavax (*Carbathiin + Thiram* ; 0.005 gL^{-1}). Germinated seeds were laid on a plastic mesh, crease down, suspended over 10 L of a nutrient solution composed of (μM): Ca, 1000; Mg, 300; NO_3, 2900; and NH_4, 300; and were grown for three days at room temperature. For the first two days the containers were covered with black plastic. On the fourth day, seedlings of similar size were selected and used for the assay.

III.2.b. Growth conditions during the assay

In each of two experiments, seedlings were grown for six days in a dilute nutrient solution containing (μM): Ca, 1000; Mg, 300; K, 800; NO_3, 3600; NH_4, 600; PO_4, 100; SO_4, 101; Cl 34; Na, 20; Fe, 10; B, 6; Mn, 2; Zn, 0.5; Cu, 0.15; and Mo, 0.1. Iron was supplied as Fe-EDTA prepared from equimolar amounts of $FeCl_3$ and Na_2EDTA. All nutrient solutions were adjusted to pH 4.8 with KOH and HCl, and solutions were constantly aerated. Treatments consisted of additions of Mn to the control solutions. Seedlings were grown in a temperature controlled growth chamber at a day/night temperature of 21.0/17.0 ± 0.5 °C and a relative humidity of 80/90 ± 5 %. The photosynthetic photon flux density (PPFD) was 307 ± 30 µmol m^{-2} s^{-1}.

III.2.c. Experiment Nº1: The effect of Mn on chlorophyll

The Mn-tolerant Norquay and Mn-sensitive Columbus were used to determine the effect of Mn on chlorophyll concentration and composition. Five seedlings of each of the two cultivars were placed on a plastic mesh suspended over each of 48 780-ml plastic

containers filled with the basal nutrient solution and one of twelve different Mn levels (*ie.* 2, 50, 100, 200, 300, 400, 500, 600, 800, 1000, 1500, 2000 μM). On the sixth day, seedlings were harvested and chlorophyll concentration was determined. The experimental design was a randomized block consisting of 2 wheat cultivars, 12 levels of Mn and 4 replicates.

III.2.d. Experiment Nº2: Differential response of five wheat cultivars to Mn

Based on the results of Experiment Nº1, a Mn concentration of 1000 μM was selected to determine the differential response of five wheat cultivars (Columbus, Katepwa, Oslo, Norquay and Laura) to Mn. Sensitivity and tolerance to manganese was evaluated by chlorophyll concentration and composition, and the leaf elongation rate (LER) of seedlings pre-treated with Mn (regrowth). Twelve seedlings of each cultivar were placed on a plastic frame suspended over each of six containers filled with 10 L of nutrient solution. Seedlings were held by polyurethane foam plugs commonly used as test tube stoppers. It was observed that some of the polyurethane foams were toxic to the seedlings causing poor seedling growth and/or death. Sterilization of the plugs for an hour in an autoclave eliminated this problem. Seedlings were grown on a 1000 μM Mn or 2 μM Mn (control) solution for 6 days under conditions described above. The experimental design was a randomized block design with 5 cultivars, 2 treatments and 3 replicates.

III.2.e. Determination of leaf area, fresh weight, and chlorophyll

On an experimental unit basis individual seedlings were cut at the collar of the primary leaf and fresh weight (FWT) and leaf area (LA) determined. Leaf area was measured on a LI-COR LI-3100 Area Meter, (LI-COR Inc., Lincoln, Nebraska, USA), and the mean value of three measurements was used. Leaf blades were then cut in small pieces (~1-2 cm), placed in 5 ml acetone and homogenized with a Polytron homogenizer (Brinkmann Instruments, Rexdale, ONT., Canada) at full speed for 10 to 15 sec. The homogenate was diluted with 5 ml of distilled water, filtered on a Nº 2 Whattman filter paper and washed three times with pure acetone (3-4 ml each wash). The filtrate was brought to volume (25 ml) with acetone making a final solution of 80% acetone. Extraction of chlorophyll was performed at room temperature, but tissues, solutions, homogenates, and extractants were kept on ice (2-4 °C) under black plastic to minimize light and temperature induced chlorophyll degradation. Absorbances were read on a

SPECTRONIC 21 Spectrophotometer, (Bausch & Lomb), at 665 and 649 *nm* and chlorophyll *'a'*, chlorophyll *'b'*, and total chlorophyll (chlorophyll *'a+b'*) were estimated using the extinction coefficient equations described by Vernon (1960). Dilutions were not necessary, as absorbance readings were below 0.7. Chlorophyll *'a'*, chlorophyll *'b'*, and chlorophyll *'a+b'* concentration were expressed on a FWT and LA basis.

Determination of chlorophyll in Experiment Nº2 was as described above, with the following changes. Seedlings were harvested, bulked and cut in small pieces (~1-2 cm). Two random samples (~0.2 g) were selected and used for chlorophyll determinations.

III.2.f. Determination of leaf elongation rate (LER)

After chlorophyll determination, seedlings from Experiment Nº2 were thoroughly washed with distilled water and transfered to a control solution (10 L) and regrown for five days under similar environmental conditions. Leaf length was measured over five consecutive days. Measurements were taken from the top of the polyurethane foam holder to the tip of the longest leaf. Leaf elongation rate (LER) was estimated by the linear regression coefficient (b) of leaf length over time.

All data were subjected to analysis of variance (ANOVA), and treatment means were compared using the least significant difference test (LSD) at $p \leq 0.05$ and $p \leq 0.01$ (SAS, 1985). Relative measurements, defined as percent of treatment compared to control, were calculated for chlorophyll and LER parameters. Arcsine transformations on percentage data of chlorophyll *'a'*, chlorophyll *'b'*, and chlorophyll *'a+b'* concentration, and LER (Experiment Nº2) were performed according to the rules outlined by Gomez and Gomez (1984). For arcsine transformation data points for some replications of Oslo over 100% were taken as 100% (Table III.2). Correlations were estimated for relationships between various parameters and significance tested at $p \leq 0.01$ (Gomez and Gomez, 1984).

III.3. Results

III.3.a. Experiment Nº1

Manganese toxicity symptoms observed were similar to those previously described by Keisling *et al.* (1984) and Ohki (1984), including leaf chlorosis,

stunting, stiffness in leaf tissue, necrotic leaf spots, white flecking, leaf tip burn and, occasionally, leaf purpling. Symptoms were most apparent on the sensitive cultivar as the level of Mn stress increased. Over the full range of Mn stress chlorophyll *'a+b'* concentration expressed on a FWT basis decreased in Norquay by only 9%, while in Columbus it was reduced by as much as 43% (*Fig. III.1*). Chlorophyll *'a'* (*Fig. III.2*) and chlorophyll *'b'* (*Fig. III.3*) concentration expressed on a FWT basis showed a similar reduction. Thus, the chlorophyll *'a/b'* ratio did not differ among Mn concentrations for either cultivar. Chlorophyll *'a+b'* concentration expressed on a FWT basis showed less variation than if expressed on a LA basis (*Fig. III.4*).

Analysis of variance (ANOVA) of chlorophyll *'a'*, chlorophyll *'b'* and chlorophyll *'a+b'* concentration indicated significant main effects attributable to cultivar and Mn concentration, as well as a significant cultivar × Mn concentration interaction at $p < 0.001$ when expressed in FWT basis, and at $p < 0.008$ when expressed on a LA basis. Separation of cultivar means (LSD at $p \leq 0.05$) for chlorophyll *'a'*, chlorophyll *'b'* and chlorophyll *'a+b'* concentration became possible at Mn concentrations of 100 μM and above.

III.3.b. Experiment Nº2

Manganese toxicity symptoms similar to those reported in Experiment Nº1 were observed on the sensitive and intermediate cultivars but not on the tolerant ones. Analysis of variance of the chlorophyll *'a'*, chlorophyll *'b'* and chlorophyll *'a+b'* concentration data from five Mn-stressed and control cultivars indicated significant main effects attributable to cultivar, Mn treatment, as well as cultivar × treatment interactions ($p < 0.001$; *Table III.1*). Significant differences were not observed for chlorophyll *'a/b'* ratio grown under either treatment.

Under control conditions a distinct pattern of chlorophyll concentration and/or composition was not observed between the five cultivars, thus the observed variability was not directly correlated to height genotype (semi-dwarf or tall) or level of Mn tolerance (tolerant, intermediate or sensitive). The LSD mean separation ranking between cultivars depended on the probability used ($p \leq 0.05$ or $p \leq 0.01$; *Table III.1*).

Tolerant cultivars grown with excess Mn contained higher contents of chlorophyll than the intermediate and sensitive cultivars. At $p \leq 0.05$, treatment with Mn separated the cultivars according to the level of tolerance determined by the RRW methodology, however at $p \leq 0.01$ the intermediate cultivar was ranked as sensitive.

This was observed for chlorophyll *'a'*, chlorophyll *'b'* and chlorophyll *'a+b'* concentration (*Table III.1*).

Cultivar ranking was the same for chlorophyll *'a'*, chlorophyll *'b'* and chlorophyll *'a+b'* concentration (expressed as a percentage of control) at both probabilities ($p \leq 0.05$ and $p \leq 0.01$). Once again, the intermediate cultivar was ranked as sensitive (*Table III.1*).

Seedling regrowth was observed for all cultivars and differential response of LER to pre-treatment was observed (*Table III.2*). Each linear regression coefficient (b) value of leaf length over time for every wheat seedling sampled had a correlation (R) of greater than 0.95. Analysis of variance of LER indicated significant main effects attributable to cultivars and treatments at $p < 0.001$, but the cultivars × treatments effect was not significant. Cultivar differences for LER under control conditions were observed between cultivar height genotypes (tall and semidwarf), but there were not significant differences within each height genotype. Leaf elongation rate of tall genotypes were about 50% greater than the semidwarf genotypes. Differences of LER between cultivars did not reflect a relationship with their Mn tolerance as determined by RRW.

Under conditions of Mn stress, cultivar differences for LER did not generally express their Mn tolerance as determined by RRW. The Mn-sensitive Katepwa, the Mn-tolerant Norquay, and the Mn-intermediate Oslo were not significantly different to each other, and thus were classified as intermediate (*Table III.2*). However, the Mn-sensitive Columbus and Mn-tolerant Laura were significantly different for LER from each other, and from all of the other cultivars (*Table III.2*). In contrast, the mean separation for LER expressed as a percent of control correctly identified the sensitive and tolerant cultivars. Contrary to the chlorophyll assay, however, in the LER assay the intermediate cultivar was grouped with the tolerant ones.

Unlike chlorophyll concentration, the LSD mean separation ranking for LER between cultivars did not depend on the probability used ($p \leq 0.05$ or $p \leq 0.01$). This was observed for LER of seedling regrowth regardless of seedling pre-treatment (Mn-stress and control), and for the relative LER (expressed as percentage of control) of seedling regrowth.

Manganese tolerance as assayed by RRW was significantly correlated ($p \leq 0.01$) with Mn tolerance as assayed by the relative chlorophyll *'a+b'* concentration (expressed as percentage of control) ($R = 0.96$; *Fig. III.5a*), but it was not correlated ($p \leq 0.01$) with the relative LER (expressed as percentage of control) of seedling regrowth ($R = 0.70$; *Fig. III.5b*). A significant correlation ($p \leq 0.01$) was obtained between the product of the relative chlorophyll *'a+b'* concentration (expressed as percentage of

control) and the relative LER (expressed as percentage of control) with RRW (R = 0.98; *Fig. III.6*).

III.4. Discussion

These results clearly indicated a differential effect of Mn on chlorophyll concentration for wheat seedlings of cultivars differing in tolerance to Mn toxicity. Reductions in chlorophyll concentration over a range of Mn concentrations have been reported for wheat (Guerrier, 1988; Ohki, 1985; Wilkinson and Ohki, 1988), but the cultivars used by these researchers were apparently sensitive or intermediate in their tolerance to Mn toxicity. Although chlorosis scores have been used to select for Mn-efficiency (Longnecker *et al.*, 1988), to my knowledge this is the first report in which chlorophyll concentration is shown to be a useful selection criteria for Mn tolerance.

The effect of Mn on chlorophyll concentration could be due to a specific effect on a common precursor for chlorophyll *'a'* and chlorophyll *'b'* biosynthesis. This is indicated by the lack of change in chlorophyll *'a/b'* ratio over the range of Mn concentrations used in both Experiment Nº1 and Experiment Nº2. It has been reported that high Mn concentrations inhibit an Fe-requiring step in chlorophyll synthesis in tobacco callus (Clairmont *et al.*, 1986) and in the cyanobacterium *Anacystis nidulans* (Csatorday *et al.*, 1984), although Nable *et al.*, (1988) failed to find a similar effect in leaves of young tobacco plants. Wilkinson and Ohki (1988) observed that chlorophyll *'a/b'*, chlorophyll *'a'*/carotenoid and chlorophyll *'b'*/carotenoid ratios were unaffected by Mn stress, but suggested the possibility of photo-oxidation of chlorophyll when carotene concentration was deficient (Krinsky, 1966).

Differences in the chlorophyll concentration of wheat cultivars grown under control conditions (*Table III.1*) need not be a problem in selecting cultivars or lines for tolerance to Mn, since a relative chlorophyll concentration can be used (expressed as percentage of control). It would, however, be a problem in selecting Mn tolerant seedlings from a segregating population where tolerance must be evaluated on a single plant in a non-destructive fashion. It is worth pointing out, however, that the lower chlorophyll concentration of Mn tolerant cultivars observed under control conditions in this study (*Table III.1*) might not be the result of genotypic differences in chlorophyll concentration. If the critical concentration for Mn deficiency in Mn-tolerant cultivars is higher than that in Mn-sensitive cultivars, then the tolerant cultivars may be experiencing Mn deficiency when grown in control solutions. Stimulation of growth of

Mn tolerant cultivars at concentrations which are toxic to Mn-sensitive cultivars has been reported (Burke *et al.*, 1990; Macfie *et al.*, 1989).

The leaf elongation rate of monocots over a period of days has been shown to be nearly linear (Edwards, 1967; Wilhelm and Nelson, 1978; Reeder *et al.*, 1984), and very sensitive to a variety of environmental influences (Terry *et al.*, 1983). It has been proven to be a good indicator for detecting differences in the response of genotypes to water stress (Cutler *et al.*, 1980) and to herbicide stress (Bowran and Blacklow, 1987). To my knowledge, this is the first report which has used LER of wheat seedlings during a period of recovery from Mn stress as a parameter for selecting Mn tolerant seedlings. It should be pointed out that a significant correlation ($R = 0.96$) exists between LER and RRW for the Mn-sensitive and Mn-tolerant cultivars. It is the grouping of the Mn-intermediate cultivar which skewed the correlation (*Fig. III.5b*). Since the rate of leaf elongation is constant, it would be possible to take only one measurement at day four or five of regrowth to determine the LER, however, problems of scale would arise if different height genotypes were used in a segregating population.

Evaluation of cultivars tolerant or sensitive to Mn stress as determined by chlorophyll *'a+b'* concentration and LER correlated well with the RRW technique at $p \leq 0.01$ (*Fig. III.5*). However, the ranking of the intermediate cultivar changed depending on the screening technique used. As pointed out by Horst (1982) for Mn tolerance of cowpea and by Taylor and Foy (1985 a,b) for Al tolerance of wheat, ranking of cultivars by a variety of different screening techniques might be affected by factors such as the level of stress, length of treatment, composition of nutrient solution, growth conditions, or genotypes. Perhaps taking both chlorophyll concentration and LER into account may provide a better selection for seedling tolerance to Mn (*Fig. III.6*). This possibility requires further study.

Regardless of the imprecision inherent in screening techniques, selection of extremes is generally not a problem. Manganese tolerance in wheat has been reported to be controlled by a few major genes (Scott and Fisher, 1989) and thus the phenotypic classes arising from a segregating population of a cross between varieties with extreme tolerance and sensitivity would be either tolerant or sensitive, not intermediate. However, since there is a wide range of response to Mn toxicity in wheat (Brauner and Sarruge, 1980; Macfie *et al.*, 1989), many genes could exist to account for this variability and it is possible that Mn tolerance might be of a quantitative nature (see Chapter IV). In this case, intermediate genotypes would arise which may have desirable characteristics.

III.5. Conclusion

To my knowledge this is the first report of a rapid technique for selection of Mn tolerant seedlings of wheat. A technique using application of Mn to the petioles and rating of Mn toxicity symptoms in the leaf has allowed a simple non-destructive screening of cowpea genotypes (Horst, 1982). This technique, however, may not be suitable for cereals. Other screening techniques which have been described are either time consuming (relative root weight, root length), expensive (aconitic acid, fluorescence) or destructive (Mn seed content), and are more suitable for selecting cultivars or lines rather than for selection in segregating populations. The chlorophyll concentration of Mn-stressed seedlings and the LER of seedling regrowth appear to be suitable techniques for screening unreplicated selections of segregating populations for tolerance to Mn. While we have not explored the possibility, these techniques should have potential for use with other cereals.

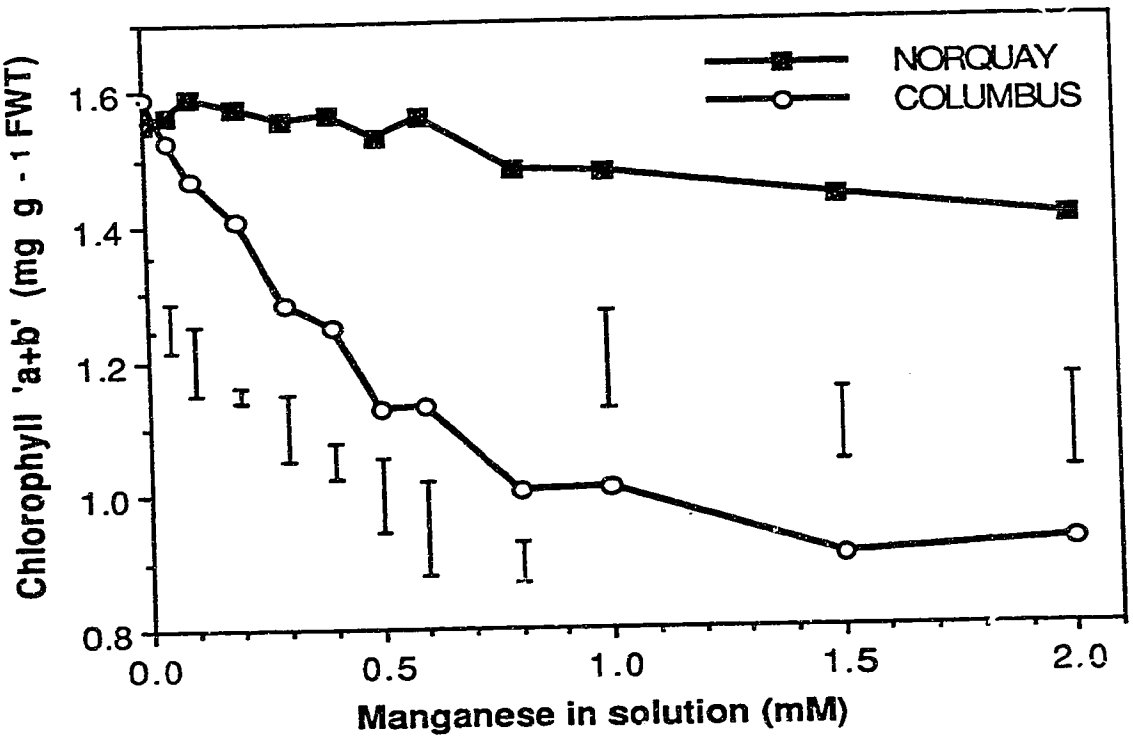

Fig. III.1. The effect of increasing Mn concentrations on chlorophyll *'a+b'* concentration expressed on a fresh weight (FWT) basis for the Mn-tolerant cv Norquay and the Mn-sensitive cv Columbus. Vertical bars represent LSD ($p \leq 0.05$).

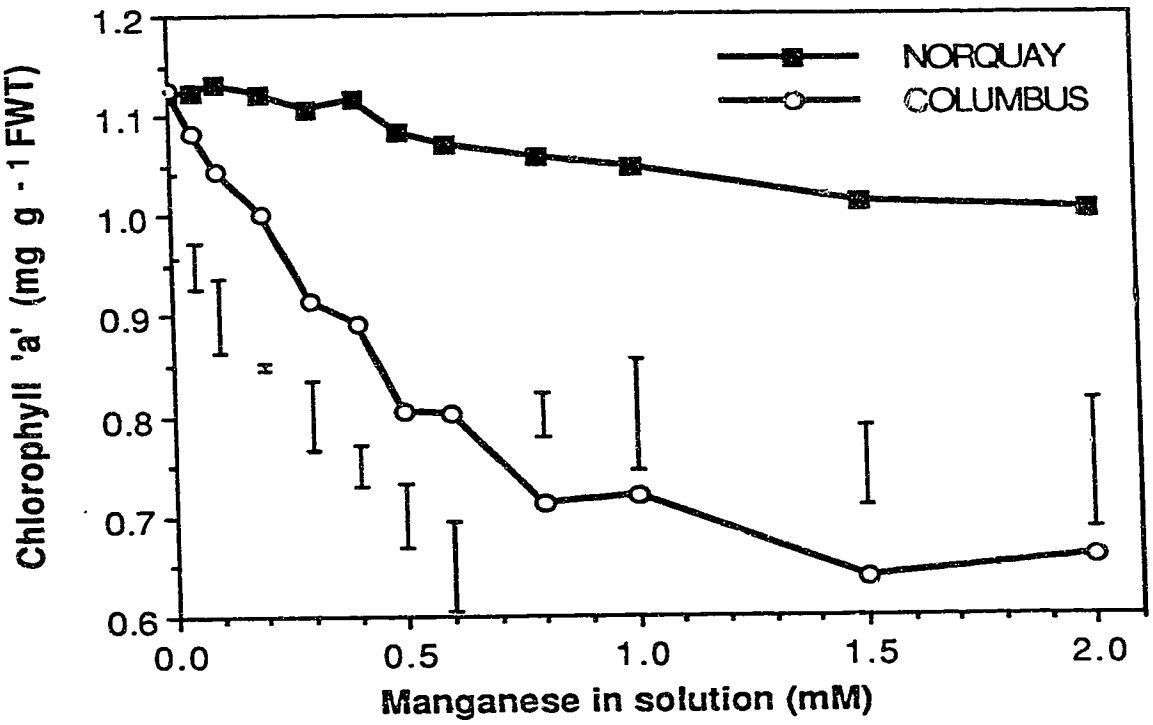

Fig. III.2. The effect of increasing Mn concentrations on chlorophyll *'a'* concentration expressed on a fresh weight (FWT) basis for the Mn-tolerant cv Norquay and the Mn-sensitive cv. Columbus. Vertical bars represent LSD ($p \leq 0.05$).

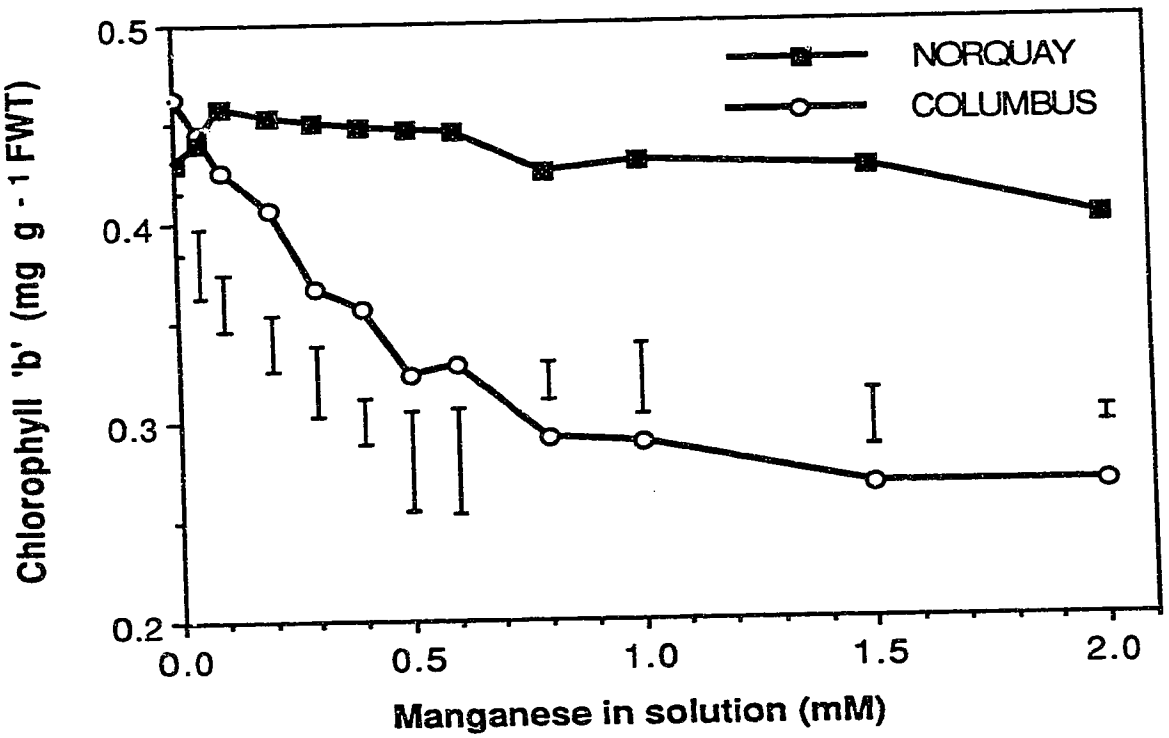

Fig. III.3. The effect of increasing Mn concentrations on chlorophyll *'b'* concentration expressed on a fresh weight (FWT) basis for the Mn-tolerant cv Norquay and the Mn-sensitive cv Columbus. Vertical bars represent LSD ($p \leq 0.05$).

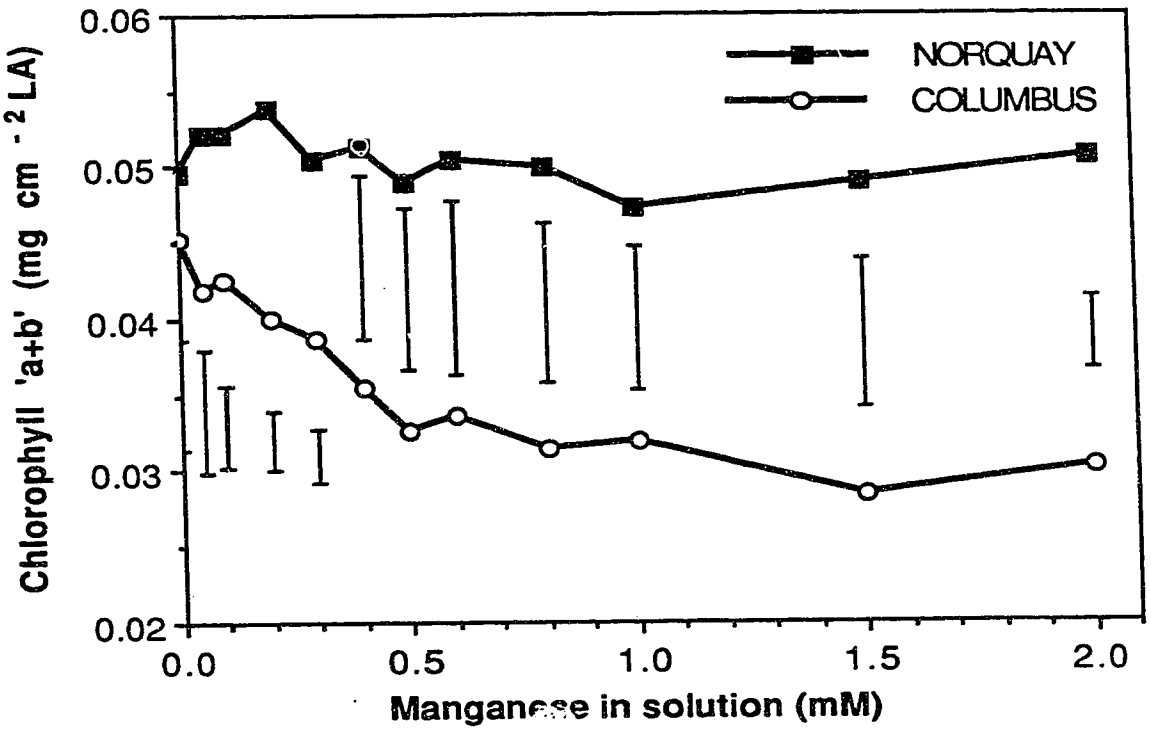

Fig. III.4. The effect of increasing Mn concentrations on chlorophyll *'a+b'* concentration expressed on a leaf area (LA) basis for the Mn-tolerant cv Norquay and the Mn-sensitive cv Columbus. Vertical bars represent LSD ($p \leq 0.05$).

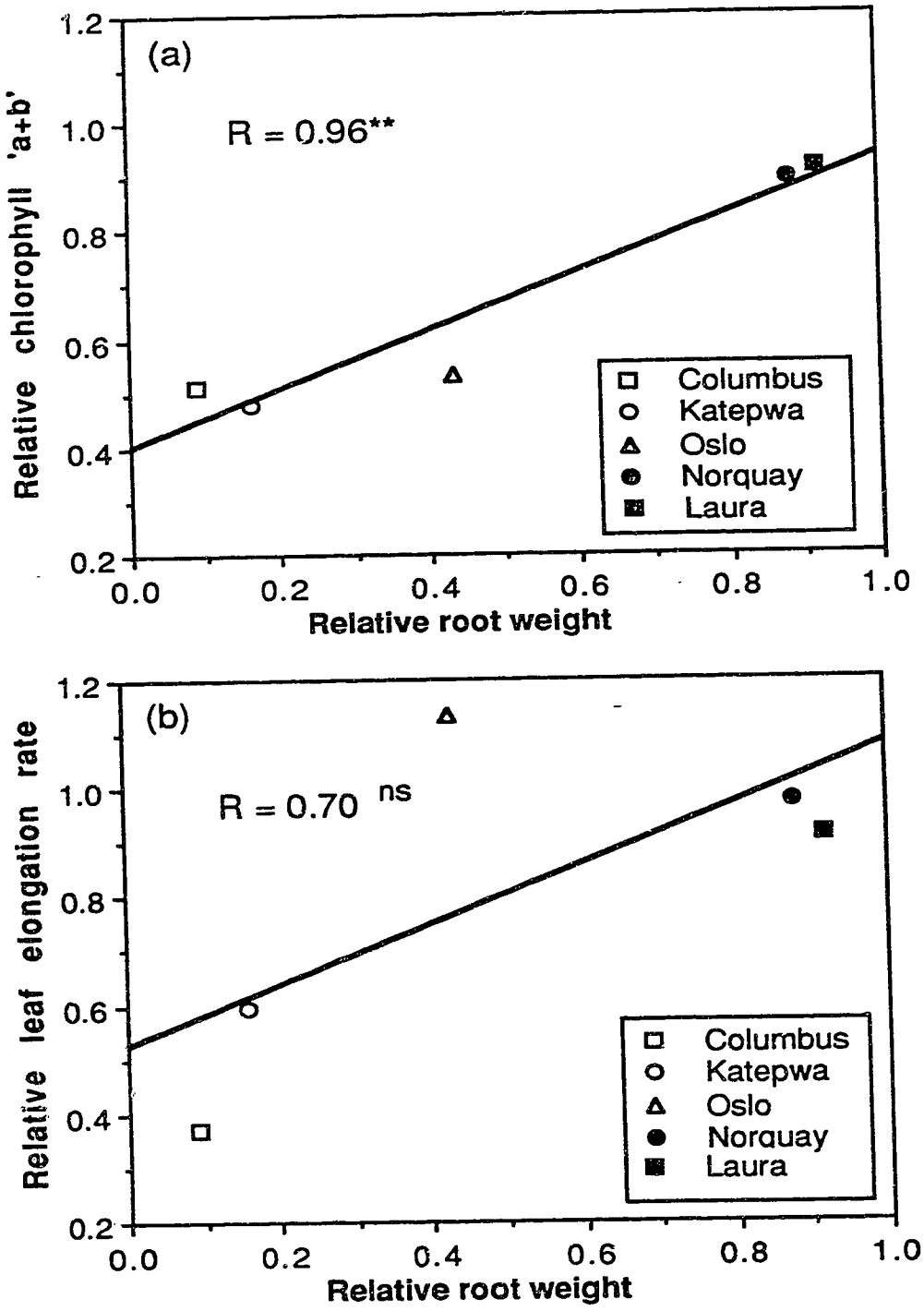

Fig. III.5. Relationship between relative root weight (RRW) and relative chlorophyll *'a+b'* (percent of control) (a) and relative leaf elongation rate (percent of control) (b). (RRW = root weight in the presence of 500 μM Mn, divided by control root weight; data from Macfie *et al.* (1989)).

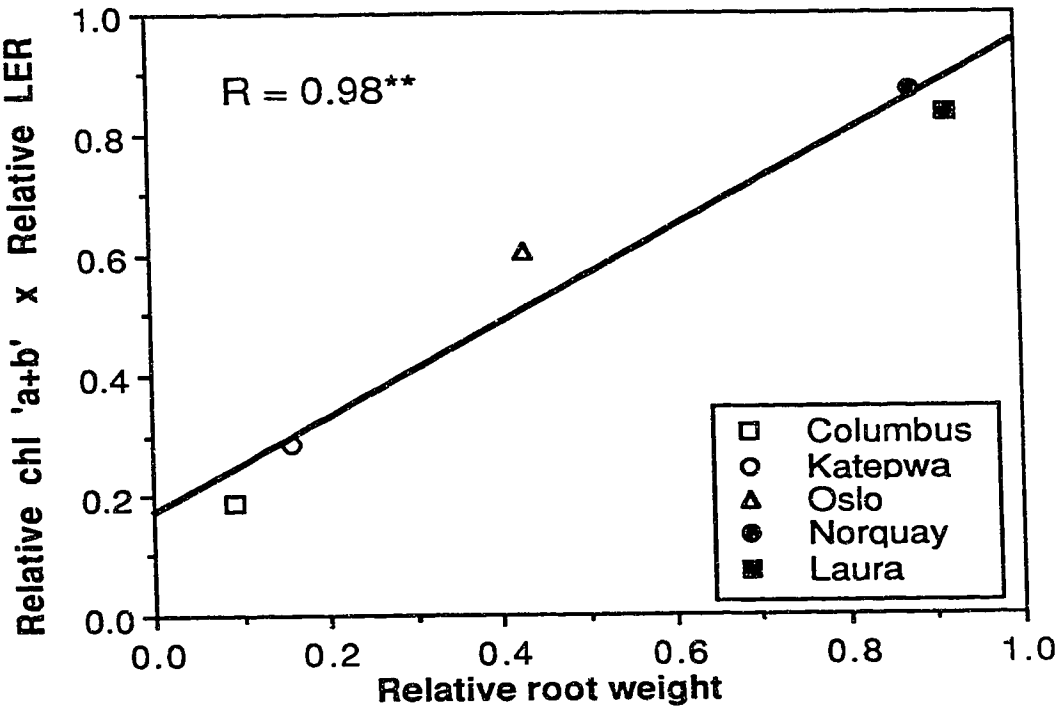

Fig. III.6. Relationship between the product of relative chlorophyll *'a+b'* concentration (percent of control) and relative leaf elongation rate (percent of control) with relative root weight (RRW). (RRW = root weight in the presence of 500 μM Mn, divided by control root weight; data from Macfie *et al.* (1989)).

Table III.1. Mean chlorophyll concentration and composition of wheat seedlings grown in control and 1000 μM Mn nutrient solutions.

Cultivar	Chlorophyll concentration 'a'	'b'	'a+b'	'a/b'
	mg g^{-1} FWT			
Control				
Columbus	1.35	0.46	1.81	2.94
Katepwa	1.43	0.49	1.93	2.93
Oslo	1.51	0.50	2.01	3.00
Norquay	1.28	0.42	1.70	3.07
Laura	1.29	0.44	1.73	2.90
LSD (0.05)	0.14	0.06	0.20	0.15
(0.01)	0.20	0.09	0.28	0.21
	mg g^{-1} FWT			
1000 μM Mn				
Columbus	0.70	0.22	0.92	3.12
Katepwa	0.70	0.23	0.92	3.03
Oslo	0.80	0.27	1.07	3.00
Norquay	1.15	0.37	1.51	3.10
Laura	1.18	0.40	1.58	2.94
LSD (0.05)	0.09	0.04	0.13	0.18
(0.01)	0.13	0.05	0.18	0.26
	%			
Percent of control				
Columbus	52	49	51	106
Katepwa	48	47	48	104
Oslo	53	53	53	100
Norquay	89	89	89	101
Laura	92	90	91	101
LSD (0.05)	10	11	10	10
(0.01)	14	16	14	15

Table III.2. Mean leaf elongation rate (LER) for regrowth of pre-treated, Mn-stressed (1000 μM) and non-stressed (control) seedlings of five wheat cultivars.

Cultivar	Leaf elongation rate — pre-treatment — Control	Mn 1000 μM	Percent of control
	mm/day		%
Columbus	38.7	14.4	36.9
Katepwa	40.5	23.9	59.5
Oslo	21.7	24.3	113.3
Norquay	22.4	21.9	97.6
Laura	38.9	35.6	91.5
LSD (0.05)	4.4	3.2	16.0
(0.01)	6.2	4.6	22.7

III.6. References

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Chapter IV

Heritability and generation mean analysis of manganese tolerance in crosses of spring wheat (*Triticum aestivum* L.) cultivars.

IV.1. Introduction

Nearly half of the world's non-irrigated arable lands are acid soils (Clark, 1982). In addition to aluminum (Al) toxicity, manganese (Mn) toxicity is one of the most important growth limiting factors for crop production in acid soils (Foy, 1984). Interspecific and intraspecific differences in tolerance to Mn have been identified among agriculturally important species, providing breeding potential to develop cultivars adapted to acid soils (Foy *et al.*, 1988). In some cases breeding for tolerance to Mn might be more feasible and economical than the use of soil amendments. This is particularly true in developing countries where more than half of the acid soils are found (Clark, 1982). A primary requirement for such a breeding effort is an understanding of the genetic factors determing Mn tolerance in crops.

To my knowledge, studies on the genetics of Mn tolerance have been reported for only four plant species; lettuce (Eenink and Garretsen, 1977), alfalfa (Dessureaux and Ouellette, 1958; Dessureaux, 1959), soybeans (Brown and Devine, 1980; Heenan *et al.*, 1981) and spring wheat (Camargo, 1983). Camargo (1983) described a cross between a Mn-tolerant × Mn-sensitive wheat cultivar from which a high broad-sense heritability of Mn-tolerance was estimated. Scott and Fisher (1989), Foy *et al.* (1988), and Scott *et al.* (1987) all commented on the genetics of Mn tolerance in wheat, but failed to provide hard data by refering to "unpublished results" and/or "personal communication".

In this report, I describe the results from a genetic study on the inheritance of Mn tolerance in crosses of spring wheat cultivars using a generation mean analysis approach, as well as estimates of dominance and heritability using chlorophyll concentration as a rapid, seedling based, screening bioassay for evaluating the tolerance of wheat seedlings to Mn toxicity (Moroni *et al.*, 1991a).

IV.2. Materials and Methods

IV.2.a. Plant material and Mn tolerance assay

The Canadian spring wheat (*Triticum aestivum* L.) cultivars Columbus and Katepwa were described as Mn-sensitive (tall genotypes), Oslo as intermediate in tolerance to Mn (a semidwarf genotype), and Norquay as Mn-tolerant (a semidwarf genotype) by Macfie *et al.* (1989). The fifth cultivar, Laura, was described as a Mn-tolerant cultivar (tall genotype) by Moroni *et al.* (1991b). These levels of tolerance were determined by the relative root weight method (RRW = root weight in the presence of 500 μM Mn, divided by control root weight; Macfie *et al.* ,1989). In this study the five cultivars were crossed in a half-diallel design with no reciprocals to develop six generations per cross, the parents (P_1, P_2), F_1, F_2 and two backcrosses of the F_1 to the parents (BC_1, BC_2). The crosses were as follows: four Mn-tolerant × Mn-sensitive crosses (Norquay × Columbus, Norquay × Katepwa, Laura × Columbus, and Laura × Katepwa); two Mn-tolerant × Mn-intermediate crosses (Norquay × Oslo, and Laura × Oslo); two Mn-intermediate × Mn-sensitive crosses (Oslo × Columbus, and Oslo × Katepwa); one Mn-tolerant × Mn-tolerant cross (Norquay × Laura); and one Mn-sensitive × Mn-sensitive cross (Katepwa × Columbus).

Sensitivity and tolerance to Mn toxicity were evaluated by chlorophyll concentration of seedlings grown in nutrient solutions as described in Chapter III (Moroni *et al.*, 1991a). Based on earlier results (Moroni *et al.*, 1991a), a Mn concentration of 1000 μM was used as the stress level to determine the differential response to Mn among seedlings. For the assay, each replicate per population consisted of 20 seedlings each of P_1, P_2, F_1, BC_1 and BC_2 and 50 seedlings of F_2. Seeds were surfaced sterilized in 1.2% sodium hypochlorite for 20 minutes and germinated overnight at room temperature in an aerated solution of the systemic fungicide Vitavax (*Carbathiin + Thiram* ; 0.005 gL^{-1}). Germinated seeds were laid on a plastic mesh, crease down, suspended over 10 L of a nutrient solution composed of (μM): Ca, 1000; Mg, 300; NO_3, 2900; and NH_4, 300; and were grown for three days at room temperature. The nutrient solutions were adjusted to pH 4.8 with KOH and HCl and were constantly aerated. For the first two days the containers were covered with black plastic. On the fourth day seedlings were transfered for measuring of Mn tolerance.

Seedlings were mounted in polyurethane foam plugs (which had been pre-sterilized for an hour in an autoclave to prevent seedling toxicity; Moroni *et al.*, 1991a) on a plastic frame suspended over a container filled with 20 L of an aerated nutrient

solution. The nutrient solution contained (μM): Ca, 1000; Mg, 300; K, 800; NO_3, 3600; NH_4, 600; PO_4, 100; SO_4, 101; Cl 34; Na, 20; Fe, 10; B,6; Mn, 2; Zn, 0.5; Cu, 0.15; and Mo, 0.1. Iron was supplied as Fe-EDTA prepared from equimolar amounts of $FeCl_3$ and Na_2EDTA. 1000 µM Mn was superimposed over the nutrient solution, and adjusted to pH 4.8 with KOH and HCl. Seedlings were grown in a temperature controlled growth chamber for six days at a day/night temperature of 21.0/17.0 ± 0.5 °C and a relative humidity of 80/90 ± 5 %. The photosynthetic photon flux density was 307 ± 30 µmol m^{-2} s^{-1}. The experimental design was a randomized block design with 6 generations, and 2 replicates per population tested.

Chlorophyll was determined according to the method of Hiscox and Israelstam (1979). Individual seedlings were cut at the collar of the primary leaf and fresh weight (FWT) was determined. Leaf blades were then cut in small pieces (~1-2 cm), placed in test tubes containing 10 ml of dimethyl sulphoxide (DMSO), and chlorophyll was extracted without maceration by incubating for 3 hrs in a water bath at 65 °C. Tissues, solutions, and extractants were kept under black plastic to minimize light induced chlorophyll degradation. Absorbances were read on a SPECTRONIC 21 Spectrophotometer, (Bausch & Lomb), at 645 and 663 *nm*, and total chlorophyll (chlorophyll *'a+b'*) was estimated using the extinction coefficient equations described by Arnon (1949). Chlorophyll was expressed on a FWT basis.

IV.2.b. Analysis Procedures

The chlorophyll concentration from all generations for each population were subjected to analysis of variance (ANOVA), and generation means were compared using Tukey's test at $p \leq 0.01$ (SAS, 1985). The potence ratio (h), estimated according to Petr and Frey (1966), was used as a net measure of phenotypic dominance (Mather and Jinks, 1982) as:

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

where $\mathbf{X}F_1$, $\mathbf{X}MP$, and $\mathbf{X}HP$ are the means of the F_1, the 2 parents, and the high parent, respectively. Broad-sense heritability (H) was estimated according to Mahmud and Kramer (1951) as:

$$H = [(VF_2^2 - \sqrt{VP_1^2 \times VP_2^2}) / VF_2^2] \times 100$$

where VF_2, VP_1, and VP_2 are the phenotypic variances of the F_2, P_1, and P_2 generations, respectively. Narrow-sense heritability (h^2) was estimated according to Warner (1952) as:

$$h^2 = [2VF_2 - (VBC_1 + VBC_2)] / VF_2$$

where VF_2, VBC_1, and VBC_2 are the phenotypic variances of the F_2, BC_1, and BC_2 generations, respectively. Phenotypic variances were estimated by the corresponding variances within the experimental units. Narrow-sense heritability was estimated on arithmetic and logarithmic transformed data to udjust for unequal variances.

Generation means were subjected to the analysis suggested by Mather and Jinks (1982) to asses inheritance of Mn tolerance. Such an analysis required fulfillment of certain assumptions: 1) homozygosity of the parents, 2) no reciprocal effects, 3) no linkage, and 4) no differential intra-plot competition among generations. Parental cultivars have been subjected to several generations of selfing and the breeder's seed used was assumed to be homozygous and homogeneous. Although reciprocal differences for quantitative characters have been reported for wheat in some studies, in this study they were assumed to be negligible. Three- and six-parameter model procedures were used as outlined by Mather and Jinks (1982). According to Mather (1949), and described by Mather and Jinks (1982), each of the six mean phenotypes per cross (P_1, P_2, F_1, F_2, BC_1, and BC_2) can be described in terms of the midparent (m) which depends on the general conditions of the observations, the additive component [d] and the dominance component [h] where:

[d] = the sum over loci of all d's which measure departure of each homozygote from the midparent m and

[h] = the sum over loci of all h's which measure the departure of the heterozygote from midparent m.

The scaling test for additive gene action developed by Mather (1949) and described by Mather and Jinks (1982) was conducted using the following formulae:

$$A = 2BC_1 - P_1 - F_1$$
$$B = 2BC_2 - P_2 - F_1$$
$$C = 4F_2 - 2F_1 - P_1 - P_2$$

where P_1, P_2, F_1, F_2, BC_1, and BC_2 are the generation means of P_1, P_2, F_1, F_2, BC_1, and BC_2, respectively. If the gene effects are additive on the average, the values should not be significantly different from zero. Inadequacy of the scale is indicated if one or more of the values deviates significantly from zero. The standard errors of the scaling tests were calculated from the within generation variances, deriving sampling variances according to the formulae described by Mather and Jinks (1982) as follows:

$$VA = 4VBC_1 + VP_1 + VF_1$$
$$VB = 4VBC_2 + VP_2 + VF_1$$
$$VC = 16VF_2 + 4VF_1 + VP_1 + VP_2$$

where VP_1, VP_2, VF_1, VF_2, VBC_1, and VBC_2 equal the within generation variances of P_1, P_2, F_1, F_2, BC_1, and BC_2, respectively. The significance ($p \leq 0.05$ and $p \leq 0.01$) of the scaling tests were obtained from a table of normal deviates (*ie.* '*c*' test) (Mather, 1972).

The Joint scaling test proposed by Cavalli (1952) and described by Mather and Jinks (1982) was used to test the fit of the additive-dominance model and to compare with the results of the individual scaling tests. The Joint scaling test is more powerful than the individual tests proposed by Mather (1949) in detecting epistatic effects, since it uses data from all six generations to provide estimates for the mean, additive, and dominance effects. Estimates of the model parameters (m, $[d]$, $[h]$) were obtained from the six equations describing the mean phenotypes by a weighted least squares solution. The six means were weighted by the reciprocal of their corresponding variance. Goodness of fit of the three-parameter model was tested by chi-square with three degrees of freedom. Failure of the model (m, $[d]$, $[h]$) indicates the possibility of epistasis. For crosses where epistasis is implicated, Jinks and Jones (1958) proposed a six-parameter model, as described by Mather and Jinks (1982), to determine the adequacy of a digenic epistatic model. The six estimates of the model are defined as follows:

m	=	midpoint (between *AA* and *aa*)
$[d]$	=	difference of *AA* and *aa* from midparent value
$[h]$	=	difference of *Aa* from midparent value
$[i]$	=	homozygote × homozygote interaction
$[j]$	=	homozygote × heterozygote interaction
$[l]$	=	heterozygote × heterozygote interaction

and are estimated based on the formulae:

$$m = {}^1/_2P_1 + {}^1/_2P_2 + 4F_2 - 2BC_1 - 2BC_2$$
$$[d] = {}^1/_2P_1 - {}^1/_2P_2$$
$$[h] = 6BC_1 + 6BC_2 - 8F_2 - F_1 - 1{}^1/_2P_1 - 1{}^1/_2P_2$$
$$[i] = 2BC_1 + 2BC_2 - 4F_2$$
$$[j] = 2BC_1 - P_1 - 2BC_2 + P_2$$
$$[l] = P_1 + P_2 + 2F_1 + 4F_2 - 4BC_1 - 4BC_2$$

where P_1, P_2, F_1, F_2, BC_1, and BC_2 are the generation means of P_1, P_2, F_1, F_2, BC_1, and BC_2, respectively. The sampling variances of these estimates were obtained by squaring the corresponding standard errors of the within generation variances. Significance of gene effects ($p \leq 0.05$ and $p \leq 0.01$) were determined by *t*-test. Calculations for the Joint scaling test and for the estimates of the three- and six-parameter models were performed with a Lotus 1-2-3 (Lotus Dev. Corp., 1987) generation mean analysis microcomputer program kindly provided by Dr T.J. Ng (Ng, 1990).

IV.3. Results and Discussion

IV.3.a. Populations response to Mn stress

Manganese toxicity symptoms were observed primarily in seedlings of Mn-sensitive cultivars, and in seedlings of segregating generations. Symptoms observed included leaf chlorosis, stiffness of leaf tissue, white flecking, leaf tip burn and, occasionally, leaf purpling. These symptoms were similar to those previously described by Keisling *et al.* (1984) and Ohki (1984).

Generation means for chlorophyll concentration of the ten wheat crosses are shown in *Table IV.1*. Analysis of variance indicated that significant differences existed among generation means for chlorophyll concentration for the ten crosses of five wheat cultivars differing in Mn tolerance (*Table IV.1*). In the four Mn-tolerant × Mn-sensitive crosses (Norquay × Columbus, Norquay × Katepwa, Laura × Columbus, and Laura × Katepwa) and in the two Mn-tolerant × Mn-intermediate crosses (Norquay × Oslo, and Laura × Oslo), the chlorophyll concentration of the Mn-tolerant parent was always significantly higher ($p \leq 0.01$) than the Mn-sensitive and Mn-intermediate parent (*Table IV.1*). Furthermore, in the two Mn-intermediate × Mn-sensitive crosses

(Oslo × Columbus, and Oslo × Katepwa) the chlorophyll concentration of the Mn-intermediate parent was always significantly higher ($p \leq 0.01$) than the Mn-sensitive parent (*Table IV.1*). In the Mn-tolerant × Mn-tolerant cross (Norquay × Laura) and Mn-sensitive × Mn-sensitive cross (Katepwa × Columbus) the chlorophyll concentration of the parents were not significantly different ($p \leq 0.01$) (*Table IV.1*). In this case, non significance may be ascribed to the narrow ranges between parental values for chlorophyll concentration. These results indicate the validity of using chlorophyll concentration as a measure of Mn tolerance in wheat (Moroni *et al.*, 1991a), since this method is able to differentiate between the cultivars differing in Mn tolerance as determined by the relative root weight method (Macfie *et al.*, 1989; Chapter II).

Progeny distribution for the six generations of the Mn-tolerant × Mn-sensitive, Mn-tolerant × Mn-intermediate and Mn-intermediate × Mn-sensitive crosses indicated a continuous distribution of Mn tolerance as assayed by chlorophyll concentration. This is exemplified by the frequency distribution of the six generations of the crosses Norquay × Columbus (*Table IV.2*) and Laura × Columbus (*Table IV.3*), (Mn-tolerant × Mn-sensitive crosses). In these two crosses where the cultivars have extreme Mn tolerance and Mn sensitivity, distinct reaction classes were not observed. This is in contrast to other reports which indicated that variation in Mn tolerance is controlled by a few genes (Foy *et al.*, 1988; Scott and Fisher, 1989). For example, a cross of Carazinho × Teal (Mn-tolerant × Mn-sensitive; Foy *et al.*, 1988) indicated that the Brazilian cultivar Carazinho had two major genes for Mn tolerance when compared to the cultivar Teal, with the appearance of distinct reaction classes in the frequency distribution. Unfortunately no indication as to the screening technique used, no details of the frequency distributions were given by the authors. It should be pointed out that one of the ancestral parents of Laura is Carazinho, and that the progenitors of Mn tolerance of Laura, Norquay, and Carazinho may be the Brazilian land races Polysuu and/or Alfredo Chavez 6.21 (Chapter II).

IV.3.b. Dominance estimates

Estimates of dominance for the ten crosses are presented in *Table IV.4*. Negative or positive dominance values (potence ratio) indicate the direction of dominance towards the respective parent. Dominance values for three of the four Mn-tolerant × Mn-sensitive crosses (Norquay × Columbus, Norquay × Katepwa, and Laura × Katepwa) were low and positive, while for the other Mn-tolerant × Mn-sensitive cross (Laura × Columbus) the dominance value was also low, but negative (*Table IV.4*). The two Mn-

tolerant × Mn-intermediate crosses showed differing dominance values; the Norquay × Oslo cross showed positive overdominance, the Laura × Oslo cross showed negative negligible dominance (*Table IV.4*). For the two Mn-intermediate × Mn-sensitive crosses, the Oslo × Columbus cross showed partial dominance while the Oslo × Katepwa cross showed low dominance, both values being positive (*Table IV.4*). The dominance for the Norquay × Laura cross (Mn-tolerant × Mn-tolerant) showed overdominance and positive, while for the Katepwa × Columbus cross (Mn-sensitive × Mn-sensitive) it showed high and positive dominance (*Table IV.4*).

The indication of overdominance as indicated by the potence ratio method for the Norquay × Oslo and Norquay × Laura crosses may not be realistic, since differences in magnitude between the F_1 generations and parentals are small (*Table IV.1*), suggesting overdominance, but not as high as the potence ratio method showed. As described in Materials and Methods, the potence ratio method is the ratio of the two differences (XF_1 - XMP) to (XHP - XMP). In crosses where the parental and F_1 generation means were nearly equal, a relatively small error in estimation could magnify the potence ratio value (Petr and Frey, 1966). This appeared to be the case for the two crosses were overdominance was observed. It should be pointed out that dominance values for quantitative attributes give only an average effect of the genes involved in expression. In general, F_1 generations were not higher than the parent with more tolerance to Mn (*Table IV.1*), indicating that heterosis did not occur. An exception to this observation is the Mn-tolerant × Mn-tolerant cross (Norquay × Laura) where the F_1 generation is higher then either parent (*Table IV.1*). The magnitude of the differences, however, is insignificant from the stand point of breeding. Furthermore, the low dominance estimates for the crosses where the parental had extreme tolerance and sensitivity indicate that dominance is not a major effect in the inheritance of tolerance in this genetic material, and might not be an obstacle in a breeding program for Mn tolerance.

IV.3.c. Broad-sense heritability estimates

Heritability percentages in the broad-sense are also shown in *Table IV.4*. The broad-sense heritability percentages were high for the four Mn-tolerant × Mn-sensitive crosses (Norquay × Columbus, Norquay × Katepwa, Laura × Columbus, and Laura × Katepwa), and the two Mn-tolerant × Mn-intermediate crosses (Norquay × Oslo, and Laura × Oslo) ranging from 49% to 78% (*Table IV.4*). For one of the Mn-intermediate × Mn-sensitive crosses (Oslo × Columbus) the broad-sense heritability was low and positive, but for the other Mn-intermediate × Mn-sensitive cross (Oslo ×

Katepwa) the broad-sense heritability was negligible (*Table IV.4*). In the case where the parents of the cross did not differ in Mn tolerance ($p \leq 0.01$), broad-sense heritabilities were opposite in value and sign; for the Norquay × Laura cross it was negligible and positive (6%), for the Katepwa × Columbus cross it was large and negative (-57%; *Table IV.4*).

As expected, were the parentals differed widely in Mn tolerance, broad-sense heritabilities were high. Where parentals showed similar tolerance characteristics, broad-sense heritability estimates were low and even negative. These results suggest that the variability found in these populations were in great part due to genetic origin. Camargo (1983) reported also high broad-sense heritability of a cross of BH-1146 × Siete Cerros (Mn-sensitive × Mn-tolerant) using the length of the central primary root of plants to evaluate the levels of tolerance to Mn. The Mn-tolerance of Siete Cerros can be traced back to the same progenitors of the Mn tolerance observed in Laura, Norquay, and Carazinho, namely the Brazilian land races Polysuu and/or Alfredo Chavez 6.21 (Chapter II).

IV.3.d. Narrow-sense heritability estimates

Narrow-sense heritability estimates calculated on the basis of original scale and logarithmic transformation are shown in *Table IV.4*. Several trends are noteworthy. In the Mn-tolerant × Mn-sensitive crosses were Norquay is the Mn-tolerant parent, (Norquay × Columbus, and Norquay × Katepwa), and in both Mn-tolerant × Mn-intermediate crosses (Norquay × Oslo, and Laura × Oslo), the narrow-sense heritability estimates were very high (for some even greater than the theoretical, *ie.* 1.00) and did not differ much, regardless of the scale used (*Table IV.4*). Thus, additive gene action is a major component of the variability found for this gerplasm, indicating that it should be suitable for selection in a breeding program. This is in contrast to the Mn-tolerant × Mn-sensitive crosses were Laura was the Mn-tolerant parent (Laura × Columbus, and Laura × Katepwa). In this case, the narrow-sense heritability estimates were low regardless the scales used. Moreover, in the logarithmic scale, the estimates were about 100% more than in the arithmetic scale (*Table IV.4*). These results indicate that non-additive gene action might be of great importance in the variability found in these populations, making it difficult to select for Mn tolerance in a breeding program. For the remainder crosses (Mn-tolerant × Mn-tolerant, Mn-intermediate × Mn-sensitive, and Mn-sensitive × Mn-sensitive) the narrow-sense heritability estimates were low and mostly negative (*Table IV.4*).

As observed by Ketata *et al.* (1976) heritability estimates greater than the theoretical limit, *ie.* greater than 1.00, as observed in some of the crosses, *eg.* Norquay × Katepwa, Norquay × Oslo, may be ascribed to several causes. Sampling errors, differential responses of the F_2 *vs.* the backcrosses to the environment, and non-allelic interactions can result in an upward bias of the narrow-sense heritability estimates as measured by Warner's method (Warner, 1952). Furthermore, heritability estimates tend to be high for characters which display wide differences between parental means (Ketata *et al.*, 1976; Merrit, 1988), as was the case in some of the crosses of this study. Furthermore, genotype-environment interactions, which could not be evaluated in this study, may have biased the estimates of heritability. To my knowledge there are no reports of narrow-sense heritability estimates for Mn tolerance of wheat or any other crop species.

IV.3.e. Generation mean analysis estimates

Generation mean analysis is normally used to analyze crosses between two parents which differ widely in the characters under analysis. Those crosses where the tolerance of the parents did not differ significantly ($p \leq 0.01$) were not subjected to the scaling tests or to generation mean analysis (Mn-tolerant × Mn-tolerant cross (Norquay × Laura) and Mn-sensitive × Mn-sensitive cross (Katepwa × Columbus); *Table IV.1*). For the remaining eight crosses, the results of the A, B, and C individual tests, and joint scaling test, are shown in *Table IV.5*, and the estimates of gene effects using a three- and six-parameter model are shown in *Table IV.6* and *Table IV.7*, respectively.

Significance of any of the individual scaling tests (A, B, or C) indicates epistasis on the scale of measurement used. However, since each test has its own expectations in terms of type and magnitude of epistasis effects, agreement should not necessarily be expected among tests. The joint scaling test is more powerful than the other tests in detecting epistasis, since it uses information from all six generations. In this study, the joint scaling test detected epistasis whenever it was declared significant by any of the individual scaling tests (*Table IV.5*).

The joint scaling test, and one or more of the individual scaling tests were statistically significant for all but the Norquay × Columbus cross, where neither test was statistically significant (*Table IV.5*). Thus, based on the individual and joint scaling tests (*Table IV.5*), a three-parameter model proved to be satisfactory in explaining the genetic differences for Mn-tolerance of the Norquay × Columbus cross (*Table IV.6*). This indicates that epistasis is not involved in the inheritance of Mn-tolerance of this cross.

In this study, additive, [*d*], gene effects for the Norquay × Columbus cross seemed to be the most important factor (significant at $p \leq 0.01$) contributing to the genetic control of Mn tolerance, while dominance, [*h*], gene effects were smaller and positive ($p \leq 0.01$) (*Table IV.6*).

Because of the presence of epistasis as detected by the significance of the scaling tests, the three-parameter model was not sufficient to explain the genetic variation for the other crosses. Therefore, the six-parameter model was invoked to determine the type and magnitude of the gene action involved in the inheritance of Mn tolerance in those crosses (*Table IV.7*). In the Norquay × Katepwa cross (Mn-tolerant × Mn-sensitive) only additive, [*d*], and additive × dominance, [*j*], gene effects were statistically significant ($p \leq 0.01$), while, in the Norquay × Oslo cross (Mn-tolerant × Mn-intermediate) additive, [*d*], dominance, [*h*], and additive × additive, [*i*], gene effects were significant ($p \leq 0.01$). Furthermore, in this cross (Norquay × Oslo) there was also significant dominance × dominance, [*l*], gene effects with negative sign (*Table IV.7*). For the Norquay × Oslo cross dominance, [*h*], towards the Mn-tolerant parent and significant negative dominance × dominance, [*l*], gene effects indicated a duplicate type of epistasis for Mn tolerance, suggesting that difficulty would be encountered in selecting for Mn tolerance from this cross.

In the three crosses were Laura was the Mn-tolerant parent, (Laura × Columbus, Laura × Katepwa, and Laura × Oslo), significant gene effects were detected for all but the additive × dominance, [*j*], parameter; all parameters were positive except for dominance × dominance, [*l*], gene effects which was negative. In general, dominance, [*h*], gene effects were larger in magnitude than additive, [*d*], gene effects for all three crosses (*Table IV.7*). For these crosses dominance, [*h*], towards the Mn-tolerant parent and significant negative dominance × dominance, [*l*], gene effects indicated a duplicate type of epistasis for Mn tolerance, again suggesting that difficulty would be encountered in selecting for Mn tolerance from these crosses.

In the Mn-intermediate × Mn-sensitive crosses (Oslo × Columbus, and Oslo × Katepwa) significant additive, [*d*], dominance, [*h*], and additive × dominance, [*j*], gene effects were detected for both crosses, and all were positive. Only for the Oslo × Katepwa cross was there significant additive × additive, [*i*], and dominance × dominance, [*l*], gene effects. In this cross, the dominance × dominance, [*l*], gene effect was, as for previous crosses, negative (*Table IV.7*). This, combined with the dominance, [*h*], towards the more Mn-tolerant parent, indicates that a duplicate type of epistasis exists in this material. Again, difficulty may be encountered in selecting for Mn tolerance from this cross.

The prevalence of non-allelic interactions where Laura (Mn-tolerant) and Oslc (Mn-intermediate) served as a tolerant or intermediate parent, and the minimal detection of non-allelic interactions where Norquay (Mn-tolerant) served as the tolerant parent, may suggest a different genetic control for Mn tolerance between these cultivars. Although the progenitors for Mn tolerance of Laura and Norquay can be traced back to a common source, this character have been inherited through different lineage (Chapter II).

In general the more important epistatic effect detected by the six-parameter model was of the dominance × dominance, [*l*], type. The general nonsignificance in this study of the additive × dominance, [*j*], effects, may be due to the canceling of positive and negative effects from different loci. The estimates of epistasis as well as dominance and additive gene action may have been influenced by genotype-environment interactions in both the three- and six-parameter models. The possible importance of genotype-environment interactions could be determined by conducting tests in several environments. Foy *et al.* (1988) previously suggested that the level of dominance of Mn tolerance in wheat should be used cautiously because of possible variation in performance of the F_1 at different levels of Mn stress.

The few reports in the literature on the inheritance of Mn tolerance in plants indicate that this character is quantitative in nature with additive gene effects playing a major role. Manganese tolerance in lettuce seems controlled by one to four genes, depending the species used (Eenink and Garretsen, 1977). In lucerne, Mn tolerance has been attributed to additive gene effects with little or no dominace (Dessureaux, 1959), and in soybean Brown and Devine (1980) concluded that Mn tolerance was multigenic rather than controlled by one single locus. Heenan *et al.* (1981) also concluded that minor genes were important in the inheritance of Mn tolerance in soybeans, but they suggested that a single gene may influence Mn tolerance in the Bragg × Amredo cross (Mn-sensitive × Mn-tolerant). Furthermore, reciprocal differences in progeny suggested that cytoplasmic inheritance may also influence Mn tolerance in soybeans (Brown and Devine, 1980).

In wheat a wide range of tolerance to Mn toxicity (Macfie *et al.*, 1989; Chapter II) and symptoms of Mn toxicity (Keisling *et al.*, 1984; Ohki, 1984) indicate that many genes with small effects could exist to account for this variability. Nontheless, Foy *et al.* (1988), and Scott and Fisher (1989), citing unpublished data, have reported that several major genes can account for a substantial proportion of the variation in Mn tolerance. Results from this study appear contradictory to this view, and indicate that Mn tolerance in spring wheat is quantitatively inherited.

IV.4. Conclusion

The results of this study indicate that Mn tolerance in wheat is a heritable character which could be selected successfully in a backcrossing program, providing that the appropiate parent for Mn tolerance is used. Furthermore, Mn tolerance would appear to be quantitatively inherited in the crosses reported here. This is supported by (1) the continuous frequency distribution of seedlings in tolerance to Mn toxicity as determined by the seedling chlorophyll concentration technique of segregating generations and exemplified by the Norquay × Columbus cross (*Table IV.2*) and Laura × Columbus cross (Table *IV.3*), (2) the similarity of the F_1 and F_2 means, and (3) the high and significant additive, [*d*], gene action.

A preponderance of additive effects coupled with high heritability and small dominance (potence ratio) estimates obtained in this study indicate that selection for this character should be highly effective in early generations, particularly where Norquay was the Mn tolerant parent.

Table IV.1. Generation means for chlorophyll concentration of ten crosses of five wheat cultivars differing in tolerance to Mn.

	cultivar crosses									
Generation	Norquay (T§) × Columbus (S)	Norquay (T) × Katepwa (S)	Norquay (T) × Oslo (I)	Norquay (T) × Laura (T)	Laura (T) × Columbus (S)	Laura (T) × Katepwa (S)	Laura (T) × Oslo (I)	Oslo (I) × Columbus (S)	Oslo (I) × Katepwa (S)	Katepwa (S) × Columbus (S)
	chlorophyll content (μg g^{-1} FW)									
P_1	1016.7 *a*‡	1028.1 *a*	1066.6 *bc*	1082.2 *c*	999.9 *a*	1091.4 *a*	1135.7 *ab*	1060.1 *a*	1100.5 *a*	810.3 *bc*
BC_1	972.5 *a*	1049.8 *a*	1135.1 *a*	1168.9 *ab*	902.8 *b*	1083.7 *a*	1188.0 *a*	1080.1 *a*	1131.1 *a*	876.9 *a*
F_1	883.2 *b*	931.4 *b*	1122.5 *ab*	1184.0 *a*	753.5 *c*	988.9 *b*	1088.3 *bc*	993.3 *b*	1006.9 *b*	806.3 *bc*
F_2	861.8 *b*	923.4 *b*	1024.6 *cd*	1103.9 *c*	742.6 *c*	941.2 *b*	1042.2 *c*	952.4 *b*	991.4 *b*	795.3 *c*
BC_2	778.0 *c*	826.0 *c*	1117.1 *ab*	1198.7 *a*	705.3 *c*	955.0 *b*	1136.1 *ab*	860.6 *c*	957.2 *b*	853.1 *ab*
P_2	658.1 *d*	711.9 *d*	993.6 *d*	1125.6 *bc*	599.6 *d*	819.1 *c*	1047.1 *c*	783.4 *d*	871.5 *c*	781.4 *c*

§, T = Mn-Tolerant; I = Mn-Intermediate; S = Mn-Sensitive.
‡, Means within a column of each cross followed by the same letter are not significantly different at the 0.01 probability level according to Tukey's Studentize multiple range test.

Table IV.2. Frequency distribution of chlorophyll concentration of wheat seedlings grown in 1000 µM Mn for six generations of the population derived from the wheat cross of Norquay (P_1) × Columbus (P_2) (Mn-tolerant × Mn-sensitive, respectively).

	range of chlorophyll content (µg g^{-1} FW)																							
Generation	550 575	575 600	600 625	625 650	650 675	675 700	700 725	725 750	750 775	775 800	800 825	825 850	850 875	875 900	900 925	925 950	950 975	975 1000	1000 1025	1025 1050	1050 1075	1075 1100	1100 1125	1125 1150
P_1												1	2	6	2	2	4	9	13	4	4	2	1	1
BC_1												4	9	6	8	7	4	3	5	4	1	3	1	1
F_1					1					2	1	7	12	6	6	5	3	1						
F_2				1		3	4	4	4	12	7	7	12	6	6	7	7	2	1	3	1	1	1	
BC_2			1	1		2	8	5	6	4	2	4	2	2	1	1					1			
P_2	3	2	6	4	6	12	4	2		1														

Table IV.3. Frequency distribution of chlorophyll concentration of wheat seedlings grown in 1000 µM Mn for six generations of the population derived from the wheat cross of Laura (P_1) × Columbus (P_2) (Mn-tolerant × Mn-sensitive, respectively).

	range of chlorophyll content (µg g^{-1} FW)																													
Generation	450 475	475 500	500 525	525 550	550 575	575 600	600 625	625 650	650 675	675 700	700 725	725 750	750 775	775 800	800 825	825 850	850 875	875 900	900 925	925 950	950 975	975 1000	1000 1025	1025 1050	1050 1075	1075 1100	1100 1125
P_1								1								1		2	1	3	2	7	6	7	5	3	2
BC_1										1	2	1		3	2	1	5	5	5	2	2	3	4		2	1	1
F_1									2	8	7	5	5	2	4	4	3										
F_2	1			1		6	4	7	8	7	12	12	8	6	6	7	1	6	2	2	3	1					
BC_2				1	1	7		2	4	4	4	4	4	3	1	3	2										
P_2			2	3	5	9	10	5	3	2																	

Table IV.4. Potence ratio (h) (Petr and Frey, 1966), broad-sense (H) heritability (Mahmud and Kramer, 1951), and narrow-sense (h^2) heritability (Warner, 1952) estimates for ten crosses of five wheat cultivars differing in tolerance to Mn.

	cultivar crosses									
	Norquay (T§) × Columbus (S)	Norquay (T) × Katepwa (S)	Norquay (T) × Oslo (I)	Norquay (T) × Laura (T)	Laura (T) × Columbus (S)	Laura (T) × Katepwa (S)	Laura (T) × Oslo (I)	Oslo (I) × Columbus (S)	Oslo (I) × Katepwa (S)	Katepwa (S) × Columbus (S)
	dominance estimates									
h	0.26	0.39	2.53	3.68	-0.23	0.25	-0.07	0.52	0.18	0.72
	heritability estimates									
H	78	73	49	6	64	58	62	28	-8	-57
h^2										
Original scale	0.79	1.09	1.14	-0.49	0.18	0.16	0.72	-0.04	-0.98	-1.07
Log scale	0.78	1.01	1.32	-0.11	0.40	0.45	1.02	0.16	-0.41	-0.57

§, T = Mn-Tolerant; I = Mn-Intermediate; S = Mn-Sensitive.

Table IV.5. Individual tests (A B C) (Mather, 1949) and Joint scaling test (as proposed by Cavalli (1952) and described by Mather and Jinks (1982)) of the fit of the additive-dominant genetic model for ten crosses of five wheat cultivars differing in tolerance to Mn.

	cultivar crosses							
	Norquay (T§) × Columbus (S)	Norquay (T) × Katepwa (S)	Norquay (T) × Oslo (I)	Laura (T) × Columbus (S)	Laura (T) × Katepwa (S)	Laura (T) × Oslo (I)	Oslo (I) × Columbus (S)	Oslo (I) × Katepwa (S)
	Individual scaling tests							
A	45.0 (±26.5)	140.2** (±37.0)	81.0* (±37.2)	52.2 (±37.1)	87.0* (±36.3)	152.1** (±35.3)	106.7** (±31.8)	154.7** (±53.3)
B	14.7 (±29.4)	8.7 (±39.5)	118.1** (±44.1)	57.5 (±30.9)	102.0** (±34.5)	136.7** (±39.5)	-55.5* (±25.9)	36.0 (±41.2)
C	5.9 (±48.3)	90.6 (±71.2)	-206.7** (±76.1)	-136.1** (±47.8)	-123.4* (±48.6)	-190.8** (±64.5)	-20.6 (±39.9)	-20.2 (±63.5)
	Joint scaling test							
$\chi^2_{[3]}$	3.0	14.8	29.3	17.2	25.2	67.1	17.9	11.3
p	0.50-0.30	0.01-0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.02-0.01

§, T = Mn-Tolerant; I = Mn-Intermediate; S = Mn-Sensitive.
*, **, Significantly different from zero at the 0.05 and 0.01 probability level according to *c*-test.

Table IV.6. Estimates of the gene effects using a three-parameter model (as proposed by Cavalli (1952) and described by Mather and Jinks (1982)) on means of parents, F_1, F_2, and backcrosses for ten crosses of five wheat cultivars differing in tolerance to Mn.

	cultivar crosses							
Parameter	Norquay (T§) × Columbus (S)	Norquay (T) × Katepwa (S)	Norquay (T) × Oslo (I)	Laura (T) × Columbus (S)	Laura (T) × Katepwa (S)	Laura (T) × Oslo (I)	Oslo (I) × Columbus (S)	Oslo (I) × Katepwa (S)
m	839.3** (±5.1)	879.5** (±8.2)	1035.6** (±11.3)	797.7** (±6.9)	957.3** (±7.2)	1093.8** (±7.5)	918.3** (±6.7)	989.1** (±10.8)
[*d*]	180.6** (±5.1)	169.2** (±8.1)	35.5** (±11.0)	198.2** (±7.0)	134.4** (±7.4)	47.2** (±7.3)	150.6** (±6.9)	119.0** (±10.7)
[*h*]	49.9** (±10.2)	81.7** (±17.7)	98.1** (±21.0)	-46.5** (±11.9)	33.5** (±11.3)	20.2 (±17.5)	74.9** (±12.0)	30.2 (±21.9)

§, T = Mn-Tolerant; I = Mn-Intermediate; S = Mn-Sensitive.
**, Significantly different from zero at the 0.01 probability level according to *c*-test.

Table IV.7. Estimates of the gene effects using a six-parameter model (as proposed by Jinks and Jones (1958) and described by Mather and Jinks (1982)) on means of parents, F_1, F_2, and backcrosses for ten crosses of five wheat cultivars differing in tolerance to Mn.

	Wheat crosses							
Parameter	Norquay (T§) × Columbus (S)	Norquay (T) × Katepwa (S)	Norquay (T) × Oslo (I)	Laura (T) × Columbus (S)	Laura (T) × Katepwa (S)	Laura (T) × Oslo (I)	Oslo (I) × Columbus (S)	Oslo (I) × Katepwa (S)
m	791.7** (±56.5)	811.7** (±74.5)	620.1** (±78.1)	554.0** (±60.6)	642.8** (±63.6)	611.8** (±60.6)	849.9** (±48.2)	775.2** (±69.7)
[*d*]	179.3** (±5.4)	158.1** (±8.8)	36.5** (±12.5)	200.2** (±7.5)	136.2** (±7.9)	44.3** (±7.8)	138.3** (±7.6)	114.5** (±11.9)
[*h*]	196.9 (±139.4)	326.8 (±181.2)	1111.4** (±189.2)	554.9** (±157.2)	847.5** (±164.9)	1244.9** (±183.3)	266.6* (±126.5)	633.3** (±189.5)
[*i*]	45.7 (±56.3)	58 (±74.0)	410.0** (±77.1)	245.8** (±60.2)	312.4** (±63.1)	479.6** (±60.1)	71.9 (±47.5)	210.8** (±68.6)
[*j*]	30.3 (±37.4)	131.5** (±47.9)	-37.1 (±51.8)	-5.4 (±46.4)	-15.0 (±48.8)	15.3 (±43.0)	162.2** (±38.7)	118.7* (±60.2)
[*l*]	-105.4 (±86.5)	-207.1 (±114.1)	-609.0** (±118.4)	-355.5** (±99.9)	-501.4** (±104.3)	-768.2** (±102.8)	-123.2 (±81.6)	-401.5** (±127.7)

§, T = Mn-Tolerant; I = Mn-Intermediate; S = Mn-Sensitive.
*, **, Significantly different from zero at the 0.05 and 0.01 probability level according to *t*-test.

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Chapter V

Summary

V.1. General discussion

Devine (1982) has described four prerequisites to undertake a breeding program for specific edaphic adaptation. These four prerequisites are as follows: (a) that techniques are available to assay plant response to the particular edaphic stress, (b) that there is useful genetic variation for the plant characteristics needed either in agronomically suited cultivars or in noncultivated forms of the crop species or in related species, (c) that the character is heritable, and finally (d) that the estimated degree of improvement in adaptation (determined from the range of variation, and heritability) is sufficient to be of applied use (Devine, 1982). The results obtained in this study indicated that these four prerequisites have been fulfilled for the development of a breeding program of wheat cultivars tolerant to Mn toxicity.

The chlorophyll concentration and leaf elongation rate (LER) of seedling regrowth techniques were shown to be suitable plant parameters in determing Mn tolerance of wheat seedlings (Chapter II). Both techniques are relatively rapid, easy, inexpensive, and most importantly, they are seedling based. In segregating populations used in breeding programs each seedling is of a different genotype. Furthermore, the chlorophyll technique was significantly correlated with Mn tolerance as assayed by the relative root weight methodology (RRW). Nevertheless, a more convenient way would be necessary to handle thousand of seedlings in a breeding program. For example, visual score of chlorosis, and other Mn toxicity symptoms, might be used to develop a chart to determine seedling classes with tolerance to Mn toxicity. Likewise, the LER technique could be adapted to make one measure of regrowth. It should be pointed out that LER might not be proper in segregating populations where the parentals are of different height (*eg.* semidwarf × tall); differential LER response to Mn stress might be confounded by differential LER due to the height genotype of the parentals. A combination of both techniques would probably be more accurate; it has already been shown to correlate with RRW (Chapter II). A similar procedure has already been developed for Mn efficiency of barley (Longnecker *et al.*, 1990).

The screening of Canadian and foreign cultivars by RRW methodology indicated a large variation for Mn tolerance is available for selection in a breeding program (Chapter III). In general, the Canadian cultivars which were screened were mostly Mn-

sensitive, yet some of the cultivars were shown to have Mn-intermediate tolerance. Only three cultivars were shown to be Mn-tolerant (Norquay, Laura, and Biggar). Manganese tolerance appears to have originated from the Brazilian land races Polyssu (= Ponta Grossa 142) and/or Alfredo Chavez 6.21. The demonstration that Mn tolerance came from a narrow range source may facilitate the testing and selection in the field of this germplasm for use in a breeding program.

The results from the inheritance study (Chapter IV) indicated quantitative inheritance of Mn tolerance. This is supported by: (1) the continuous frequency distribution of segregating generations indicating differential tolerance to Mn toxicity, (2) the similarity of the F_1 and F_2 means, and (3) the high levels of additive gene action. That Mn tolerance of wheat might be quantitatively inherited was not surprising, what was surprising was that heritability estimates and gene effects estimates indicated that the genetic control of Mn tolerance in Norquay and Laura may be different. Whether this is true cannot be said for certain. As pointed out in the literature review (Chapter I), the level of Mn stress on plants is dependent on environmental conditions, particularly light intensity and temperature. Furthermore, Foy *et al.* (1988) suggested that the level of dominance of Mn tolerance in wheat should be used with caution because of possible variation in performance of the F_1 at different levels of Mn stress. In this study, genotype × environment interactions were not tested. Until such tests are conducted, the conclusion that cv Norquay and cv Laura may have different genetic control for Mn tolerance should be considered as speculation. Nevertheless, a preponderance of additive effects coupled with high heritability and small dominance (potence ratio) estimates and the wide range of variation available indicate that selection for Mn tolerance should be effective in early generations.

The results obtained in this study would certainly facilitate the development of a breeding program for Mn tolerance in countries such as Brazil and Australia where acid soil problems (*eg.* Mn and Al toxicity) is a constraint for wheat production. A review of the literature indicates that Mn toxicity in soils of the wheat growing regions of Western Canada is not a problem. Thus, at this time, to recommend the development of a breeding program for Mn tolerance in Canadian germplasm would be inappropriate. Nevertheless, this and other research (Macfie *et al.*, 1989) on Mn tolerance of wheat, as well as research on aluminum (Al) tolerance of wheat (Zale, 1987; Briggs *et al.*, 1989), has focussed attention on a variety of questions concerning Mn tolerance as it relates to Canadian agriculture. Two of these may serve as focus for future research in this area. First, there is a need to determine whether selection of these traits (Al and Mn tolerance) observed in Canadian wheat cultivars are directly related to changes in the

soil environment due to modern agricultural practices (*ie.* soil acidification) or whether these traits confer the cultivars certain physiological advantages under field conditions; that is, efficiency of some physiological mechanisms and/or enzymes.

Secondly, there is a need to study the effects of Mn toxicity on the root system. Failing to observe Mn toxicity symptoms in the root system does not necessarily mean they do not occur. It has become a common feature in the literature to dismiss Mn toxicity effects on the root system by pointing out that (a) most Mn toxicity symptoms are observed in the shoots and not in the roots (Bould *et al.*, 1983), and (b) that tolerance to Mn in wheat operates by tolerance of shoots to high internal Mn concentrations (Foy *et al.*, 1973). Differential root-tip diameter of cv Columbus (Mn-sensitive) and cv Norquay (Mn-tolerant) to toxic levels of Mn, and observations of differential production of exudate (*ie.* guttation) upon cutting the shoots (Moroni *et al.*, unpublished), together with the observed range of RRW among wheat cultivars (Chapter II) would indicate a major effect of Mn toxicity on the root systems. To my knowledge, there is no evidence which would indicate that the control mechanism of Mn tolerance in wheat could not be located in the root system.

V.2. Conclusions

It can be concluded from this study that: (1) the source of Mn tolerance observed in Mn-tolerant Canadian cultivars is of Brazilian origin, (2) manganese tolerance appears to have originated from the Brazilian land races Polyssu (= Ponta Grossa 142) and/or Alfredo Chavez 6.21, (3) chlorophyll concentration of Mn-stressed seedlings, and leaf elongation rate (LER) of seedling regrowth are suitable parameters for screening seedlings tolerant to Mn toxicity, (4) there may be different genetic controls of Mn tolerance, (5) manganese tolerance is quantitatively inherited, and (6) selection for Mn tolerance should be effective in early generations.

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