AN ABSTRACT OF THE THESIS OF

ALFONSO LOPEZ-BENITEZ for the degree of DOCTOR OF PHILOSOPHY

in AGRONOMIC CROP SCIENCE presented on December 10, 1976

TITLE: INFLUENCE OF ALUMINUM TOXICITY IN INTERGENERIC CROSSES OF WHEAT AND RYE

Abstract approved: Redacted for Privacy

Warren E. Kronstad

A technique previously designed to screen wheat cultivars and segregating populations for tolerance to Aluminum utilizing nutrient solutions in growth chambers was found to be efficient in differentiating among cultivars of wheat, rye, and triticale for tolerance to Al under greenhouse conditions. A 5°C increase in temperature, from 25°C to 30°C of the nutrient solutions increased the toxicity of a given Al concentration.

Screening of 263 entries including diploid, tetraploid and hexaploid wheat species and diploid rye provided evidence that adequate tolerance levels to Al in triticale can only be derived from some hexaploid wheats and some diploid ryes.

The level of tolerance present in both wheat and rye was expressed in the F_1 hybrids but did not interact to produce hybrids that were more tolerant than the more tolerant parent. When the rye was more tolerant than the wheat and the Al concentration exceeded the level of tolerance provided by the wheat, the heterogencity of rye for Al tolerance was expressed in the resulting F_1 hybrids. When a tolerant wheat was crossed to a sensitive

rye and the Al concentration exceeded the tolerance level of the rye, the F_1's were uniformly tolerant indicating the parent wheat was homozygous for tolerance of Al. Chromosome doubling of F_1 hybrids did not cause any dosage effects in the reaction of amphiploids to Al.

The F_1 progeny from the Penjamo 62 x Rye 1003 cross was tolerant to 1 ppm while sensitive plants to this Al concentration were observed in the amphidiploids derived from this F_1 possibly resulting from chromosome(s) loss or aneuploidy.

The level of tolerance in tolerant wheats was somewhat reduced in the F_1 hybrids when crossed to sensitive ryes, but was fully recovered in the amphidiploids derived from these F_1's. The opposite was true when a tolerant rye was crossed to a sensitive wheat. The F_1 hybrid was almost as tolerant as the rye parent but this level of tolerance was not recovered in the amphidiploid. If aneuploidy was present it did not involve rye chromosomes because rye was the only tolerant parent and the amphidiploid was uniformly as tolerant as Atlas 66.

Although the amphidiploid derived from the cross involving tolerant wheat x tolerant rye showed the highest level of tolerance, this was not as tolerant as the rye parent. It was believed that modifier genes from the wheat genotype influenced the expression of the full level of tolerance contributed by the rye parent in the amphidiploids. Nevertheless, it provided the resulting amphidiploids with a level of tolerance approximately twice that

of Atlas 66. When tolerant rye was crossed to a sensitive wheat, the amphidiploid obtained had a level of tolerance similar to the tolerant wheat cultivar, Atlas 66.

Aluminum affected mitotic activity of wheat, rye and triticale within the first three hours of Al treatment. Cell division decreased as exposure to Al increased; however, the mitotic activity of tolerant plants never reached zero. It appeared that a protective mechanism was induced and allowed mitotic activity to continue progressively causing roots to continue to elongate in the Al solution.

It appears that Al affects some processes at interphase. Binucleated cells were observed in both Al treatments and controls. Therefore, Al alone cannot be responsible for inducing this type of cell.

INFLUENCE OF ALUMINUM TOXICITY IN INTERGENERIC CROSSES OF WHEAT AND RYE

by

Alfonso Lopez-Benitez

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1977

APPROVED:

Redacted for Privacy

Professor of Agronomy
in charge of major

Redacted for Privacy

Head of Department of Agronomic Crop Science

Redacted for Privacy

Dean of Graduate School

Date thesis is presented December 10, 1976

Typed by THE COPY SHOP for Alfonso Lopez-Benitez

ACKNOWLEDGEMENT

I wish to express my appreciation to Dr. W. E. Kronstad, my major professor for his interest and guidance.

Appreciation is also expressed to Dr. R. Metzger and Dr. D. P. Moore for their valuable assistance and suggestions.

A special thank you goes to Nan Scott, staff, fellow graduate students and secretaries for their fellowship.

Financial support during my study at Oregon State University is gratefully acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

DEDICATED

TO

MY PARENTS, MY

WIFE AND CHILDREN

INFLUENCE OF ALUMINUM TOXICITY IN INTERGENERIC CROSSES OF WHEAT AND RYE

INTRODUCTION

Cereals will continue to play a major role in attempting to meet world food requirements as the population continues to increase. At the present time about half the plowed land of the world is given to cereal production. However, in some of the less developed countries food production is not keeping pace with population requirements because of marginal land and inadequate technology to solve the associated problems limiting maximum production.

A new cereal grain capable of performing well in crop lands where other cereals are not adapted would be extremely valuable to help meet these food needs. Scientists have synthesized triticale, a new cereal obtained by an integeneric cross between wheat and rye. It is hoped that in triticale, the desirable biological characteristics of rye and wheat can be combined.

Rye is characterized by its ability to do well under a wide range of soil moisture, acidity and fertility conditions. Rye can tolerate excessive soil moisture better than wheat can and at the same time it is more drought resistant than wheat. This may be attributed to the more vigorous and extensive root development which gives rye a greater ability to grow and produce under a limited moisture supply.

Rye also has a wider potential adaptation in the temperate areas than wheat due to its greater winter hardiness. Rye can

germinate and endure lower winter temperatures, can stand alternate freezing and thawing of soils and can survive better than wheat under limited snow cover. It can also do well in the subtropical zones of the world. Rye also has the ability to perform satisfactorily on poor, acid and sandy soils, and has a broad spectrum of disease resistance.

Although rye is superior to wheat in many attributes it is inferior in most major cereal producing regions for such factors as percentage of flour yield, milling and baking properties, gluten protein and grain yields. Therefore, it would be very desirable if through intergeneric hybridization the good attributes of wheat and rye could be combined thus providing wider adaptation and higher grain yields in the resulting triticale.

Although triticale is still in its experimental stage, there is evidence (Osorio et al, 1973) that certain strains of triticale perform better than wheat under certain soil conditions. In fact, soil problems are responsible in a large part for the low crop yields in many parts of the world. Soil acidity and the associated toxicity has been recognized for a long time as one of the most important soil factors limiting crop yields. A triticale highly tolerant to soil acidity would make strongly leached soils more productive and would increase substantially the amount of land planted to cereals.

If such triticales are to be developed it will require an efficient, economical and practical technique to screen breeding

materials for reaction to such limiting factors as Al toxicity which is associated with acid soils. Consequently the objectives of this study were:

1. To assess the effectiveness of a seedling screening technique in evaluating wheat, rye and triticale in the greenhouse utilizing water baths for temperature control rather than growth chambers.
2. To identify sources of tolerance to Al in wheat, wheat related species and rye for synthetizing triticale.
3. To identify cultivars of wheat and rye which are compatible in wheat-rye crosses and have different levels of tolerance to Al.
4. To determine the expression of tolerance to Al of F_1 hybrids derived from parents with different degrees of tolerance.
5. To determine whether upon colchicine treatment chromosome doubling of the F_1 hybrids has any effect on the reaction of the amphidiploids thus obtained to Al.
6. To study the effect of Al toxicity in wheat, rye and triticale on the rate of mitotic cell division and the specific stages of mitosis.

II. LITERATURE REVIEW

Acid soils are characterized by: 1) increased concentrations of soluble Aluminum (Al), 2) increased availability of Iron (Fe) and Manganese (Mn), lower levels of exchangeable bases, and 4) relative low pH. Soil acidity also alters the population and activities of microorganisms responsible for transformations involving Nitrogen (N), Sulfur (S), and Phosphorus (P), thus indirectly affecting the availability of these elements to higher plants.

In addition, a toxic condition produced by excess soluble or exchangeable Al develops which has a detrimental effect on root development.

Aluminum Toxicity of Plants in Acid Soils

Investigations directed to understand plant reaction to aluminum toxicity were first undertaken by Way (1850). Subsequently a great deal of work has been done to identify specific factors associated with acid soils that are toxic to plants.

Aluminum toxicity is considered to be a major factor limiting plant growth and thereby crop yield in acid soils. Aluminum injury in plants has been described as a reduction of the main root axis and a restriction of root systems. These conditions render plants inefficient in taking up nutrients, and as a result are more susceptible to winter and drought injury.

Reports suggesting that Al in sufficient concentrations was toxic to plants were made as early as 1901 (Pratt 1966). Later,

reports by Miyake (1916) suggested the possibility of a relationship between Al and the infertility of acid soils. He demonstrated that 1.2 ppm Al in solution was toxic to rice. This relationship between Al and infertility in acid soils was also suggested by Hartwell and Pember (1918) who attributed the differences in performance between cultivars of barley and rye to the presence of Al in acid soils.

Magistad (1925) demonstrated the importance of the relationship between pH and solubility of Al. He showed that the curve for solubility of Al in soil at various pH values practically coincided with the curve for soluble Al in water at the same pH values. Minimum Al solubility occurred at pH values from 5.8 to 7.0, while at pH values of 4.7, more than 3 ppm of soluble Al were obtained. Experiments in water culture clearly showed that the toxicity of acid soils resulted from the presence of Al ions (Vlamis 1953).

By the 1960's it was well established that Al was a major factor affecting plant growth in acid soils (Jenny et al. 1950; Yuan and Fiskell 1959; Lin and Coleman 1960; Harward and Coleman 1954; Low 1955; and Bhumbla and McLean 1965).

Crop Responses to Liming Acid Soils

The development of shallow root systems is a common occurrence in plants when grown in acid soils. Research by Armiger et al. (1968), Adams and Lund (1966), Foy et al. (1965), McLeod and Jackson

(1967), and others, suggest that Al toxicity limits the penetration of roots in certain acid soils. This restriction of plant roots to the surface soil means that plants cannot exploit the subsoil for water and nutrients needed for maximum yields. It has been suggested that this problem may be lessened by using lime to raise the pH of the soil.

Duggar and Funches (1914) were among the first researchers to show that agricultural lime was beneficial to cotton, corn, cowpeas, peanuts, velvet beans, soybeans and sorghum. Due to the positive results of early experimentation and economical support by the federal government, farmers in 1930 started to make extensive use of lime. However, in spite of its extensive use and research being carried on there was little agreement as to the mechanism by which lime improved crop yields in acid soils. Among the first investigators to look into the alterations of different soil parameters brought about by liming was Schmehl et al. (1950). They used soil (pH 4.7) to study the effect of exchangeable calcium (Ca), Mn, Al, and Fe on the growth of Alfalfa. They found that, by liming the soil to a pH of 6.5 alfalfa yields were increased threefold and the amount of readily soluble Al in the soil was reduced. Similar results were obtained by Wright (1937) who suggested that low pH is not the limiting factor for plant growth and concluded that Al was precipitated after coming in contact with phosphorus and thus made insoluble. A similar conclusion was reached by Moschler et al. (1960), from their experiments with Alfalfa. Reduction of exchange-

able Al in the soil is one of the principal benefits from liming acid soils.

The importance of an adequate supply of nutrients in acid soils was indicated by Aslander (1952). He showed that it was possible to have high yields of wheat, red clover and sugarbeets at a relatively low pH provided plant nutrients in the soil were readily available to plants.

Differential responses between crop species have been reported by Adams and Pearson (1967). Soybeans, in general, were considered by Foy and Brown (1964) to be more tolerant to Al than barley and cotton and, therefore, moderately responsible to liming. Potatoes were considered by Hewitt (1947) as one of the crops more tolerant to soil acidity. However, Lee (1971) showed that Al affected the growth and tuber yield in sand culture. Responses of potatoes to liming have been reported by Carolus and Brown (1935) at soil pH of 4.7 and by Volk and Gamon (1954) at pH 5.0.

In addition to interspecific differences in tolerance to Al toxicity, intervarietal differences have also been reported. Armiger et al. (1968), found that varietal differences in soybeans for tolerance to Al toxicity in acid soil (pH 4.0) were minimized or eliminated by liming the soil to pH 5.5. Similar results were obtained in wheat by Foy et al. (1965).

Effect of Al on Plant Growth

Although a large part of the literature dealing with effects of Al on plant growth refers to Al as a non-essential and toxic element, there have been some reports on its beneficial effects on plant growth.

For example, McLean and Gilbert (1927) grew different plant species in nutrient cultures to study the relationship between Al and Phosphorus as a preventive of Al injury. They found that low concentrations of Al had a stimulatory effect, while high concentrations were toxic to rye, corn, alfalfa, buckwheat, oats, onion, and Red Top. Lipman (1938) also observed corn ear production was enhanced by 1 ppm of Al in culture media. Increases in leaf and root weights of lettuce at low levels of Al have also been reported by Harward et al. (1955). Beneficial effects of Al were also reported by Hass (1936) with lemon. Aluminum also indirectly affects the sepal color of Hydrangea macropylla as demonstrated by Asen et al. (1963).

Favorable effects of Al have also been reported on potatoes by Lee (1971, 1972). He found that low concentrations stimulated vegetative growth of several cultivars and number and size of tubers were increased. Other crops such as cranberry, according to Medappa and Dana (1968), not only are adapted to very acid soils but actually stimulates their root development. Sivasubramaniam and Talibudeen (1971) concluded that leaves of tea can accumulate up to 20,000 ppm of Al.

However, as indicated by the literature, a large proportion of the important man-cultivated crops are badly injured by high concentrations of Al in acid soils (Pearson and Adams 1967).

The best descriptive symptom of Al toxicity in plants as indicated by Foy (1974) appears as inhibition of root growth. Aluminum injured roots are characteristically stubby and spatulated in appearance. The root tips stop growing and turn brown. The root system as a whole is collaroid in appearance, having many inhibited and thickened lateral roots but lacking in fine branching.

Aluminum toxicity in the above ground portion of the plant is often characterized by symptoms resembling those of P deficiency, including over-all stunting, small and abnormally dark green leaves; purpling of stems, leaves and veins; yellowing and death of leaf tips. Aluminum toxicity may also appear as Ca deficiency, for example, cupping or rolling of young leaves and collapse of plant apex (Foy 1974).

The toxic effects of Al in plants have been studied at a cellular and physiological level. Clarkson (1965) concluded that morphological abnormalities of root systems treated with Al are such that they can only be explained by an inhibitory effect of Al on cell division. He showed that cessation of root elongation was closely correlated with disappearance of mitotic figures. Aluminum induced polynucleated cells were reported by Rios and Pearson (1964) in cotton and by Fleming and Foy (1968) in wheat

as a consequence of inhibition of cell division. However, Henning (1975) studied the effect of Al on cells of primary meristems of wheat roots and indicated that no polynucleated cells were ever observed in Al-injured root tips.

The inhibitory effect of Al on cell division of Al treated roots of barley was attributed by Sampson et al. (1965), to an action of this element on DNA. They found two physiologically distinct fractions of double stranded DNA as reported previously in wheat by Sampson et al. (1963). One had a low molecular weight and was metabolically stable. The second was identified as genetic DNA by Sampson and Davis (1966).

Clarkson (1968) also showed that Al affected the genetic DNA fraction, and according to Sampson and Davis (1966), the failure of genetic DNA synthesis prohibits cells from passing through the S-period. As a result, cell division is halted. However, when cell division in barley was halted by Al treatment, DNA synthesis continued, but the type of DNA synthesized had an unusual base composition and was metabolically labile.

Aluminum has also been suggested by Wright (1943), to act on the metabolism of P in plants. He advanced the hypothesis that Al was precipitated as aluminum phosphate within the roots and suggested that this precipitation of P by Al accounts for the observed reduction of P transport to the shoots.

Wallihan (1958) suggested that the Al-P precipation was more likely to occur in the roots. Rasmussen (1968), however, concluded that Al was precipitated on the surface of the epidermal cells of

corn roots. The rate at which P increased in Al-treated roots was demonstrated by Clarkson (1966) to be greater than in controls. He also showed that this P was inorganic and exchangeable. Aluminum has been also found to bind P on the cell walls. In this respect Clarkson (1967) suggested that the free carboxyl groups of galacturonic acid chains in the middle lamella seem likely sites for absorption of Al. Thus, it appears that there are two reactions between Al and P that result in an apparent increase in the rate of P uptake by plants (Clarkson 1966): at the cell surface or in the free space, which results in the fixation of P by an absorption-precipitation reaction; and within the cell, possibly within the mitochondria, which results in a marked decrease in the rate of sugar phosphorylation. However, Randall and Vose (1963) studied this stimulation of P uptake in the presence of Al and concluded that the Al-induced P uptake was largely a metabolic process, although precipitation in the root cannot be ruled out. This conclusion was drawn from the fact that cyanide caused a marked inhibition of the Al-induced phosphorus uptake.

Aluminum toxicity in plants has also been associated by Johnson & Jackson (1964) with reduced Ca uptake. They studied uptake and transport of Ca as affected by Al and they found Ca uptake was decreased by decreasing pH from 6.0 to 4.0. Similar results were observed by Lance and Pearson (1969) in cotton. They found that inhibition of Ca uptake was avoided by increasing the Ca concentration in the culture and concluded that the permeability

of the plasmalemma must have been reduced since reduction of all types of uptake occurred.

Reaction of Wheat to Al

The wheat plant has been subjected to a great deal of research regarding tolerance to acid soils. Differential tolerance to Al was first shown by Neenan (1960). He suggested the possibility of testing for tolerance and developing lines adapted to high soil concentrations of Al. Varietal responses to liming have also been reported by Foy et al. (1965). They noted that wheat varieties developed in the eastern United States and Brazil were exceptionally tolerant and responded the least to liming, while those developed in the plains of the western states were very sensitive to Al and had the best response to liming acid soils.

Ikeda et al. (1965), also found varietal differences in barley and wheat. Association of morphological damage of root tips and lateral roots with degrees of tolerance, by Fleming and Foy (1968), established the basis for cytological and histological study of the wheat reaction to Al.

Research on the level of tolerance in wheat led Mesdag and Slootmaker (1969) to conclude that five different groups of wheat varieties exist with regard to tolerance to Al.

Further evidence for varietal differences was given by Kerridge et al. (1971), who grouped more than fifty cultivars of wheat into three different classes depending on their tolerance.

One important contribution made to the study of Al toxicity in wheat was that by Kerridge and Kronstad (1968). They provided a genetic interpretation for the differences in tolerance of wheat, and concluded that the wheat cultivar, Druchamp differs from Brevor by one pair of genes, with tolerance being dominant. Genes for tolerance were found by Slootmaker (1974) to be located on chromosomes of the "D" genome of hexaploid wheats.

Rorison (1958) and Clarkson (1965), suggested that the restriction in the development of roots is probably due to the inhibition of cell division that results in an abnormal and undifferentiated tumor-like tissue. In addition, Al appears to destroy both cytoplasm and nuclei as suggested by Henning (1975). He concluded that the primary effect of Al was death of cells which occurred within the first 24 to 48 hours of exposure to Al. However, the mitotic cycle was affected almost at the onset of the Al treatment.

Reaction of Rye and Triticale to Al

Nuttonson (1958) indicates that rye is the only cereal crop able to perform well in poor, sandy and podzolized soils of Northern Europe, Northern Asia and areas adjacent to the Great Lakes in North America. Similar observations were made by Hartwell and Pember (1918) who showed that rye was more tolerant to Al acid soils that many other cereals, and by McLean and Gilbert (1927) who classified rye as moderately tolerant to Al toxicity.

There is some evidence that some strains of triticale are highly tolerant to Al toxicity (Slootmaker 1974). This investigator suggested that this high level of tolerance in triticale is due to the addition of the rye genome. These findings are consistent with Osorio et al. (1973), who reported that under acid soil conditions in Brazil, triticale yielded nearly as much as the local wheat cultivars. This brings up the question as to how the tolerance of Al in wheat and rye, respectively contribute to the tolerance to Al in triticale and whether the chromosome doubling of the sterile wheat-rye F_1 has any effect on the reaction to Al of the new amphidiploid.

III. MATERIALS AND METHODS

Screening Techniques

McLean and Gilbert (1927) and Millikan (1949) and many other investigators have shown that Al injury is often observed in plant roots before any top damage is evident and indicated that root growth appears to be a better index of tolerance to Al than top growth.

Basically, the procedure of exposing wheat seedling roots to Al and evaluating their subsequent recovery in Al-free solution has been used by Ali (1973), Moore (1974), Henning (1975), and Rhue (1976), in wheat, and by Martinez (1976), in rice. Except for two modifications, the same procedure was used in this study. All the experiments carried out were conducted in the greenhouse in 50 cm x 70 cm x 150 cm water baths where temperature fluctuation was always within one degree celcius. Air temperature varied from 150°C at night to 30 or 35°C in the afternoon. A description of this technique is provided.

Two different nutrient solutions were used: 1) full strength nutrient solution (Hoagland's solution), and 2) one-tenth strength nutrient solution to which Al treatments were added. Table 1 shows the mineral composition of the full strength nutrient solution. In solution two, the mineral composition was basically the same as solution one but the concentration of salts was reduced to 1/10th, phosphorus was omitted and iron was added as iron chloride ($FeDl_3$) in place of Fe-CyDTA as indicated by Moore (1974).

Table 1. Chemical composition of the full strength nutrient solution used in these experiments.

SALT	CONCENTRATION
$Ca\ (NO_3)_2$	4.0 mM
$Mg\ SO_4$	2.0 mM
$K\ NO_3$	4.0 mM
$(NH_4)2\ SO_4$	0.435 mM
$KH_2\ PO_4$	0.5 mM
$Mn\ SO_4$	2.0 μ M
$Cu\ SO_4$	0.3 μ M
$Zn\ SO_4$	0.8 μ M
NaCl	30.0 μ M
Fe- CYDTA	10.0 μ M
$Na_2\ MoO_4$	0.1 μ M
$H_3\ BO_3$	10.0 μ M

CyDTA Cyclohexanediamine Tetraacetic Acid

Seeds were surface sterilized with 10 percent Sodium hypochloride to reduce possible contamination by microorganisms and then sprouted in petri dishes. Sprouted seeds were placed on the bottom of a polyvinylchloride resine screen of acrilic trays, screens were then placed on top of twenty-five litre black polyethelene wastebaskets containing the full strength nutrient solution. The level of this solution was such that it always touched the resine screen to make sure sprouted seeds would not dehydrate and provided emerging radicles with a good supply of nutrients.

Seedlings were allowed to grow on this solution for 48 hours at which time they had developed three primary roots of about 4 to 5 cm long in the case of wheat and some triticales and 5 to 7 cm long in the case of rye and other triticales. These seedlings were then transferred to the 1/10th nutrient solution containing Al. Prior to this, roots were rinsed in distilled water to avoid possible transference of phosphorus to the Al solution. Seedlings were allowed to grow in this solution for 48 hours and then rinsed with distilled water again and transferred back to the Al free solution for 48 hours. The tolerant class was noted by those seedlings which recovered from the Al treament. The length of the root regrowth in the Al free solution after the Al treatment was recorded and taken as criterion of estimating the level of tolerance to a given level of Al.

Both solutions, Al-free and Al-containing were continously aerated during the experiments. A pH of 4.0 $\pm$ 0.05 was maintained and, except where indicated, the temperature of both solutions was 25°C $\pm$ 1. The number of seedlings tested varied according to the amount of seed available of each cross and amphidiploid. Root regrowth in Al-free solution after a short exposure to Al was considered a reliable criterion of estimating the level of tolerance of a given cultivar. This criterion was based on studies made by several investigators, Ali (1973), Moore (1974), Henning (1975), and Martinez (1976).

Using this method, preliminary screening for tolerance was made to determine the range of tolerance available among varieties of rye and triticale. It was clearly seen that as the Al concentration increased, the length of root regrowth decreased until it was irreversibly stopped. These results were always reproducible provided pH, temperature, Al concentration, time of exposure to Al and nutrient concentration were rigorously controlled.

Selection of Parents

Two hundred twenty six entries of wheat and diploid related species and 50 varieties of diploid rye from different countries of the world were screened for tolerance to Al toxicity. A list of these materials is given in Appendix, Table 1 to 4. The cultivars of hexaploid wheat Brevor, Druchamp, Chinese Spring and Atlas

66, whose reaction to Al toxicity had already been established by Ali (1973), were used as differential varieties.

Two levels of reaction of wheat and rye were selected, sensitive and tolerant. Seedlings from this material were selected and transplanted to pots in the greenhouse where they were grown to maturity. A series of preliminary crosses were made to determine the sensitive and tolerant cultivars of wheat that would cross easily with cultivars of rye so that cultivars representing the following four types of crosses were utilized.

Group I	Sensitive wheat x Sensitive rye
Group II	Tolerant wheat x Sensitive rye
Group III	Sensitive wheat x Tolerant rye
Group IV	Tolerant wheat x Tolerant rye

Seed harvested from the wheat and rye plants that had been selected for their reaction to Al was germinated, retested, grown out and used as parents in crosses. Because rye is a self sterile crop, each cultivar previously screened was isolated and pollination within the cultivar was allowed.

Cultivars of wheat and rye used in wheat-rye crosses are shown in Table 2. The cultivars of wheat and rye involved in each group of crosses are shown in Table 3.

F_1 seeds were harvested and tested for reaction to Al toxicity along with their parental material in the same nutrient solutions. The cultivars of wheat, Brevor, Druchamp, Chinese Spring and Atlas 66 were always included in each experiment as controls.

Table 2. Varieties of wheat and rye used in wheat-rye crosses, their origin and concentration of Al required to inhibit root regrowth.

Cultivar of Wheat	Al ppm	Origin
Brevor	0.4	U.S.A.
Daws	1.0	U.S.A.
Inia 66	1.0	Mexico
Druchamp 8925	1.5	France
Chinese Spring	2.5	China
Penjamo	2.5	Mexico
Hope 7D	2.5	U.S.A.
Atlas 66	20.0	U.S.A.
Preludio	20.0	Brazil
Carazinho	20.0	Brazil

Cultivar of Rye			PI
Rye 37	5.0	Afghanistan	00037 (CI)
Rye 1003	5.0	Turkey	---
Rye 1133	5.0	Turkey	---
Rye 5	20.0 at 30°C	Spain	323356
Rye 1443	20.0 at 30°C	Brazil	239580

Table 3. Cultivars of wheat and rye involved in each of the four groups of F_1's and level of Al used to test them.

Cross	Al concentration in ppm
Group I. Sensitive Wheat x Sensitive Rye	
Inia 66 x Rye 1133	1 and 4
Penjamo 62 x Rye 1003	1 and 4
Hope 7D x Rye 1133	1 and 4
Group II. Tolerant Wheat x Sensitive Rye	
Atlas 66 x Rye 37	5 and 10
Carazinho x Rye 1133	5 and 10
Group III. Sensitive Wheat x Tolerant Rye	
Daws x Rye 1443	5, 10 and 15 at 30°C
Hope 7D x Rye 1443	5, 10 and 15 at 30°C
Group IV. Tolerant Wheat x Tolerant Rye	
Atlas 66 x Rye 1443	5, 15 and 20 at 30°C
Carazinho x Rye 1443	5, 15 and 20 at 30°C

Two cultivars of rye, Rye 1443 and Rye 5 from Brazil and Spain respectively, showed consistent tolerance to 35 ppm Al. At 40 ppm the length of root regrowth was longer than that at 35 ppm. This could have been due to Al precipitation in the 40 ppm Al solution so that the actual concentration of Al in solution could have been less than 35 ppm. Two modifications to this technique were tried to find the Al concentration that would inhibit root regrowth of these ryes without using such high levels of Al: 1) Concentration of nutrients in the Al solution was reduced to 1/20th, and 2) temperature of nutrient solutions was increased by 5°C. Both modifications were effective in inhibiting root regrowth on these two tolerant cultivars of rye at 20 ppm of Al. To make sure seedlings would have a satisfactory supply of nutrients, the second modification was used. Tolerant varieties of wheat, when tested under this modification, turned out to be completely sensitive to 4 ppm Al rather than 20 ppm at 25°C as previously.

Since Rye 5 turned out to be a tetraploid rye, no F_1 seeds were obtained when used as a parent in crosses with wheat. However, it was used as a control to test tolerant ryes. The Al concentration used to test the reaction of the F_1 was chosen based on the reaction given by parental materials. In Group I, one ppm Al inhibited root regrowth of Inia 66 and Daws but not on Penjamo 62 and Hope 7D. This concentration of Al established statistical differences between the first two wheats and the second two.

Rye 37 and Rye 1133 were completely sensitive to 5 ppm Al. However, 4 ppm Al concentration was chosen to test the reaction

of these cultivars of rye which were used in wheat-rye crosses as sensitive rye parents.

In Group II, F_1's were tested at 5 and 10 ppm Al. Five ppm would indicate whether the hybrids were more or less sensitive than the rye parent, and 10 ppm was used because it would provide the same information with respect to Atlas 66 and Carazinho.

Since tolerant rye roots were inhibited at 20 ppm Al and 30°C temperature, it was decided to test the F_1's from tolerant ryes at this temperature using 5, 10 and 15 ppm for Group III. This would provide a method of determining how effectively the tolerance in rye was transmitted to the F_1's. In Group IV, F_1's were tested at 5, 15 and 20 ppm and 30°C. This would indicate whether a cross between a tolerant wheat and a tolerant rye would result in F_1's being more tolerant than the tolerant rye. To test for statistical differences between cultivars and amphidiploids, a randomized block design was used and the Honest Significant Difference (H.S.D.) was determined at the 5 percent level.

F_1's from all groups previously tested for reaction to Al toxicity were transplanted into pots and placed on greenhouse benches under high light intensity and sufficient fertilizer to stimulate tillering. When three to five vigorous tillers had been produced, a colchicine treatment was applied.

Colchicine Treatment

Plants produced from seed harvested from doubled sectors of colchicine treated F_1's are referred to as amphidiploids. Since F_1's and amphidiploids were not available at the same time, both F_1's and amphidiploids were tested in different experiments and compared to their respective wheat and rye parents to evaluate their reaction to Al.

Amphidiploid seedlings along with their respective parents were tested together in the same nutrient solutions for their reaction to Al in an effort to determine if a dosage effect in the reaction to Al resulted by doubling the chromosome number.

The technique used for colchicine treatment was to take F_1 seedlings that had been previously tested for their reaction to Al and allow them to grow on greenhouse benches at 15 to 18°C temperature under high light intensity and suitable fertilizer to promote tillering. When three or more vigorous tillers developed, one or two of the strongest tillers were cut at about 2 cm from the crown and an eye dropper containing 0.1 percent colchicine in aqueous solution was inverted over the cut stem. Eye droppers were prepared by widening their tips to fit the cut stems. The rubber cup of the eye dropper retained the colchicine solution in the eye dropper and generally no leakage occurred. This allowed the cut stems to take up colchicine for 72 hours. The droppers were refilled as needed. On the second or third day, a swelling of the cut stem was evident indicating that the colchicine was being taken up.

The colchicine solution was removed after 72 hours and the plants were thoroughly watered for two days to promote a faster recovery. They were then placed in a growth chamber at 5°C and 8 hours light at 2000 fc for 3 to 6 weeks depending on the growth habit of the parental material and need for vernalization. Plants were then returned to the greenhouse under a 12-hour light cycle. Later, when plants were at the flag leaf stage, the light period was extended to 16 hours to promote fast flowering and ripening.

About 25 percent of the surviving plants had doubled sectors that produced seeds. These were harvested and tested for reaction to Al.

Cytological Examination of Root Tips

To study the effect of Al toxicity on the mitotic cycle of root tips in relation to reaction to Al, both sensitive and tolerant wheat, rye and triticale seedlings were grown and exposed to an Al treatment of 7 ppm. This concentration of Al is seven times the amount required to inhibit root regrowth of Daws (sensitive wheat). It is also almost twice the level needed to inhibit root recovery in Rye 37 and Rye 1133 (sensitive ryes). However, this level of Al was chosen because it insures that 100 percent of the roots of the sensitive wheat and rye would be irreversibly damaged, root tips of tolerant wheats would be reversibly damaged and the tolerant rye would not show any injury.

Root tips were collected from Daws, Atlas 66, Rye 1133, Rye 1443 and amphiploids Daws x Rye 1133, and Daws x Rye 1443. Root tip collections were made at 3, 6, 9, 12, 24, 36, and 48 hours of exposure to Al and at the same intervals of recovery period. Three root tips of each cultivar were collected at every sampling time. Root tips were collected and fixed for two hours in a fixative solution consisting of 3 parts alcohol, 1 part Glacial Acetic Acid, then transferred to 70 percent alcohol solution and storaged at 4°C until studied. Acetocarmine squashes were prepared from individual root tips and then covered with a cover glass. Mitotic figures were counted in the entire slide and recorded with regard to the stages of prophase, metaphase, anaphase and telophase. A mitotic count included any dividing cell whose chromosomal material lay between early prophase and late telophase before a cell plate separating the two daughter cells had begun to form.

IV. EXPERIMENTAL RESULTS

Hexaploid wheats previously identified by Ali (1973) as sensitive, moderately sensitive, moderately tolerant and tolerant, were used as controls. Aluminum concentrations reported by Ali (1973) to identify tolerance levels for these cultivars did not agree with those found in these experiments. Ali reported that the cultivar Brevor was sensitive to 0.4 ppm Al, Druchamp tolerant to 0.4 ppm but sensitive to 4 ppm, Chinese Spring was tolerant to 4 ppm but sensitive to 6, while Atlas 66 was tolerant to 6 but sensitive to 30 ppm.

In these experiments, (Table 4) Brevor was sensitive to 0.3 ppm, Druchamp was tolerant to 0.3 but sensitive to 1.5 ppm, Chinese Spring was tolerant to 1.5 ppm but sensitive to 2.5 ppm, while Atlas 66 was tolerant to 2.5 ppm but sensitive to 20 ppm.

These results were repeatable over time and indicated that substituting water baths in the greenhouse for growth chamber was suitable for identifying different degrees of tolerance to Al in wheat even though levels of tolerance were different than those identified by Ali (1973) using the same cultivars.

Results shown in Table 4 for seven wheat and three rye cultivars were recorded on the basis of root regrowth in Al-free solution after the Al treatment. It can be observed that a particular Al concentration was needed to inhibit root regrowth of a given cultivar. However, when root regrowth was not completely inhibited, statistical differences in tolerance between Brevor, Druchamp and Chinese Spring were noted and presented in Table 5.

Table 4. Reaction of seven varieties of wheat and three varieties of rye to Al toxicity. (S = sensitive, T = tolerant).[1]

	Aluminum level in ppm														
	0.3		1.0		1.5		2.0		2.5		3.0		5.0		
Cultivar	T	S	T	S	T	S	T	S	T	S	T	S	T	S	TPO[2]
Wheat															
Brevor	0	200	0												200
Inia	78	50	0	158											286
Daws	45	67	0	87											199
Druchamp 8925	100	0	9	49	0	158									316
Ch. Spring	152	0	108	14	48	45	8	154	0	147					676
Panjamo	100	6	16	62	7	60	0	96							347
Hope 7D	73	1	52	28	10	65	0	103							332
Rye															
Rye 37	24	56	20	60	---		---		6	24	4	24	0	100	318
Rye 1003	36	44	26	74	---		---		5	25	2	20	0	82	314
Rye 1133	20	60	30	87					10	45	5	19	0	93	369

[1] Results pooled from six experiments.

[2] Total Plants Observed

Table 5. Mean root regrowth in mm of the wheat cultivars Brevor, Druchamp 8925, and Chinese Spring at two aluminum concentrations. n = 50 (number of observations).

Al Conc. ppm	Cultivar	Root Regrowth*	Standard Error	H.S.D.**
0.5	Chinese Spring	25.3a	1.67	2.79
	Druchamp 8925	16.6b		
	Breavor	0.0d		
1.0	Chinese Spring	17.8b		
	Druchamp 8925	4.0c		
	Brevor	0.0d		

* Means noted by the same letter are not significantly different.

** Honestly significant difference at 5% level.

To determine the range of tolerance to Al in selected cultivars of wheat, rye, triticale and related species, a series of experiments were designed with the results shown in Table 6. Diploid species tested representing genomes AA, BB, and DD, as well as tetraploid wheats with genomes AABB were as sensitive as Brevor.

Hexaploid wheats tested showed a wide range of variability, from sensitivity to 0.3 ppm to tolerate at 15 ppm Al. Daws from the United States (US) was the most sensitive hexaploid wheat (Table 7), while Atlas 66 from US and Carazinho and Preludio both from Brazil were the most tolerant (Table 8).

Rye showed a much wider range of variability to Al reaction than hexaploid wheats. In addition to the variability between cultivars, large variations within cultivars were observed. This could be expected since rye is a cross pollinated species.

Table 6. Reaction to Al toxicity of wheat related species and rye. n = Number of observations.

Species	n	Genome	No. of Entries	Al Concentr. ppm	Reaction
T. monococcum	10	AA	5	0.4	Sensitive
Ae. speltoides	10	BB	5	0.4	Sensitive
Ae. squarrosa	10	DD	5	0.4	Sensitive
Tetraploid	10	AABB	143	0.4	Sensitive
Hexaploid	20	AABBDD	45	1-15	Variable
Triticale 6x	20	AABBRR	50	5.0-30	Variable
Triticale 8x	20	AABBDDRR	10	5.0-30	Variable
Diploid Rye	20	RR	50	3.0-30	Variable

Table 7. Mean root regrowth (mm) of five cultivars of wheat and two of rye at three Al concentrations. n = 50 (number of seedlings tested)

Al Concentration ppm	Cultivars	Root Regrowth*	Standard Error	H.S.D.**
0.5	Chinese Spring	23.5a	1.67	2.30
	Hope 7D	17.6b		
	Penjamo 62	16.6b		
	Rye 1003	6.0c		
	Rye 1133	5.6c		
	Inia 66	3.0c		
	Daws	1.8d		
1.0	Chinese Spring	17.8a	2.4	3.15
	Hope 7D	14.2ab		
	Penjamo 62	11.8b		
	Rye 1003	5.9c		
	Rye 1133	4.3c		
	Inia 66	0.0d		
	Daws	0.0d		
2.5	Rye 1003	2.3c		
	Rye 1133	3.1c		
	Chinese Spring	0.0d		
	Hope 7D	0.0d		
	Penjamo 62	0.0d		
	Inia 66	0.0d		
	Daws	0.0d		

* Means noted with the same letter were not significantly different.

** Honestly significant difference at 5% level.

Table 8. Mean root regrowth in mm of three tolerant cultivars of wheat and one tolerant cultivar of rye when exposed to three concentrations of Al at 25°C±1. Number of seedlings = 50

Al Concentration ppm	Cultivar	Root Regrowth*	Standard Error	H.S.D.**
3	Rye 1443	39.8a	5.8	5.17
	Preludio	30.6b		
	Atlas 66	27.8bc		
	Carazinho	23.4bc		
7	Rye 1443	30.0b		
	Preludio	14.9d		
	Atlas 66	8.5c		
	Carazinho	7.5c		
10	Rye 1443	31.0a	1.8	1.54
	Preludio	9.0c		
	Atlas 66	7.6c		
	Carazinho	5.3c		

* Means noted with the same letter were not significantly different.

** H.S.D. Honestly Significant Difference at 5% level.

Approximately 30 percent of Rye 37 from Afghanistan and Rye 1003 and Rye 1133 from Turkey were sensitive to 5 ppm Al and 70 percent were tolerant, but sensitive to 30 ppm. Seedlings sensitive to 5 ppm were transplanted and isolated by varieties to allow cross pollination. Seed harvested from these plants produced seedlings which were uniformly sensitive to 5 ppm Al. Concentrations utilized below 4 ppm Al indicated that variability within cultivars was still present as seen in Table 4. Plants were grown from these seeds and utilized in wheat-rye crosses as sensitive rye parents. Rye 5 obtained from Spain and Rye 1443 from Brazil were tolerant to 30 ppm.

Considerable variability for Al reaction was also observed with triticale (Table 6). The reaction varied from sensitivity at 5 ppm Al to tolerance to 30 ppm depending on the particular triticale. Sensitive wheats and ryes selected for their compatibility in intergeneric wheat-rye crosses are shown in Table 7. The actual level of sensitivity among these cultivars was different as seen from the mean root regrowth at 0.5 and 1.0 ppm Al.

The mean root regrowth of the cross between compatible tolerant wheat and rye cultivars can be observed in Table 8. Rye 1443 was significantly more tolerant than any wheat tested and showed almost no visible damage at Al concentrations that inhibited root regrowth of the most tolerant wheats. The wheat cultivar Preludio, was significantly more tolerant than Atlas 66 and Carazinho, with no difference observed between the latter two.

However, all three tolerant wheat cultivars were sensitive to 20 ppm Al at 25°C.

Increasing the temperature of the nutrient solution from 25°C to 30°C increased the toxicity of a given amount of Al. Root regrowth of Rye 1443 which had tolerated 35 ppm Al was inhibited at 20 ppm Al, while that of tolerant wheats was inhibited at 4 ppm Al without altering any other variable. Statistical differences between Rye 1443, Preludio, Atlas 66 and Carazinho at 3, 7 and 10 ppm at 25°C were also found at 0.5, 1.5 and 2.0 ppm Al at 30°C as shown in Table 9. Mean root regrowth of Preludio, Atlas 66, Carazinho and Rye 1443 were plotted in Figure 1 to show the difference in reaction between tolerant wheats and tolerant rye. The effect of temperature on the reaction of rye and wheat to different concentrations of Al is shown in Figures 2 and 3.

Group I. Sensitive Wheat x Sensitive Rye

Penjamo 62 and Hope 7D were not significantly different at 1 ppm Al, but were more tolerant than Daws and Inia 66 which were sensitive at 1 ppm. Eighty percent of the seedlings of the sensitive rye cultivars Rye 37, Rye 1003 and Rye 1133 were sensitive at 1 and 4 ppm Al but all seedlings were sensitive to 5 ppm. The frequency distribution of root regrowth of Inia 66, Rye 1133 and resulting F_1 hybrid is shown in Figure 4 at 1 and 4 ppm Al. One ppm of Al inhibited root regrowth of 100 percent of the Inia 66 seedlings, but only 82 percent of the Rye 1133 seedlings failed to regrow. The F_1 showed two types of reaction, sensitive and

Table 9. Mean root regrowth in mm of three tolerant cultivars of wheat and one of rye at three levels of Al when grown at 30°C ± 1. Number of seedlings = 50

Al Concentration ppm	Cultivar	Root Regrowth*	Standard Error	H.S.D.**
0.5	Rye 1443	39.1a	2.9	2.3
	Preludio	29.3a		
	Atlas	22.1c		
	Carazinho	20.8c		
1.5	Rye 1443	32.4a	5.6	2.17
	Preludio	14.1b		
	Atlas	9.2c		
	Carazinho	7.3c		
2.0	Rye 1443	31.0a	5.0	1.70
	Preludio	13.0b		
	Atlas	5.0c		
	Carazinho	3.8c		

* Means noted with the same letter were not significantly different.

** Honestly significant difference at 5% level.

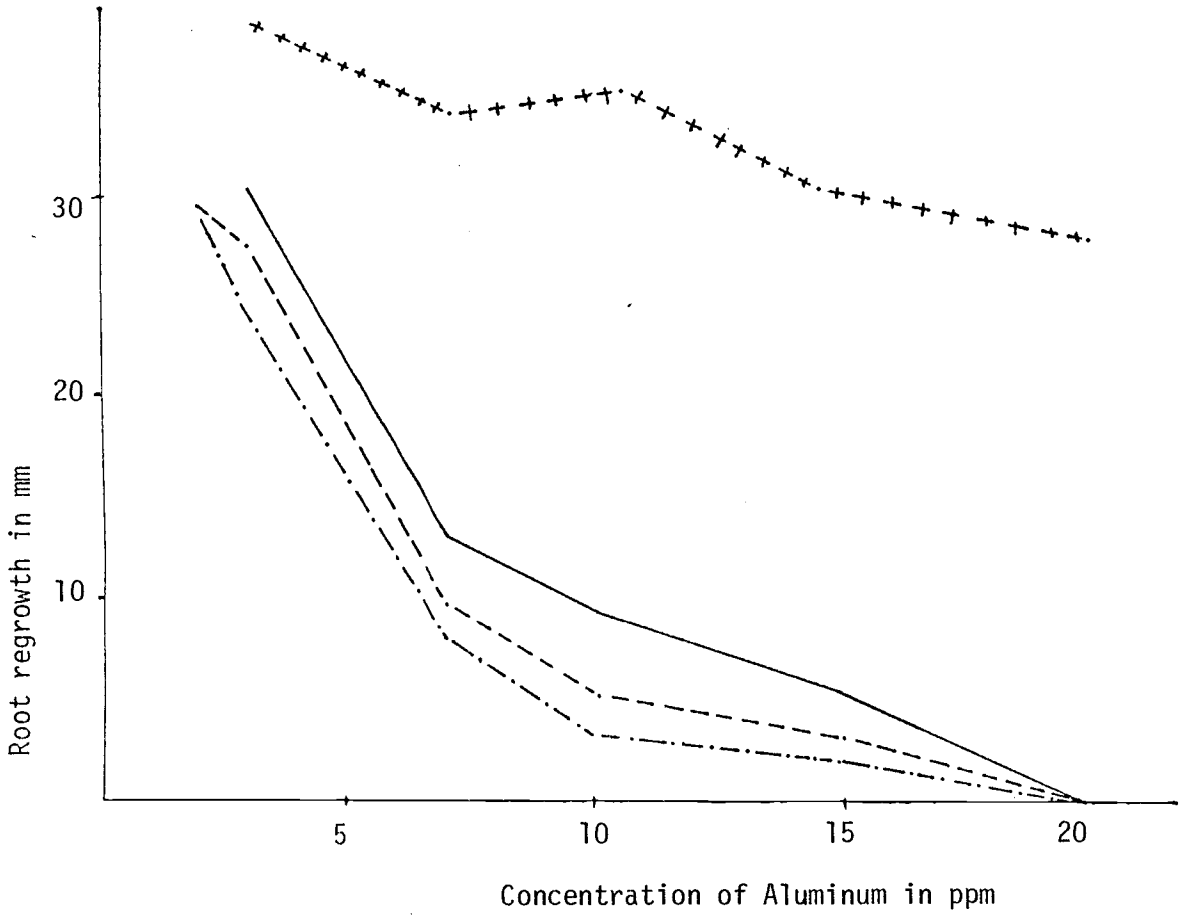

Figure 1. Mean root regrowth of Rye 1443 and wheats Atlas 66, Preludio and Carazinho of different Al concentrations and 25°C temperature.

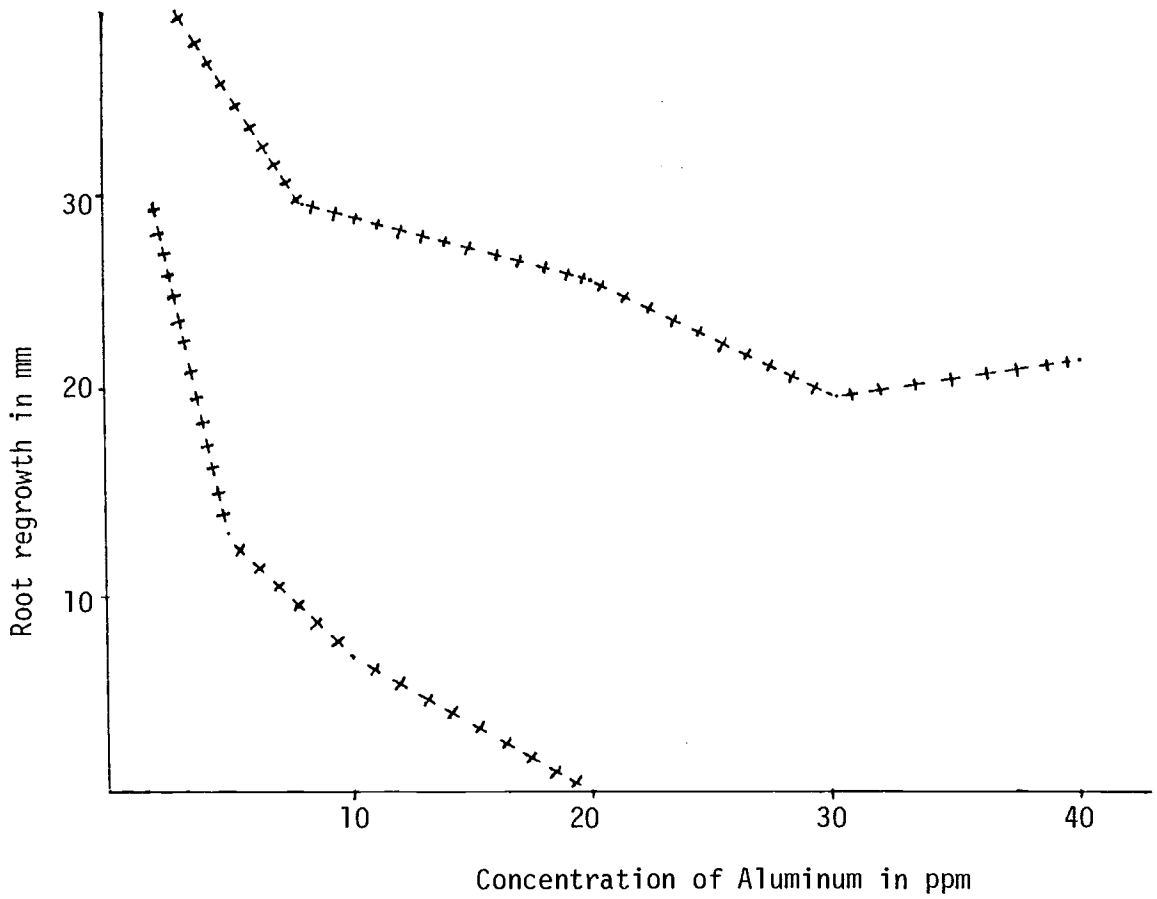

Figure 2. Effect of temperature on the reaction of a tolerant rye (Rye 1443) to different concentrations of Aluminum.

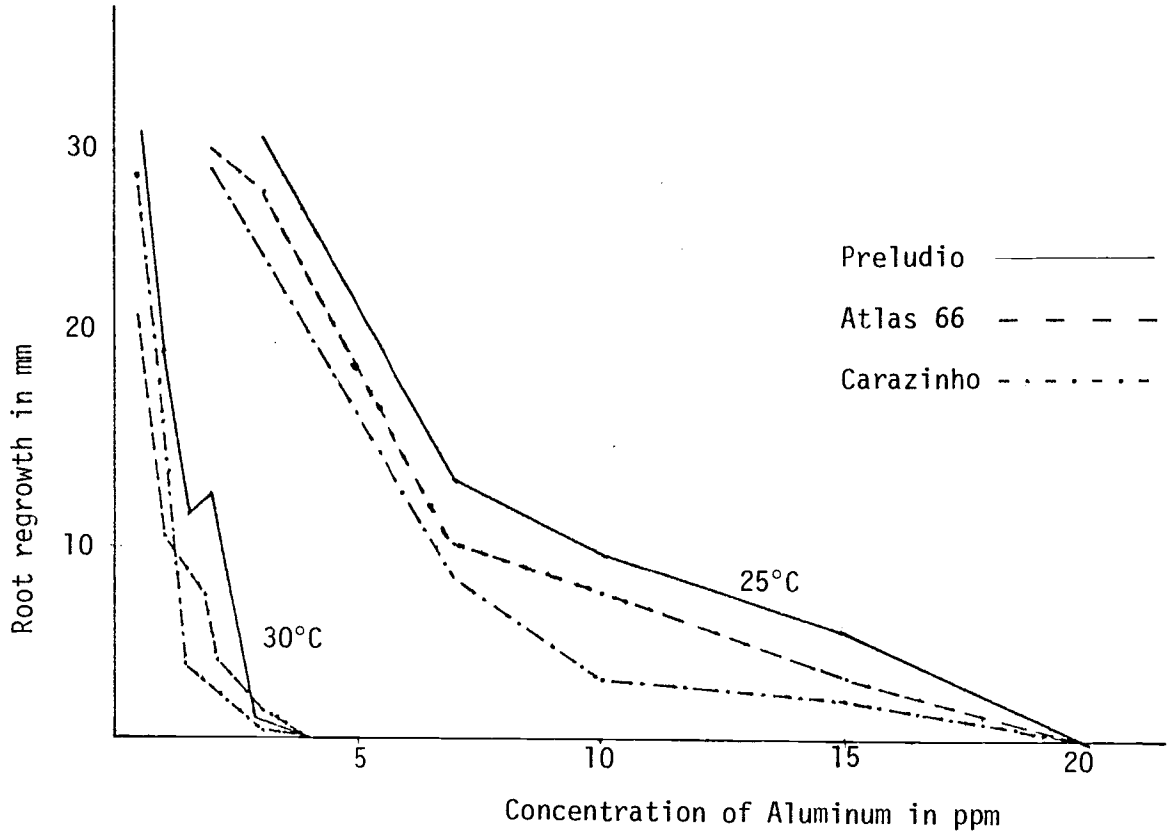

Figure 3. Effect of temperature on the reaction of Preludio, Atlass 66 and Carazinho to different Al concentrations.

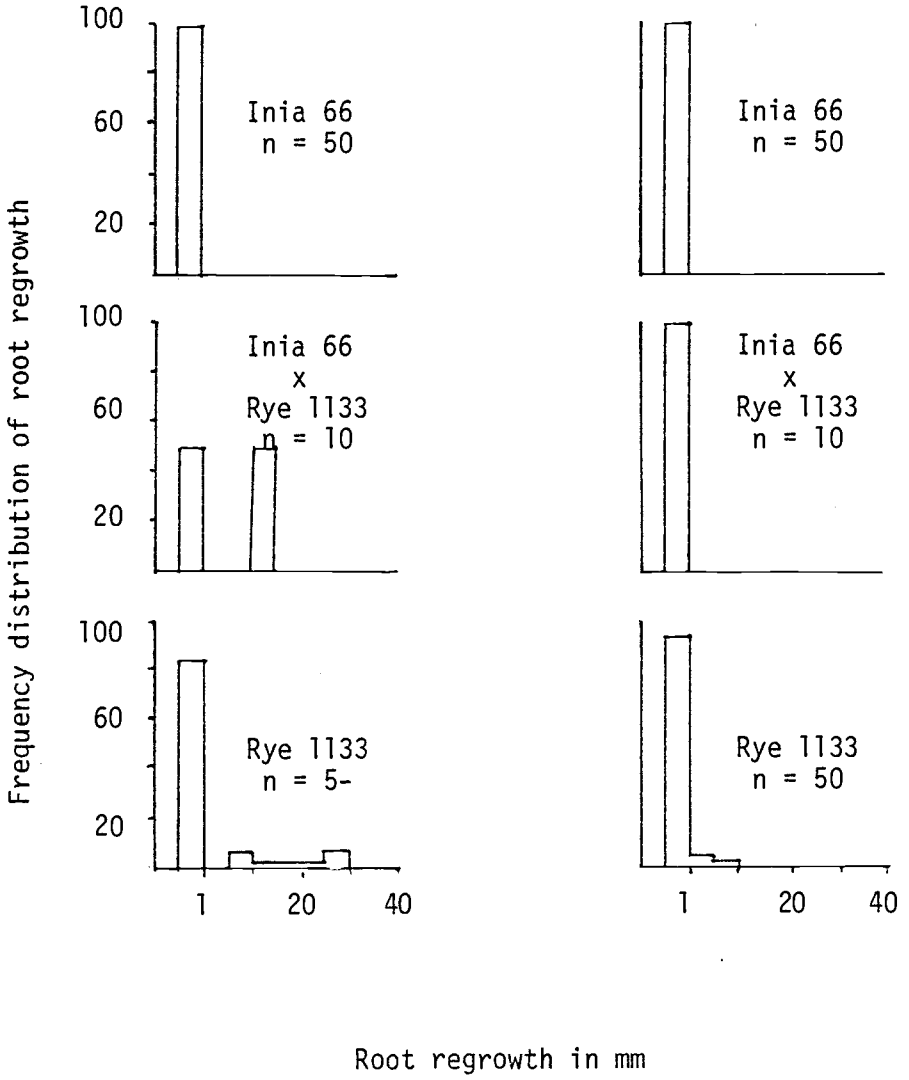

Figure 4. Root regrowth in mm of Inia 66, Rye 1133, and the resulting F_1 hybrid at 1 ppm (left) and 4 ppm (right) and 25°C temperature.

tolerant. The length of root regrowth of the tolerant F_1's did not approach the root length of the tolerant seedlings of rye. At 4 ppm Al, as expected, Inia 66 showed complete sensitivity, while Rye 1133 exhibited 12 percent tolerant seedlings with less root regrowth than at 1 ppm. The F_1 was completely sensitive.

Frequency distribution of root regrowth of Penjamo 62, Rye 1003 and the F_1 derived from the cross at 1 and 4 ppm Al is presented in Figure 5. Ninety-four percent of the Penjamo 62 seedlings were tolerant to 1 ppm with only 4 percent being sensitive. This was expected since Penjamo 62 was more tolerant at 1 ppm Al than Inia 66 even though in this study it was classified as being sensitive. Maximum root regrowth was 30 mm. Seventy-eight percent of seedlings were sensitive and 22 percent tolerant for the rye parent with a maximum root regrowth of 50 mm. Only tolerant seedlings were observed for the F_1 with root regrowth ranging from 5 to 40 mm. At 4 ppm, Penjamo 62 was completely sensitive, Rye 1003 showed 16 percent tolerant seedlings with a maximum root regrowth of 25 mm. The F_1 showed two classes, sensitive and tolerant. Maximum root regrowth was also 25 mm.

Frequency distribution of root regrowth of Hope 7D, Rye 1133 and the resulting F_1 at 1 and 4 ppm Al is presented in Figure 6. Hope 7D was tolerant to 1 ppm with a maximum root regrowth of 20 mm. Rye 1133 showed 22 percent tolerant seedlings with a maximum root regrowth of 30 mm. F_1 seedlings were all tolerant with a maximum root regrowth equal to that of the rye parent. At 4 ppm, Hope 7D was completely sensitive and about 12 percent of the Rye

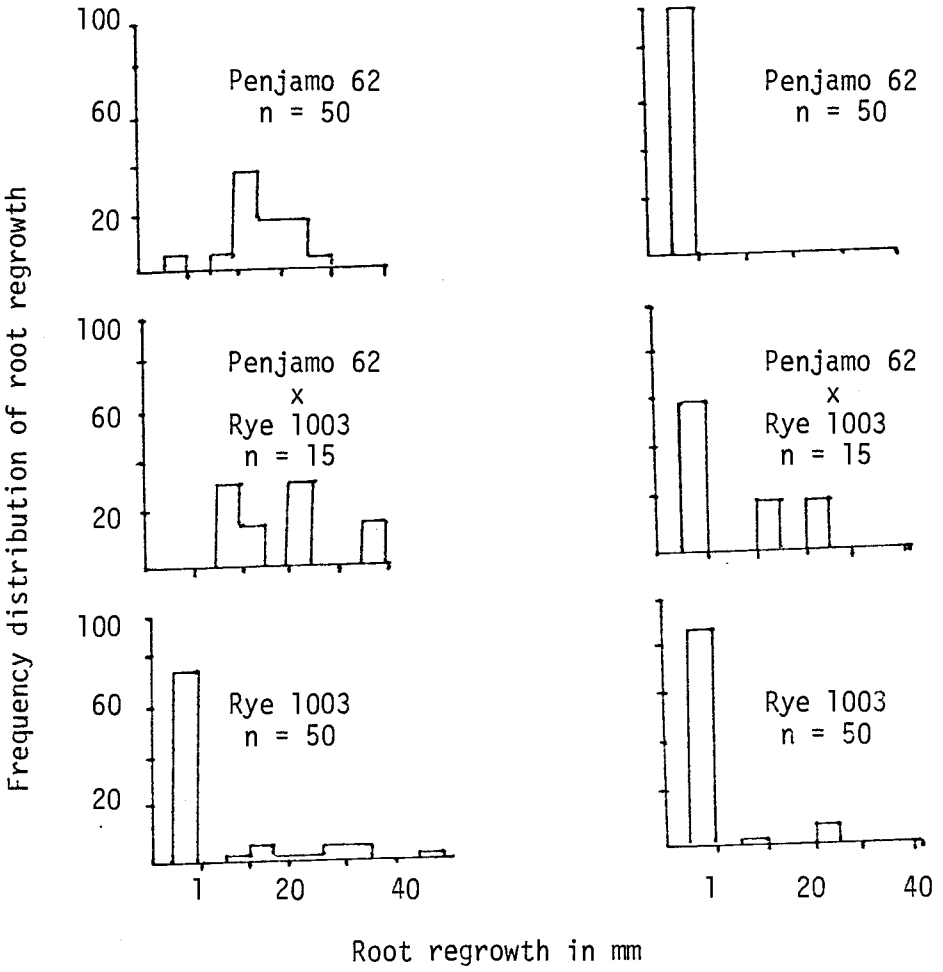

Figure 5. Root regrowth in mm of Penjamo 62, Rye 1003 and the F_1 hybrid at 1 ppm Al (left) and 4 ppm (right) and 25°C temperature.

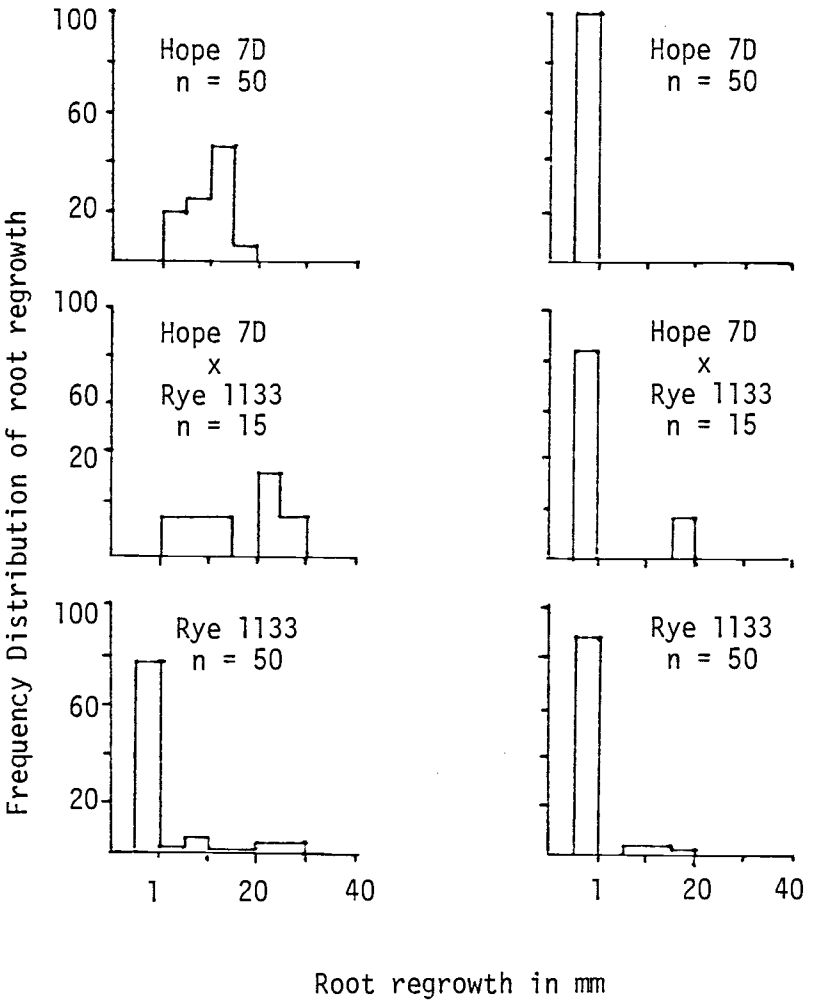

Figure 6. Root regrowth in mm of Hope 7D, Rye 1133, and the F_1 at 1 ppm (left) and 4 ppm (right) and 25°C temperature.

1133 seedlings were tolerant. Maximum root regrowth was 20 mm. Eighty-four percent of the F_1 seedlings were sensitive and 16 percent were tolerant with a maximum root regrowth equal to the rye parent.

Amphidiploids

Amphidiploids derived from sensitive wheat x sensitive rye F_1's were also tested for reaction to Al at 1 and 4 ppm Al and 25°C temperature. As seen from Figure 4, the Inia 66 x Rye 1133 F_1 showed two classes of reaction to 1 ppm Al. With the colchicine treatment, only the sensitive class produced amphidiploid seeds. Seedlings derived from these seeds were similar to the F_1's in reaction to 1 ppm Al.

Amphidiploids derived from the cross Penjamo 62 x Rye 1003, in contrast to the F_1, showed two reaction classes to 1 ppm. Twenty-four percent of the amphidiploid seedlings were sensitive and 76 percent were tolerant. Maximum root regrowth was 35 mm, while that of the rye parent was 40 mm. At 4 ppm, 64 percent of the amphidiploid seedlings were sensitive and 34 percent tolerant with the root regrowth being inferior to that of the rye parent (Figure 7).

The reaction of Hope 7D, Rye 1133, and Amphidiploid Hope 7D x Rye 1133 to 1 and 4 ppm is shown in Figure 8. At 1 ppm Al, 100 percent of the amphidiploid seedlings were tolerant. Distribution of root regrowth indicated the amphidiploid was more tolerant than the parental wheat. Maximum root regrowth was equal to that of the rye parent.

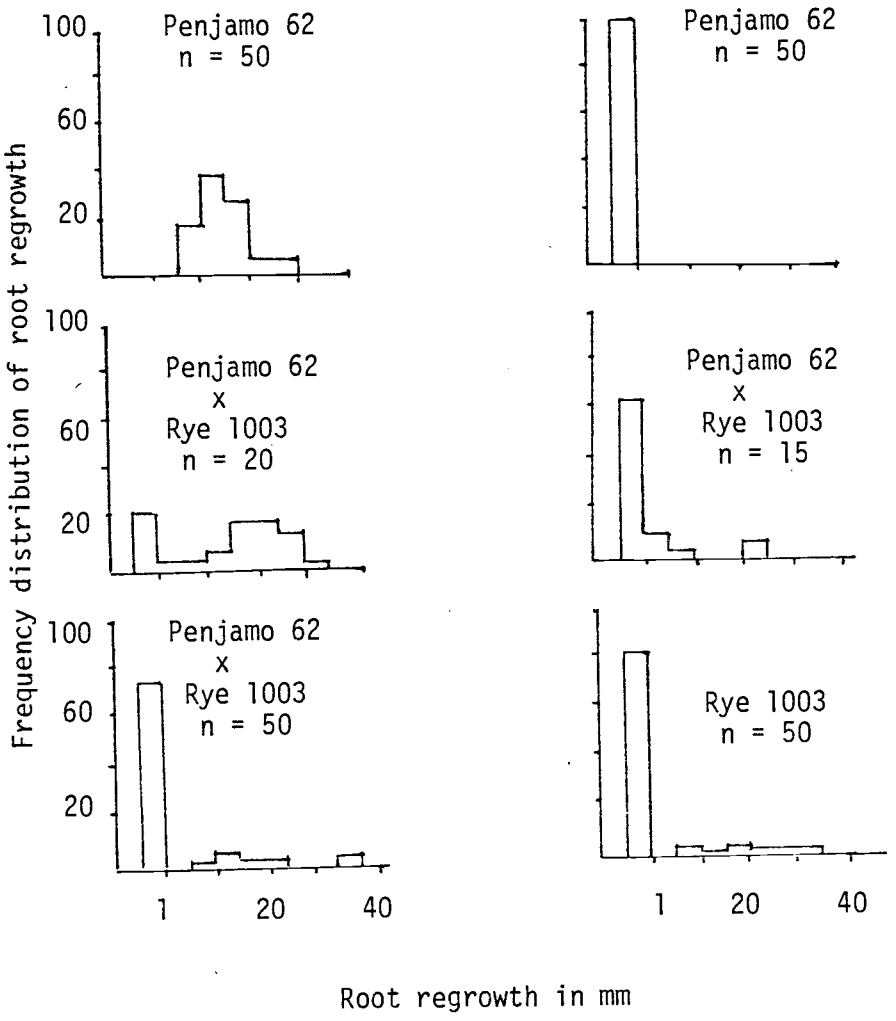

Figure 7. Root regrowth in mm of Penjamo 62, Rye 1003 and the resulting amphidiploid at 1 ppm (left) and 4 ppm (right) and 25°C temperature.

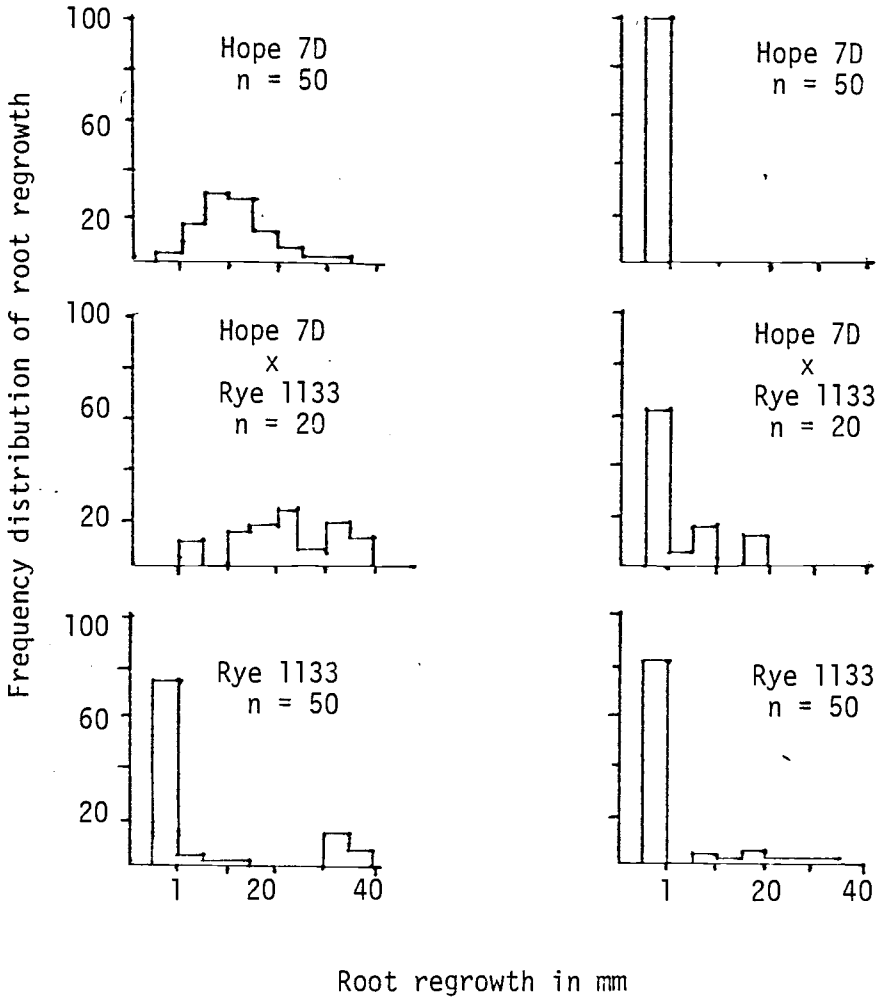

Figure 8. Root regrowth in mm of Hope 7D, Rye 1133, and the resulting amphidiploid at 1 ppm (left) and 4 ppm (right) and 25°C temperature.

In general, it appears that both F_1's and amphidiploids were somewhat more tolerant than the wheat parents to 1 ppm Al. No cultivar of wheat showed tolerance to 4 ppm Al. However, two reaction classes were found in F_1's, amphidiploids and parental rye. This suggests that genes that conferred tolerance in Rye 1133 to 1 and 4 ppm were transmitted to the F_1's and retained in the amphidiploid. Appendix Table 6 indicates that no statistical differences were found between root regrowth of sensitive ryes and amphidiploids derived from them.

Group II. Tolerant Wheat x Sensitive Rye

F_1's and amphidiploids derived from tolerant wheat x sensitive rye were tested at 5 and 10 ppm Al. As seen from Table 4, 5 ppm inhibited root regrowth of sensitive cultivars of rye; 10 ppm Al established statistical differences between tolerant wheats. This Al concentration was expected to yield information as to whether these F_1 hybrids would be more tolerant than the parental wheats.

Frequency distribution of root regrowth of Atlas 66, Rye 37 and the F_1 is presented in Figure 9. Rye 37 was sensitive to 5 ppm Al, while Atlas 66 exhibited 100 percent tolerant seedlings with a root regrowth range from 20 to 40 mm. All F_1 seedlings were tolerant, but root regrowth varied from 15 to 30 mm. Seedlings of Atlas 66 were tolerant at 10 ppm with a length of root regrowth being markedly reduced. F_1 seedlings were all tolerant, but the length of root regrowth was less than that of Atlas. Rye 37 was completely sensitive.

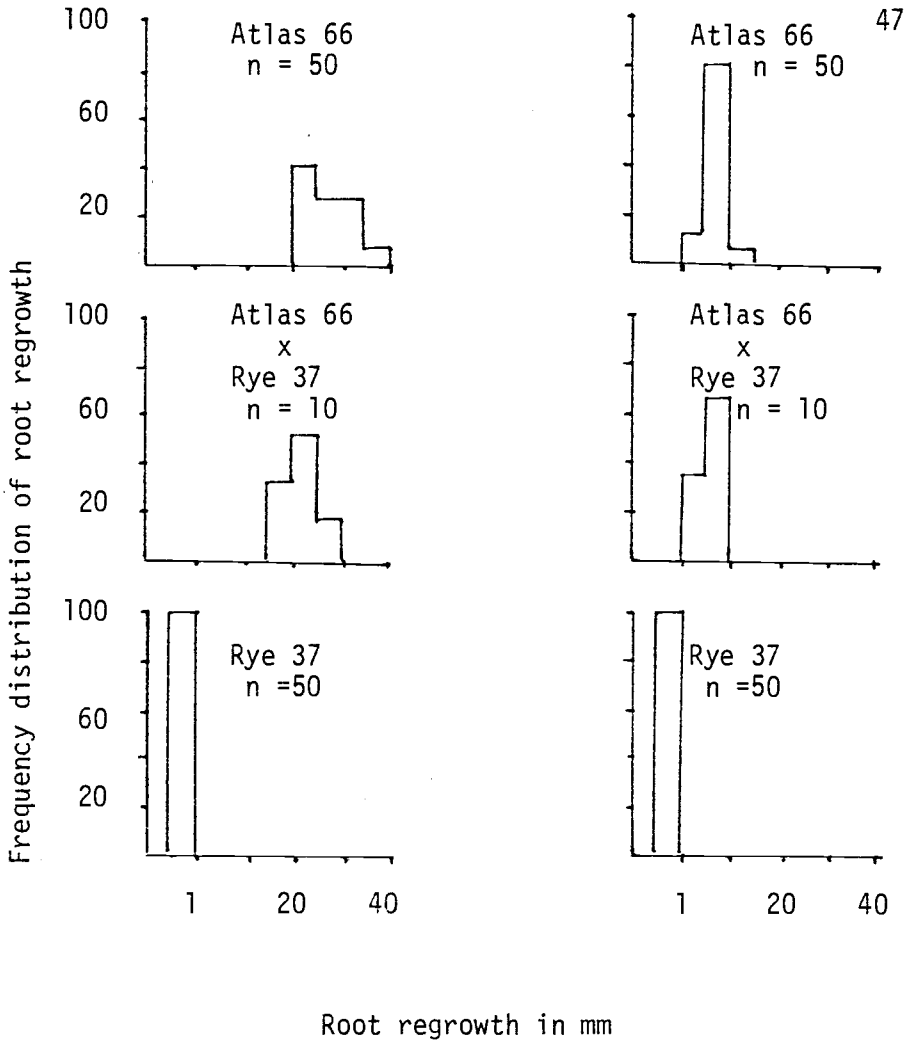

Figure 9. Root regrowth in mm of Atlas 66, Rye 37 and the resulting F_1 hybrid at 5 ppm (left) and 10 ppm (right) and 25°C.

Distribution of root regrowth of the F_1 Carazinho x Rye 1133 cross and its parents at 5 and 10 ppm is shown in Figure 10. Rye 1133 was sensitive to 5 and 10 ppm. Carazinho was tolerant to both Al concentrations. Root regrowth at 5 ppm varied from 20 to 35 mm and from 1 to 20 mm at 10 ppm. F_1 seedlings were all tolerant to both Al concentrations, but root regrowth was markedly reduced at 10 ppm.

Amphidiploids

Amphidiploids derived from tolerant wheat x sensitive rye F_1's were also tested for their reaction to Al at 5 and 10 ppm. Frequency distributions of root regrowth of the amphidiploid Atlas 66 x Rye 37, at 5 and 10 ppm is shown in Figure 11. Rye 37 was sensitive to 5 and 10 ppm while Atlas 66 was tolerant to both Al concentrations, but root regrowth was markedly reduced at 10 ppm. Seedlings of the amphidiploid did not show sensitivity to 5 or 10 ppm Al concentrations.

Amphidiploid Carazinho x Rye 1133 was tolerant to 5 and 10 ppm Al. At 5 ppm, root regrowth of Carazinho varied from 10 to 35 mm, while that of the amphidiploid varied from 1 to 35 mm. At 10 ppm root regrowth of both wheat and amphidiploid was reduced and varied from 1 to 15 mm in Carazinho and from 1 to 10 mm in the amphidiploid (Figure 12).

Mean root regrowth of Atlas 66, Carazinho, Rye 37, Rye 1133 and the resulting amphidiploids are presented in Appendix Table 7.

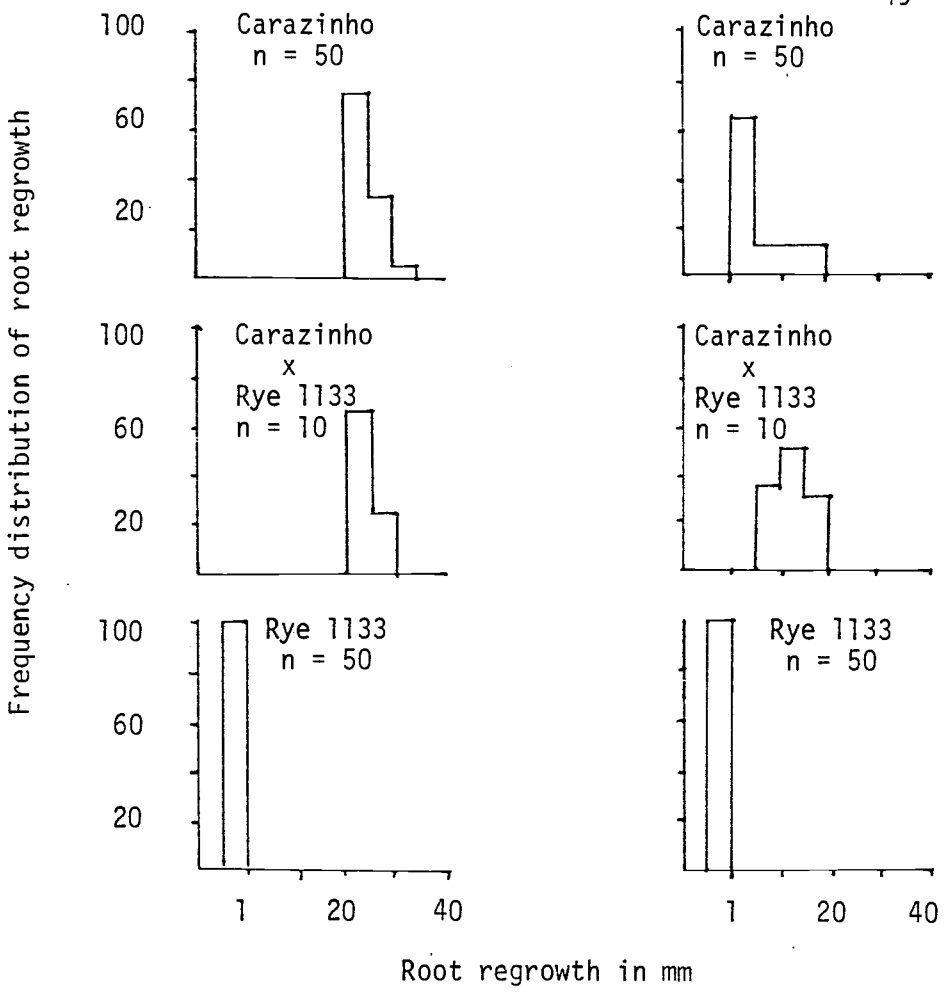

Figure 10. Root regrowth in mm of Carazinho, Rye 1133 and the resulting F_1 hybrid at 5 ppm (left) and 10 ppm (right) and 25°C temperature.

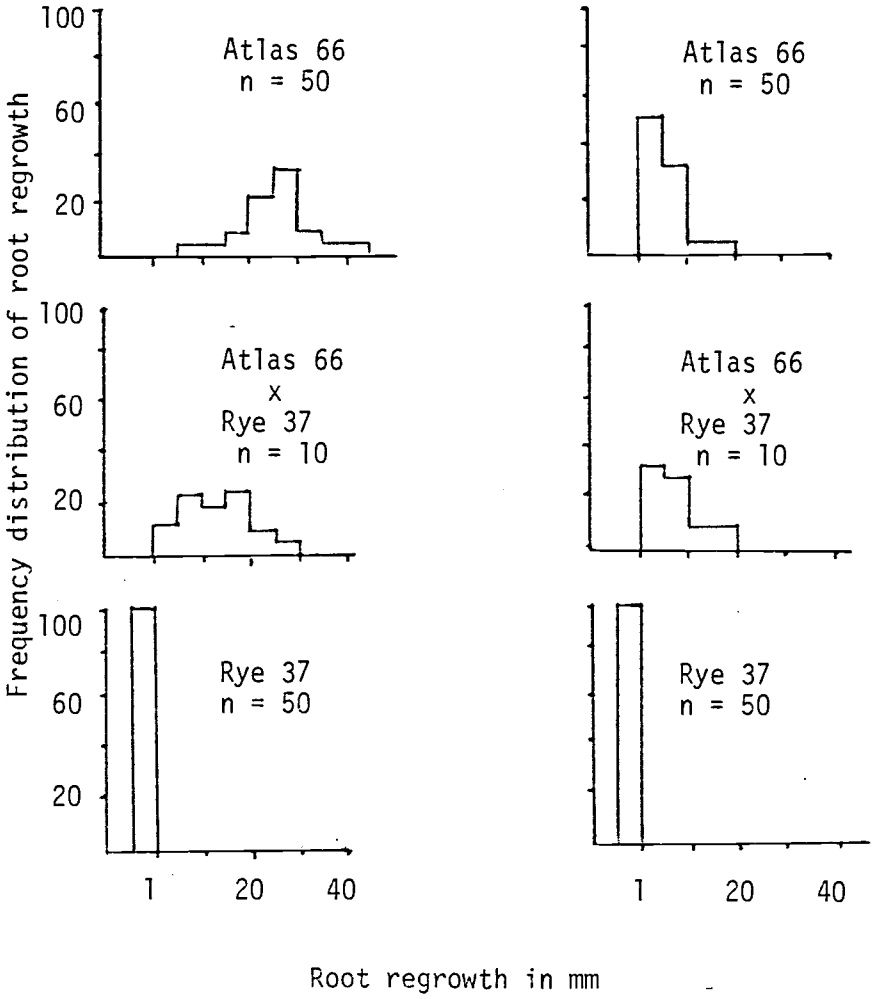

Figure 11. Root regrowth in mm of Atlas 66, Rye 37, and the amphidiploid Atlas 66 x Rye 37 at 5 ppm (left) and 10 ppm (right) and 25°C temperature.

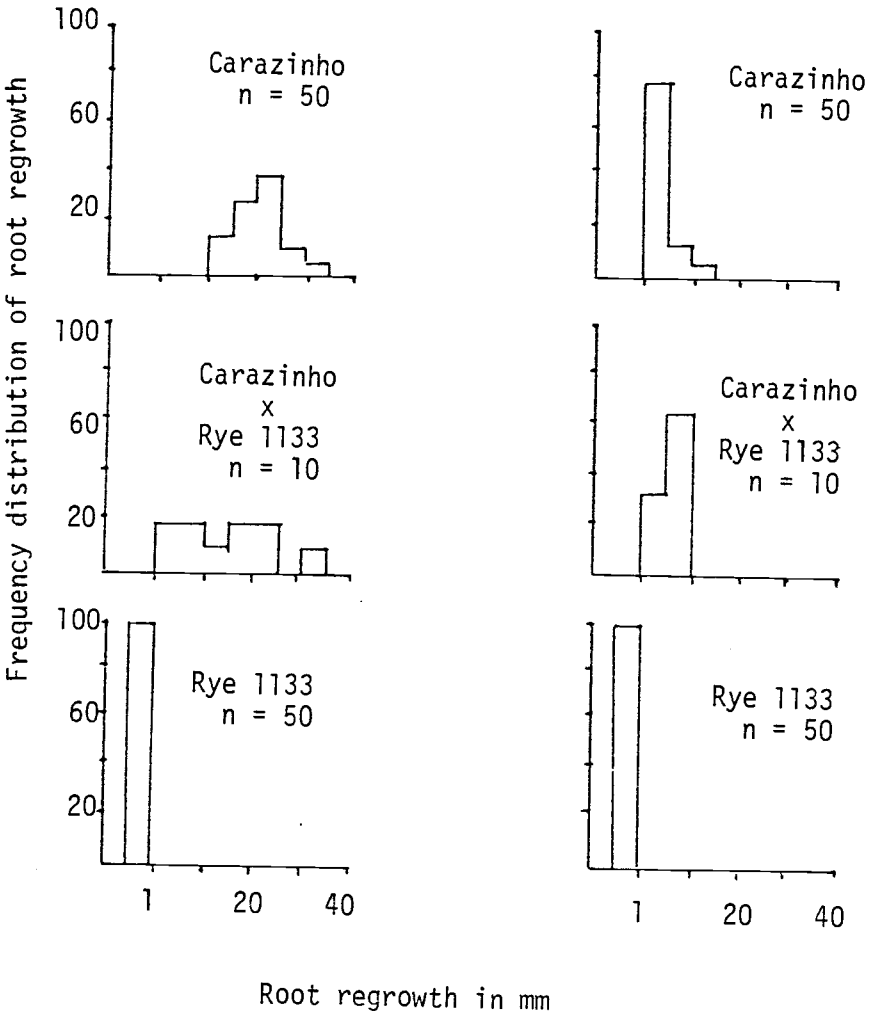

Figure 12. Root regrowth in mm of Carazinho, Rye 1133 and the amphidiploid Carazinho x Rye 1133 at 5 ppm (left) and 10 ppm (right) and 25°C temperature.

Group III. Sensitive Wheat x Tolerant Rye

Since the tolerant rye was sensitive to 20 ppm Al at 30°C temperature, F_1 progeny derived from sensitive wheat x tolerant rye was tested at 30°C using 5, 10, and 15 ppm of Al. Results of Figure 13 indicate that 15 ppm at 30°C temperature was toxic for this F_1. About 15 percent of the rye seedlings were tolerant. Although root tips of the F_1 were badly damaged by 5 and 10 ppm Al, no sensitive seedlings were recorded. Root regrowth of the rye parent was more restricted by 10 than by 5 ppm of Al. The reaction pattern of the F_1 Hope 7D x Rye 1443 to 5 and 10 ppm at 30°C can be observed in Figure 13. At 5 ppm, root regrowth of Rye 1443 varied from 1 to 20 mm and from 5 to 15 mm at 10 ppm. The F_1 did not present any sensitive seedlings at any of these Al concentrations, but root regrowth was more restricted at 10 ppm than at 5 ppm Al. No root regrowth occurred at 15 ppm.

Amphidiploid

The amphidiploid derived from Hope 7D x Rye 1443 F_1 was tested under the same conditions as the F_1 to see whether the amphidiploid would have the same degree of tolerance as the F_1. However, the amphidiploid was sensitive to 5 ppm Al at 30°C temperature. Consequently, temperature was lowered to 25°C and Al concentration increased to 10 and 20 ppm.

Frequency distributions of root regrowth of this amphidiploid and its rye parent are presented in Figure 14. Again 20 ppm Al was

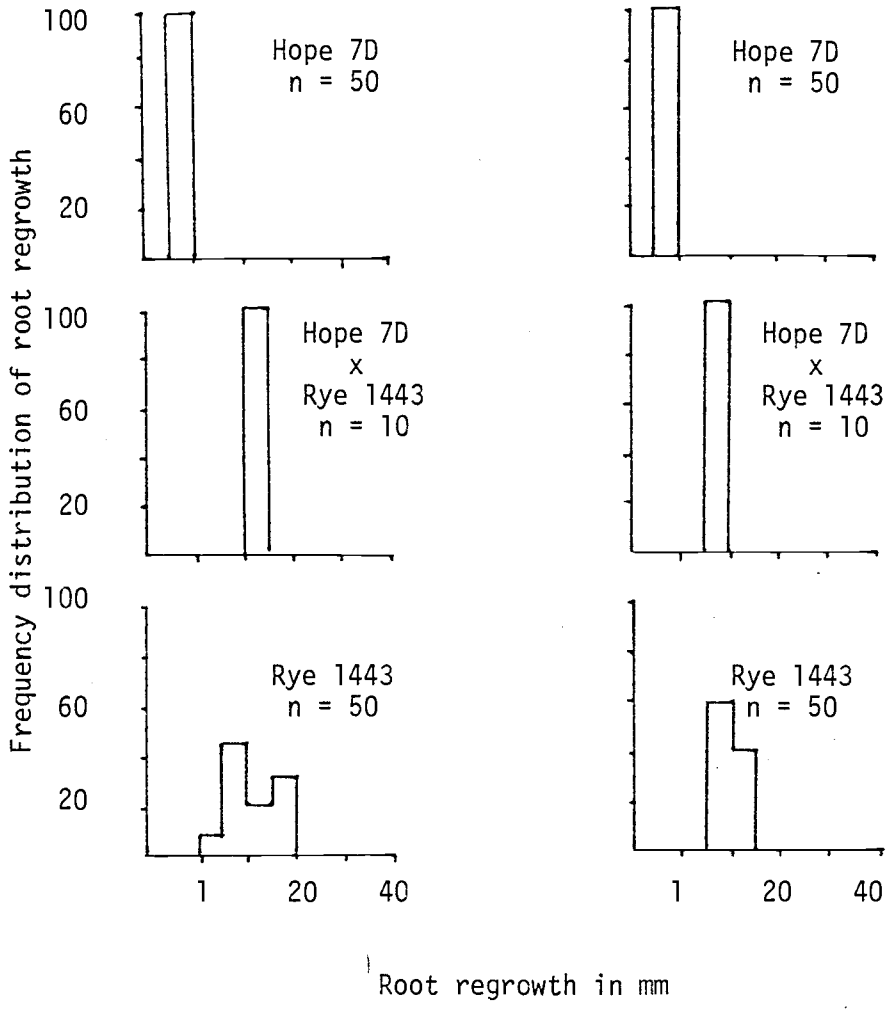

Figure 13. Root regrowth in mm of Hope 7D, Rye 1443 and the F_1 Hope 7D x Rye 1443 at 5 ppm (left) and 10 ppm (right) and 30°C temperature.

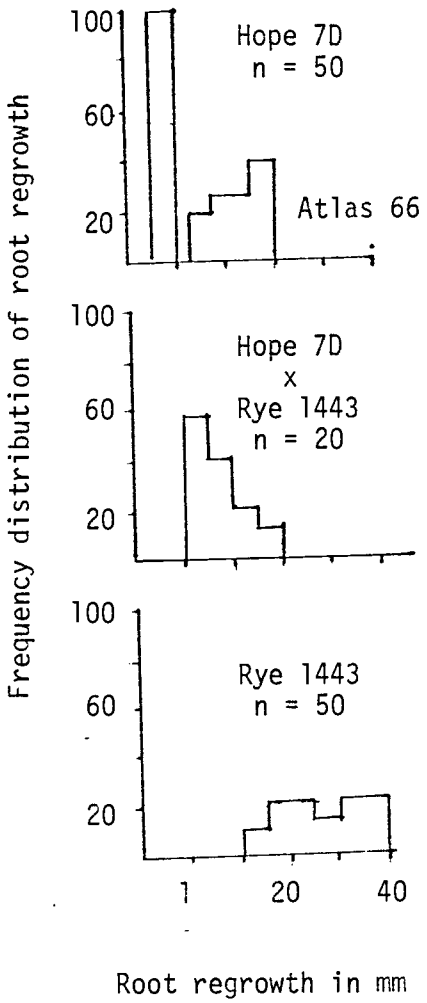

Figure 14. Root regrowth in mm of Hope 7D, Rye 1443, and the resulting amphidiploid at 10 ppm. 25°C temperature.

toxic. At 10 ppm, no sensitive seedlings were recorded.

Root regrowth varied from 1 to 20 mm. Although the amphidiploid seemed to be more sensitive than Atlas 66, which was included as control, no significant differences were found. Rye 1443 was completely tolerant. Root regrowth varied from 10 to 40 mm.

Group IV. Tolerant Wheat x Tolerant Rye

The F_1 derived from the cross Atlas 66 x Rye 1443 was tested at 5, 15 ppm and 20 ppm Al at 30°C. Distribution of root regrowth is presented in Figure 15. Atlas 66 was found to be sensitive to 4 ppm Al at 30°C temperature. Therefore the reaction shown in Figure 15 was expected. No sensitive plants were observed with Rye 1443 with root regrowth varying from 5 to 20 mm. F_1's seedlings were all tolerant with a maximum root regrowth similar to that of the rye parent. At 15 ppm, two types of reaction were recorded in the rye parent. Forty-four percent of the seedlings were tolerant and had a root regrowth of 15 mm. Thirty percent of the F_1 seedlings were tolerant with a length of root regrowth equal to that of the rye parent. No root regrowth of either the rye parent or the F_1 occurred at 20 ppm.

Amphidiploid

Since the amount of amphidiploid seeds derived from the cross Atlas 66 x Rye 1443 was limited, the experiment was conducted at 25°C to avoid the possibility of subjecting seedlings to an excessively high Al stress. Frequency distributions of root regrowth of this amphidiploid, Atlas 66, and Rye 1443 at 10 and 20 ppm are

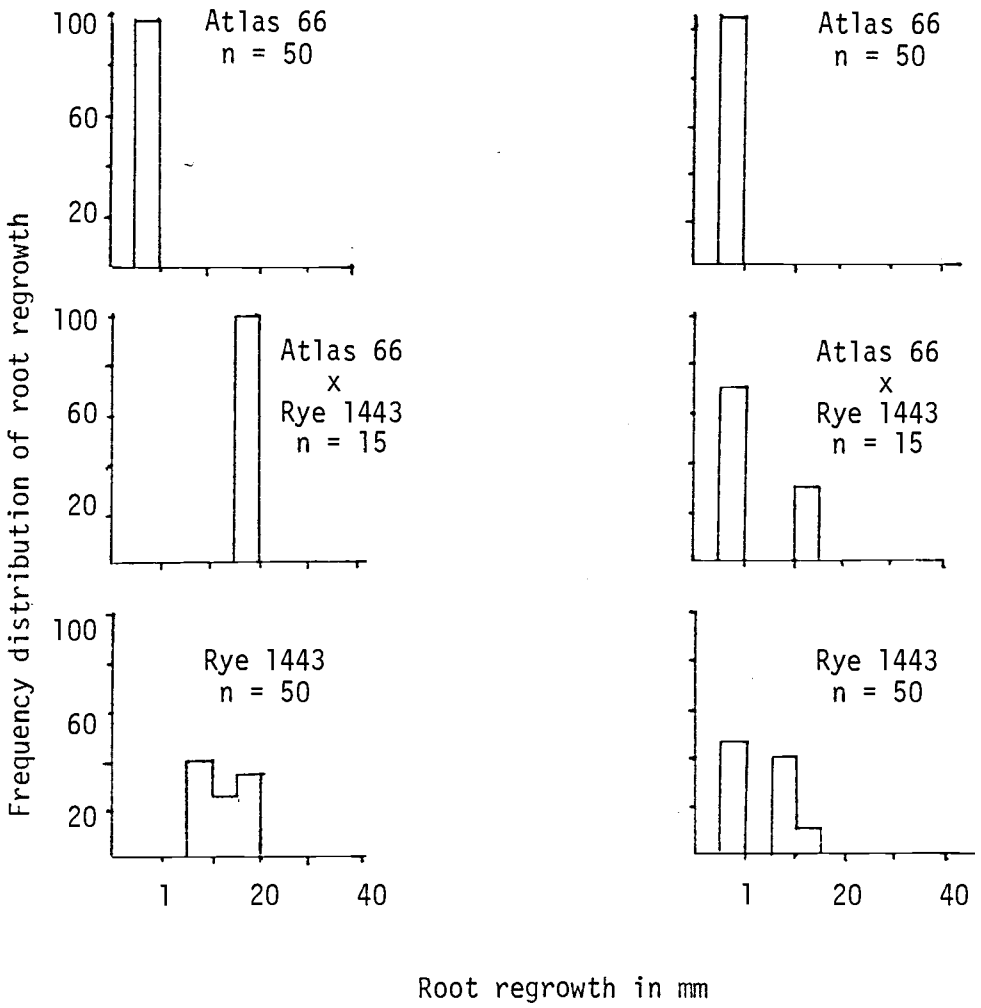

Figure 15. Root regrowth of Atlas 66, Rye 1443 and the resulting F_1 hybrid at 5 ppm (left) and 15 ppm (right) and 30°C.

shown in Figure 16. Atlas 66 was completely tolerant to 10 ppm Al, and root regrowth varied from 1 to 20 mm. Rye 1443 did not present any sensitive seedlings to this concentration of Al. Root regrowth varied from 10 to 40 mm. The amphidiploid was tolerant with root regrowth being similar to that of Atlas 66. At 20 ppm Atlas 66 was completely sensitive. Root regrowth of Rye 1443 varied from 10 to 30 mm. The amphidiploid did not exhibit any sensitive seedlings. Although root regrowth was less than that of Rye 1443, it was more than Atlas.

Effect of Al on Cell Division

The effect of Al on cell division was studied in three plant species, wheat, rye, and triticale. The number of mitotic figures of tolerant plants were compared to that of sensitive plants.

Daws, Rye 1133 and the triticale Daws x Rye 1133 were sensitive cultivars. Atlas 66, Rye 1443 and the triticale Daws x Rye 1443 were selected as tolerant types.

Mitotic figures counted for each cultivar at different times of exposure are presented in Table 10. The number of mitotic figures observed at any sampling time was referred to as Mitotic Activity and represents an average from three root tips.

Mitotic activity was always high in the control treatments, regardless of sampling time. However, except in the case of all seedlings from Atlas 66, tolerant cultivars and controls exhibited a reduction in the number of mitotic figures after 9 to 12 hours of exposure to the Al solution. Subsequently the mitotic activity

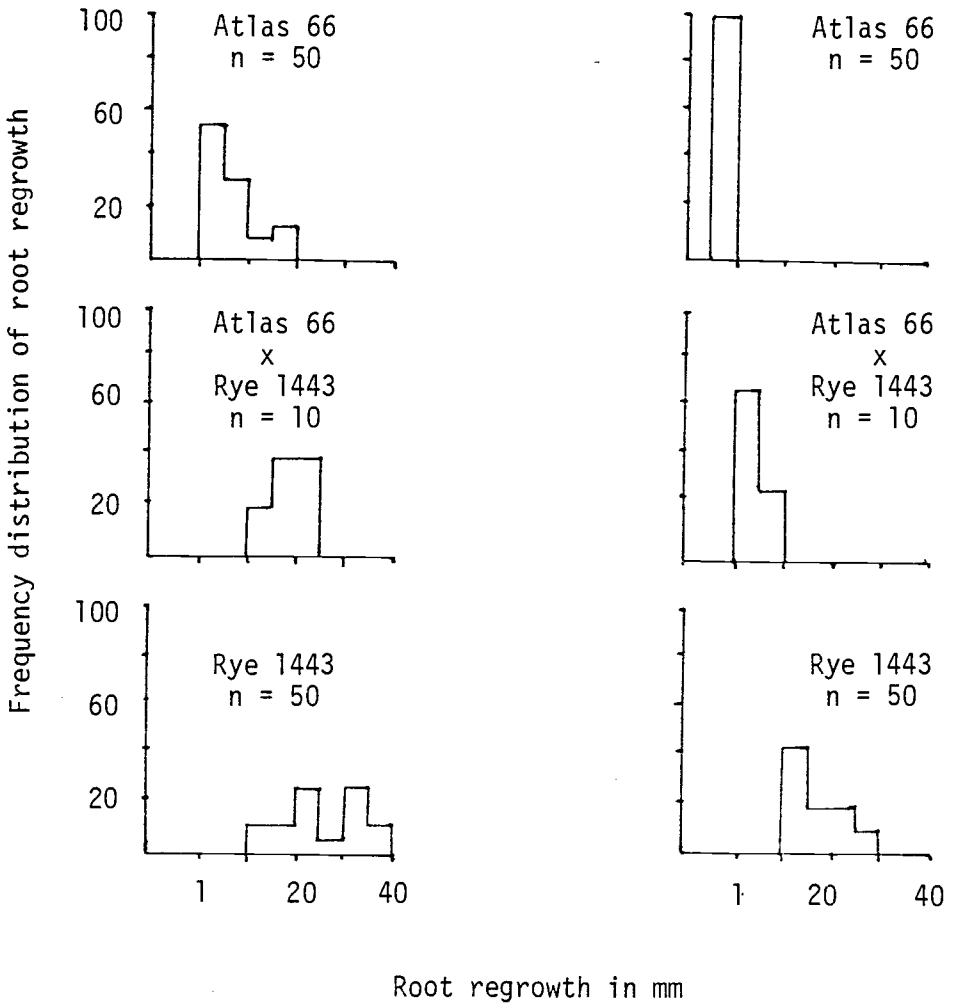

Figure 16. Root regrowth in mm of Atlas 66, Rye 1443 and amphidiploid Atlas 66 x Rye 1443 at 10 ppm (left) and 20 ppm (right) and 25°C temperature.

Table 10. Number of mitotic figures from three root tips involving sensitive and tolerant plants of wheat, rye and triticale as affected by different times of exposure to 7 ppm Al at 25°C temperature.

Sensitive Plants

Time	Daws			Rye 1133			Daws x Rye 1133		
Hours	Control*	Al**	Rec.***	Control	Al	Rec.	Control	Al	Rec.
3	76.1	17.6	0.0	100.0	18.5	1.0	150.0	74	0.0
6	90.0	9.0	0.0	71.0	18.0	0.0	160.0	30.5	0.0
9	80.0	3.3	0.0	70.0	8.5	3.0	140.0	10.6	0.0
12	74.0	3.6	0.0	83.0	8.3	2.0	112.6	1.3	0.0
24	82.0	0.3	0.0	108.0	8.6	0.0	150.0	0.0	0.0
36	66.0	0.0	0.0	140.0	3.5	0.0	149.0	0.0	0.0
48	50.0	0.0	0.0	85.0	2.0	1.0	160.0	0.0	0.0

Tolerant Plants

Time	Daws			Rye 1133			Daws x Rye 1133		
Hours	Control*	Al**	Rec.***	Control	Al	Rec.	Control	Al	Rec.
3	45.0	46.0	25.2	99	59.2	120.0	183.0	96.0	55.3
6	64.4	69.0	26.5	74.1	36.8	160.0	160.0	30.0	60.9
9	66.0	40.6	58.2	150.0	14.0	150.0	154.0	4.2	80.4
12	79.0	23.2	81.0	84.6	42.0	180.0	89.0	14.0	78.6
24	115.0	12.8	82.0	160.0	62.0	134.0	94.0	20.0	66.2
36	103.0	7.2	160.0	134.0	87.5	200.0	207.0	13.0	158.0
48	86.0	24.5	100.0	100.0	150.0	115.0	113.0	21.0	151.0

* Control without Al.
** Al treatment.
*** Recovery in Al-free solution.

increased. Mitotic activity of both sensitive and tolerant types when exposed to Al, always showed a reduction as time of exposure to Al increased. This tendency was much more pronounced in sensitive types in which mitotic activity was reduced to zero in wheat and triticale and no root regrowth occurred after the recovery period. Mitotic activity of tolerant plants exposed to Al showed a marked difference with respect to the controls. The decreasing trend from 3 to 12 hours and subsequent increase in mitotic activity from 12 to 48 hours observed in the controls also apparent in tolerant plants with Al treatment.

The marked difference in mitotic activity between the controls and Al treatments, particularly in sensitive types, can be observed in Table 10. From this table it can be noted that mitotic activity following Al treatment of both sensitive and tolerant types is affected within the first three hours of exposure to Al, and completely stopped after 24 hours of exposure in sensitive wheat and triticale. In sensitive rye, some mitotic figures were apparent after 48 hours of Al treatment. However, the number did not increase upon removal of the Al treatment and no root regrowth occurred after 48 hours of recovery in the Al-free solution.

Mitotic activity of Atlas 66 was nearly the same for both the control and Al treatment during the first 6 hours of Al treatment. After 9 hours, the number of mitotic figures decreased progressively as time of exposure to Al increased until 36 hours when the minimum number of mitotic figures was recorded.

After 48 hours of exposure to Al there was an indication that mitotic activity had begun to increase again. This, consequently, caused the affected root to continue to elongate in the Al solution. However, it took about 9 hours of recovery in the Al-free solution for mitotic activity of root tips exposed to Al to fully recover and reach a mitotic activity similar to that of the control.

Root tips from sensitive wheat and rye started to turn yellowish or light brown and somewhat thicker and softer than controls after 9 to 12 hours of Al treatment. This coincided with an appearance of early prophase of most nuclei observed. After 24 hours of Al treatment, the root tips were light brown in color, very soft with about 50 percent of the cells having been destroyed. Binucleated cells were observed after 36 hours of Al treatment, with the root tips being completely brown and thicker than the controls. Few cells and nuclei were observed during this period and for the most part, cell wall residues were observed. After 48 hours of Al treatment all the nuclei had disappeared.

After 24 hours of Al treatment, the root tips of the tolerant cultivars were slightly brown, but still root cells were well organized. After 48 hours, a large number of abnormal cells were present in addition to a great number of apparently normal cells. Mitotic activity had been markedly reduced.

Rye 1443 did not show any visible symptoms of Al injury to the root tips and no abnormal cells were observed. However, mitotic activity was reduced during the first hours of the Al treatment and root length was somewhat shorter than in controls.

The amphidiploid Daws x Rye 1443 showed a large proportion of deformed nuclei and cell wall residues after 24 hours of Al treatment. However, a large proportion of well organized cells with some mitotic activity was observed. Six hours after recovery, cell residues were still visible and after an additional three hours, roots had regrown about 1 millimeter and a larger number of normal cells were observed. Binucleated cells that first were observed in sensitive wheat and rye under Al stress were later observed in tolerant wheat and rye without Al stress. No attempt was made to determine the frequency of binucleated cells in any cultivar. Since this type of cell appeared in both control and Al treatments, it cannot be inferred that they resulted as a consequence of Al treatment.

In order to determine if Al treatment depressed any particular stage of cell division (Prophase, Metaphase, Anaphase or Telophase), an average of mitotic figures in each stage from sensitive, tolerant and control cultivars was obtained and compared. Root tips were sampled after 3, 6, 9, 12, 24, 36 and 48 hours of Al treatment and at the same intervals of time of recovery in the Al-free solution. Results are plotted in Figure 17 and presented in Appendix Table 3. It can be seen that except for the difference in the rate of mitotic activity between sensitive, tolerant and control plants, the pattern of cell division was very similar. These results indicated that after 3 hours of Al treatment, sensitive and tolerant cultivars showed a marked reduction in the number of mitotic figures in all four stages of cell division, with the reduction being more striking in sensitive types. The maximum reduction in mitotic activity of

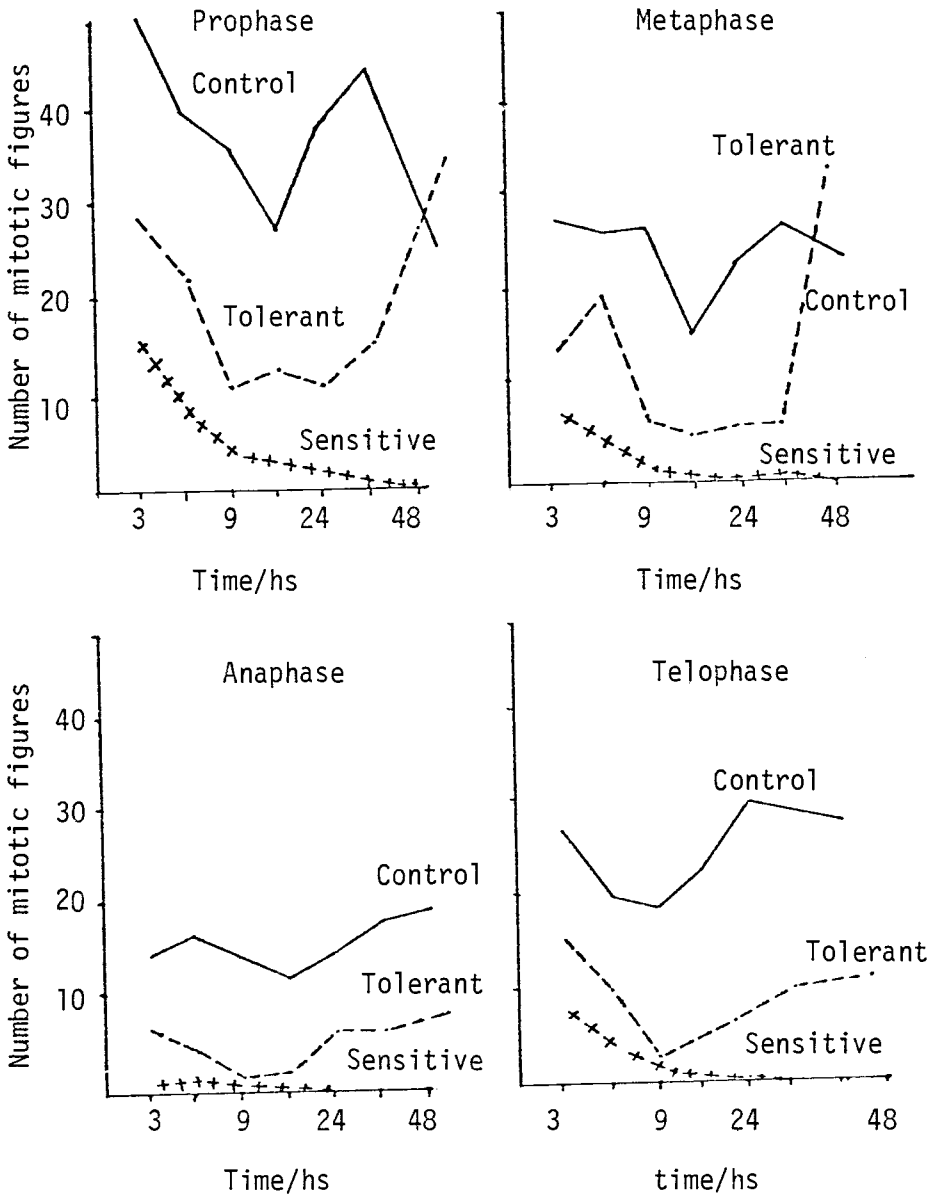

Figure 17. Effect of the Aluminum treatment on the averaged mitotic activity of two wheats two ryes and two triticales.

root tips smears from tolerant plants coincided with the reduced mitotic activity of controls at about 9 to 12 hours of Al treatment.

Rye 1443 did not show any visible damage, however root length of the control was somewhat longer after 48 hours than that of seedlings exposed to Al treatment. This difference in root length can be explained by the differences in mitotic activity between Al treatment and control. Table 10 indicates that a reduction in mitotic activity of this rye was apparent after 3 to 9 hours of Al treatment. After 9 hours, when the minimum number of mitotic figures was recorded, a marked increase was observed. However, as seen from Table 10, a clear difference in mitotic activity between control and Al treatment was apparent during the first 36 hours of Al treatment. The high rate of mitotic activity observed after 48 hours of Al treatment, as well as after 3 and 6 hours of recovery, indicated the mechanisms for tolerance of the rye had overcome the repressive effect of Al on cell division and that, as seen from the mitotic activity in the control and Al treatment, normal cell division was occurring in the Al treatment after 48 hours of exposure.

Mitotic activity of the primary triticale Daws x Rye 1443 also showed a marked difference between control and Al treatment. The number of mitotic figures decreased after 3 and up to 9 hours of Al treatment and then increased after 9 to 48 hours of Al treatment. Again, this suggested that when plants first come in contact with the Al solution, cell division may be affected within 3 hours. The

recovery of mitotic activity in the Al treatment after 9 to 48 hours also indicated that after the first 9 hours the mechanisms of tolerance introduced into triticale by rye had overcome the repressive effect of Al on cell division. However, it took approximately 36 hours of recovery in Al-free solution before mitotic activity of the exposed root fully recovered and showed a mitotic rate similar to that of the control. Mitotic activity of the primary triticale Daws x Rye 1443, where Rye 1443 was the only tolerant parent, indicated that the effectiveness of the mechanism for tolerance in the rye was reduced in the triticale.

V. DISCUSSION

Emphasis is being placed on the genetic improvement of the first man-made species, triticale. Due to the success of plant breeders, triticale has the potential of becoming a commercial crop; however, for triticale to be competitive with other cereal, it must be superior to existing cereal grains in at least one or several major attributes, or exhibit a greater degree of tolerance to various limiting factors.

Observations made by some investigators suggest that under certain soil types and climatic conditions, certain lines of triticale are superior to other cereals. Kiss (1924) reported that in experiments conducted in poor sandy soils (pH 4.0 to 5.0), triticale outyielded wheat. Srivastava (1974) also indicated that in the eastern Himalayas with over 300 cm annual precipitation and a soil pH of 4.5, triticale appears to be a better adapted crop. Further support of the tolerance of triticale to acid soils comes from the CIMMYT Fifth International Triticale Yield Nursery which points out the good performance of triticale in acid soils in Brazil.

At the present time, no specific attempts have been made to develop a triticale cultivar which is highly tolerant to soil acidity. This would require extra efforts, time and money. Since the major triticale breeding programs are not located in areas where soil acidity is a limiting factor no selection pressure is being applied to develop tolerant cultivars. The availability of a simple

and effective methodology to screen breeding material for tolerance to soil acidity would be extremely valuable for this purpose.

Screening Technique

Results obtained in this study indicated that the seedling screening technique first developed for use in growth chambers by Moore (1974) was efficient in classifying cultivars of wheat, rye, and triticale for reaction to Al when water baths for temperature control were used in the greenhouse. However, less concentration of Al was necessary to inhibit root regrowth under greenhouse conditions than when utilizing the growth chamber. The fact that this technique can be used efficiently under greenhouse conditions is significant to breeding programs where growth chambers are not available.

Factors known to affect the plants' reaction to a given concentration of Al are: nutrient concentration, pH, length of exposure to Al, Aluminum concentration and temperature. Moore (1974) showed that the pH of the nutrient solutions has a determinant effect on the wheat reaction to Al. He suggested that in the pH range from 4.0 to 5.0, two major ionic forms of Al exist in solution, Al^{+++} and $AlOH^{++}$. The relationship of one to the other depends on pH. As pH increased from 4.0 to 4.5, the Al^{+++} decreased, the $AlOH^{++}$ increased, and the toxicity of soluble Al also increased. He concluded that the toxicity of a given concentration of Al was increased by increasing the pH from 4.0 up until the solubility of

Al in solution was exceeded. Rhue (1976) speculated that the H^+ ion had a detrimental effect on the permeability of the cell membrane causing it to become less selective thereby affecting the plant reaction to a given Al concentration.

The nutrient concentrations in the Al solution also have a prominent effect on the wheat reaction to Al. Ali (1973) demonstrated that the severity of toxicity by Al was sharply increased by decreasing the nutrient concentration in the Al solution. Time of exposure to Al has also been shown to affect the reaction of wheat to Al. Henning (1975) found that the primary effect of Al was the death of cells which occurred within the first 24 to 48 hours of exposure to Al. The mitotic cycle was affected almost at the onset of the Al treatment. However, mitotic activity could proceed again if the Al stress was removed before the cell's cytoplasm was completely displaced by vacuoles. The effect of temperature on the reaction to Al has been pointed out by Clarkson (1965). He suggested that the effect of Al depends on the rate of Al uptake. The rate of the absorption process is affected by temperature (Jacobson et al 1957).

It is clear that environmental factors such as air temperature and light intensity are better controlled in the growth chamber than in the greenhouse. This factor may also affect the plant reaction to Al toxicity. Even though the nutrient solutions were maintained at 25°C $\pm$ 1 temperature, the air temperature varied from 15°C at night to 30 or 35°C at noon in the greenhouse. The light intensity

also varied from 900 fc at night to over 2000 during sunny days. These factors may have effected the rate of the absorption of Al by the plants and the physiological processes of the seedlings as compared to those in the growth chambers. This could have resulted in toxic levels of Al accumulating faster in root cells at lower Al concentrations due to a higher rate of uptake than in root cells subjected to higher Al concentrations and a lower rate of uptake. Therefore, there could have been differences in the rate of the absorption due to the higher air temperature and light intensity during the day which might have been responsible for the discrepancies in Al concentrations that inhibited root regrowth in the growth chamber compared to the greenhouse.

The fact that the tolerant cultivar of rye, Rye 1443, which tolerated 35 ppm Al at 25°C temperature, became sensitive to 20 ppm at 30°C temperature can be explained also on the basis of a higher rate of absorption and metabolic processes induced by a higher temperature of the nutrient solutions.

Reaction of Plant Species Tested

Sources of genetic variability are extremely important for a successful breeding program. This variability can be within or between species or involving different genera as with triticale.

Soil problems such as soil acidity offer triticale breeders a challenge that can only be accepted if the observed genetic variability is identified and can be utilized. Results from this study suggest that good sources of tolerance to Al toxicity for triticale

can be derived from certain hexaploid wheats and selected diploid ryes.

The progenitors of wheat which are thought to be Triticum monococcum, Aegilops speltoides, and Ae. squarrosa and represent the genomes AA, BB and DD, respectively, in hexaploid wheats. The tetraploid wheats with the genomes AABB were found to be as sensitive as Brevor in their reaction to Al. Therefore, it is unlikely that the wheat genomes AABB offer any source of tolerance for triticale to Al. This then would suggest that durum wheats would not be a promising source for tolerance to Al. This is significant when developing triticales of the hexaploid level. The fact that no tolerance was found in the cultivars of Ae. squarrosa tested does not necessarily disagree with the idea that the DD genome has contributed to Al tolerance in hexaploid wheats because, theoretically, only one or very few genotypes of Ae. squarrosa might have contributed to the original synthesis of the genomes AABBDD and a very limited number of Ae. squarrosa cultivars were tested.

Nuttoson (1958), indicated that in addition to the ability of winter rye to tolerant cold winters, it is the only cereal crop adapted to poor and podzolized acid soils. This was confirmed in this study as evidenced by the high level of tolerance to Al found in some cultivars of rye.

It can be assumed, therefore, that the DD genome from some hexaploid wheats and the RR genome from rye are the most promising source of tolerance to Al for triticale.

The strains of triticale tested in this study for Al reaction exhibited a wide degree of variability. If the 1973 tetraploid wheats tested represent the genetic variability of the genomes AABB for reaction to Al, it can be assumed that the reaction shown by purely hexaploid triticales was due solely to the addition of the RR genome and that of the octoploid triticales was due to the DD genome in addition to the RR genome. If genes for tolerance in the DD and RR genome are expressed in an additive manner in triticale, this would indicate that the octoploid level would be more tolerant than the hexaploid level; however, this possibility was not tested.

The Reaction of F_1 Hybrids

In order to gain a better understanding of the nature and source of tolerance, sensitive and tolerant wheats were crossed to sensitive and tolerant ryes.

There were two levels of sensitivity among wheat cultivars classified as sensitive in this study which were crossed to sensitive rye. Inia 66 and Daws were sensitive to 1 ppm Al while Penjamo 62 and Hope 7D were tolerant to 1 ppm Al but sensitive to 2.5 ppm.

The sensitive cultivars of rye always showed variability to Al concentration lower than 5 ppm, but were uniformly sensitive to 5 ppm. This was due to the way in which these varieties were selected. A 50-seed sample of several rye cultivars was screened

at 5 ppm Al. Sensitive seedlings were transplated and isolated by cultivars to allow cross pollination within cultivars and seed was obtained for further experiments. Sensitive cultivars of rye thus obtained showed about the same proportion of plants sensitive to 1 ppm Al as 4 ppm. Since rye is a cross pollinated crop it is possible to assume that wide heterogenity was available in the genotypes sensitive to 5 ppm. However, it was not possible to make inferences about the nature and number of genes controlling the reaction to Al in rye as no genetic data are available for this trait.

From the reaction of F_1 hybrids from crosses between sensitive wheat and rye, it can be assumed that both the wheat and rye genotypes for reaction to Al were expressed in the F_1 hybrid when tested for its reaction to Al. This can only be observed if the Al concentrations that identify those genotypes are used. One ppm of Al inhibited root regrowth of Inia 66 while two classes for reaction type were noted for the sensitive ryes. Therefore, the two classes of reaction exhibited by the Inia 66 x Rye 1133 F_1 hybrid were due solely to the variability present in the rye cultivar. When these F_1 seedlings were tested at 4 ppm they were all sensitive; however, this could not be a reliable reaction because these few seedlings developed very slowly and looked abnormal.

Penjamo 62 and Hope 7D were sensitive to 2.5 ppm Al but tolerant to 1 ppm. Sensitive ryes, Rye 1003 and Rye 1133, showed variable reaction at 1 ppm with both sensitive and tolerant types

being observed. The F_1 hybrids from these materials were all tolerant to 1 ppm which indicated that the wheat cultivars Penjamo 62 and Hope 7D were homozygous tolerant to 1 ppm and did not allow the rye variability for sensitivity to be expressed. Root regrowth of F_1's was larger than that of wheat but shorter than that of tolerant seedlings of rye. Because of the limited amount of seed available no meaningful differences could be obtained.

When the Al concentration was increased to 4 ppm, Penjamo 62 and Hope 7D were sensitive, and the two types of reaction of the sensitive rye were also observed in the F_1 hybrids but root regrowth of the tolerant class was the same as rye. This suggests that genes of both wheat and rye operate in the F_1. However, no gene interaction occurs that resulted in an F_1 being more tolerant than either parent.

The variability exhibited by the sensitive cultivars of rye was not observed in F_1 hybrids when a tolerant wheat was used instead of a sensitive type, particularly when Al concentrations were completely toxic to the sensitive ryes. The root regrowth of these F_1 hybrids at Al concentrations that inhibited root regrowth of the rye parents indicated that Carazinho and Atlas 66 were pure lines for reaction to Al. Again no gene interaction between wheat and rye genotypes occurred so that the F_1 did not exhibit more tolerance than the tolerant wheat parent. On the contrary, it seemed that the wheat genotype in a single dose is not as tolerant as the wheat cultivar per se.

A tolerant rye appeared to be an excellent source of tolerance to Al for triticale as the F_1 from a cross sensitive wheat x tolerant rye was almost as tolerant as the rye itself. The F_1 of the cross Hope 7D x Rye 1443 tolerated 10 ppm Al at 30°C temperature. This is more than two times the Al stress required to inhibit root regrowth of Atlas 66 and Carazinho cultivars. It appears that the mechanism of tolerance in rye is so effective that it can be fully expressed even when the rye genotype is in single dose in the F_1.

The cross between tolerant wheat x tolerant rye resulted in F_1 being even more tolerant than the previous crosses. Because of the level of Al stress (5, 15 ppm at 30°C) placed on this F_1 and rye parent, the rye cultivar showed two classes of reaction, sensitive and tolerant. This variability was also observed in the F_1, suggesting that the tolerance of the wheat parent had been exceeded and only the most tolerant types were expressed in the rye and F_1.

Again, root regrowth of F_1 did not exceed that of the rye parent, suggesting that the wheat and rye genes do not interact to provide a F_1 that is more tolerant than the more tolerant parent. Nevertheless it appears that gene or genes controlling reaction to Al in rye have the ability to fully express in single dose in the F_1 hybrids. This was not the case for the tolerant wheats Atlas and Carazinho when crossed to sensitive ryes.

The Colchicine Technique

Amphidiploids are derived naturally or artificially from more than one genetically differentiated species. In an amphidiploid the haploid chromosome set of one or both parental species is represented at least twice. Since amphidiploids arise from hybridization between genetically differentiated species, the initial F_1 hybrid from which they arise is invariably highly sterile. This is due to the fact that the parents are not too distantly related so as to prevent fertilization, yet are sufficiently different regarding chromosome homology to prevent pairing of chromosomes in their F_1 hybrids.

In the amphidiploid formed by doubling chromosomes of the sterile F_1 generally normal pairing and fertility is restored. Doubling of the F_1 chromosomes may occur in several ways. The most efficient agent to induce polyploidy to date has been the alkaloid colchicine. Kaltsikes (1975) mentions 10 different techniques to administer colchicine to wheat-rye F_1 hybrids. These are modifications of any of the following three basic techniques, 1) application of colchicine solution directly to seeds; 2) application to young coleoptiles, and 3) application of colchicine solution to the crown.

Since colchicine affects individual nuclei (Eigsti 1938) it is possible to assume that application of colchicine at the coleoptile stage would be the most efficient method to induce polyploidy. However, application of colchicine to the crown when plants have

developed several tillers has a great advantage over the coleoptile technique in that vegetative propagation of hybrids is possible when only a few plants are available. This technique was used in this study and about 25 percent of fertile plants were obtained from wheat-rye F_1 hybrids.

Reaction of Amphidiploids

It is possible to induce mutations other than chromosome doubling by colchicine in the wheat-rye hybrids. However, had the colchicine induced a mutation for the reaction to Al resulting in a noticeable change in the reaction of the amphidiploid, it would have had to affect genetic factors of both wheat and rye to induce a complete sensitive type. It is, however, unlikely that colchicine might have caused the same biochemical or structural changes in chromosomes of wheat and rye. Had the colchicine caused a mutation that resulted in a more tolerant amphidiploid it would have to have been on a rye chromosome since it was in a single dose. However, from the reaction shown by amphidiploids this change did not occur. Therefore, it is reasonable to believe that if any changes in the reaction to Al were shown by amphidiploids with respect to the wheat and rye parents, it must have been due to changes in the ploidy level only.

It was observed that the F_1 from the cross Inia 66 x Rye 1133 showed two classes of reaction to 1 ppm Al. This can be explained by the fact that Inia 66 was sensitive to 1 ppm while Rye 1133 exhibited some variability. This variability in rye made it possible to

observe both tolerant and sensitive classes of F_1 hybrids. Only the sensitive class at 1 ppm produced amphidiploid seeds as a result of the colchicine treatment. Seedlings from these seeds were again sensitive to 1 ppm Al indicating that the genetic material of the F_1 was doubled but no gene for tolerance to 1 ppm was present to be inherited from the rye. Consequently the Inia x Rye 1133 amphidiploid was also sensitive to 4 mmp Al.

Results obtained from the Hope 7D x Rye 1133 amphidiploid indicate that the genetic material in the F_1 hybrid was doubled by the colchicine treatment but did not affect the reaction of the amphidiploid to 1 ppm Al. The variable reaction shown by the Rye 1133 at 1 ppm did not appear in the F_1 indicating the Hope 7D was homozygous tolerant to 1 ppm and provided the F_1 with the same tolerance. Again when the level of tolerance of Hope 7D was exceeded by the Al concentration, the variability shown by rye was expressed in the F_1 and retained in the amphidiploids.

It was interesting to note that the F_1 hybrid from the cross Penjamo 62 x Rye 1003 was all tolerant to 1 ppm Al while the amphidiploid derived from it showed two classes of reaction to the same Al concentration. Apparently sensitive amphidiploids were derived from tolerant F_1's upon colchicine treatment. When root regrowth of the amphidiploid was compared to that of the wheat and rye it was clear that the rye itself was somewhat more tolerant than the amphidiploid derived from it. This indicated that although root regrowth of the F_1 was similar to that of the rye parent, chromosome doubling

did not cause any favorable changes in the reaction of the amphidiploid. This was more clearly observed when the amphidiploid was tested at 4 ppm Al and the root regrowth compared to that of the rye parent. However, when the F_1 is compared to the parental wheat, it is clear that the F_1 is somewhat more tolerant to 1 ppm than the wheat itself. Apparently this effect was retained in the amphidiploid.

It has been observed by some investigators that very little or almost no affinity exists in meiotic prophase in the sterile hybrids between wheat and rye chromosomes. Full pairing occurs in the colchicine-induced amphidiploids derived from these sterile hybrids but fertility is low. Muntzing (1939) and O'Mara (1953) indicate that considerable failure to pair of fully homologous chromosomes occur at meiosis originating, according to Riley and Chapman (1957). high frequencies of aneuploids. Merker (1971) found that aneuploidy was correlated with meiotic irregularities in triticale. Detailed karyotype analysis of aneuploid plants of triticale by Shigenaga et al. (1971), and Larter and Shigenaga (1971) indicate that chromosomes of both wheat and rye contribute to aneuploidy in triticale. The fact that the F_1 between Penjamo 62 x Rye 1003 was tolerant to 1 ppm Al and the amphiploid derived from it showed sensitive and tolerant plants to 1 ppm Al suggest aneuploidy in the amphidiploid caused chromosomes of wheat to be lost. This chromosomes undoubtedly carried tolerance to 1 ppm Al inherted from wheat. No chromosome count was made to support this hypothesis.

It appears that the F_1 hybrids of Atlas 66 x Rye 37 and Carazinho x Rye 1133 were somewhat less tolerant than parental wheats indicating the tolerance in Atlas 66 and Carazinho was expressed in these F_1 hybrids but the rye complement (sensitive ryes) did not contribute to this tolerance. Root regrowth of the amphidiploid was very similar to that of the wheat parents, suggesting that neither gene interaction nor dosage effect by chromosome doubling of the F_1 resulted in an amphidiploid being more tolerant than the tolerant parental wheat.

It was interesting to observe that the high level of tolerance to Al shown by the F_1 hybrid of Hope 7D x Rye 1443 was not recovered in the amphidiploid derived from this cross. It is not possible that aneuploidy might have been responsible for this reduction of tolerance in the amphidiploid. Had aneuploidy been responsible, the loss of the wheat chromosomes would not have any effect on the reaction of the amphidiploid because rye was the tolerant parent. Hope 7D was sensitive to 2.5 ppm while Rye 1443 was sensitive to 20 ppm at 30°C. If aneuploidy involved one rye chromosome, the level of tolerance would have been the same as that of the F_1. If aneuploidy involved both chromosomes of rye, reaction of the amphidiploid would be similar to that of Hope 7D. It can be assumed, therefore, that the genetic background of wheat in which the rye chromosomes were operating affected the rye factors controlling the reaction to Al.

Because of the limited amount of amphidiploid seed derived from the tolerant wheat x tolerant rye F_1 hybrid it was not possible

to determine whether the high level of tolerance shown by the F_1 was recovered in the amphidiploid. However, from the root regrowth of the amphidiploid at 10 and 20 ppm Al it was clear that it was markedly more tolerant than its parental wheat Atlas 66 but less tolerant than the parental rye.

This, again, indicates that if aneuploid plants were recovered, they did not involve the rye chromosomes because they would have been sensitive to 20 ppm. In this case, if chromosomes of wheat were involved in aneuploidy, it could have not been detected. From this assumption it can be again noted that the level of tolerance of the rye parent was not fully expressed in the amphidiploid. This was not the case when tolerant wheats were used as a source of tolerance. (Tables 11 and 12) It can be assumed that factors controlling tolerance in wheat were fully expressed in the amphidiploid and were not affected by introducing the genome of a sensitive rye.

It can be concluded that chromosome doubling of sterile F_1 hybrid did not result in a dosage effect for the reaction to Al in new amphidiploids. Even though the high level of tolerance to Al in rye was not fully expressed in amphidiploids, the amphidiploids which were more tolerant to Al resulted from a cross between a tolerant wheat and a tolerant rye.

Effect of Al on Mitosis of Wheat, Rye and Triticale

Clarkson (1965) suggested that the morphological abnormalities of root systems treated with Al salts are such that they may be explained by an inhibitory effect of Al on cell division. The exact mechanism by which Al stops cell division is still unclear. However, Clarkson and Sanderson (1969) and Sampson et al. (1965), speculated that Al directly affects the synthesis of DNA.

Wright (1943), Williham (1948), Rasmussen (1968) and Clarkson (1966), among others have suggested a Phosphorus-Aluminum interaction that upsets the metabolism of Phosphorus in the plant. Consequently, affecting DNA synthesis and oxydative phosphorilation that provides energy for cell division (Kihlman 1966). Cytological investigation conducted in this study indicated that aluminum affected cell division of all three plant species, wheat, rye and triticale. Regardless of the precise mechanism by which Al affected cell division, it appeared that this effect occurred within the first three hours of Al treatment in both sensitive and tolerant plants. The relative parallelism observed in mitotic activity between the controls and tolerant cultivars exposed to Al indicated that during these experiments, factors other than Al had the same effect on both controls and Al treatments. Consequently, the marked difference in mitotic activity between controls and Al treatment was due to the effects of Al.

From the results, it seems that when the roots of both sensitive and tolerant plants were exposed to Al, cell division was readily

disturbed because apparently root cells were lacking some mechanism to avoid Al injury. Cell division in sensitive plants was markedly affected upon exposure to Al and progressively decreased. Possibly the absorption process might have accumulated sufficiently high amounts of Al in the meristematic root cells that led to their destryction and prevented further cell division. Consequently, the root tip damage on sensitive varieties was irreversible.

Cell division in the tolerant plants also progressively decreased as Al was being taken up. However, it was not completely stopped and after 12 to 36 hours of exposure to Al the number of mitotic figures increased again. This indicated that damage to tolerant plants by Al to increased concentrations was reversible and allowed meristematic cells to continue to divide even though Al was present. Subsequently, root elongation took place in the same Al solution. Root elongation of tolerant plants in Al solution was not observed if the Al concentration was too high. This may be due to the fact that Al entered root cells faster than the time needed for the tolerant reaction to provide protection in the plant.

Since the first root tip samples were taken after 3 hours of Al treatment it was not possible to tell how soon Al affected cell division. If cell division was affected in sensitive and tolerant plants at the same time, this would indicate that indeed no morphological structures are involved in tolerance to Al and that

the same amount of Al enters root cells of both sensitive and tolerant plants until the plant reaction mechanism of tolerant plants occurs and inhibits or reduces the uptake of Al. Clarkson (1965) suggested that the time required for inhibition of mitotic activity was determined by the rate of Al uptake. Varietal differences in the reaction of wheat to Al were attributed by Henning (1975) to differences in the ability to exclude Al from the cytoplasm of the meristematic root cells. This ability requires the presence of Al in order to be expressed. Once expressed in tolerant plants, it affects the rate of Al uptake so that no inhibition of cell dividion occurs. It was observed that Al did not affect any particular stage of Cell division but gradually disappeared as exposure to Al increased. This accounted for the decreasing number of mitotic figures observed. These observations are consistent with Clarkson (1965) and Henning (1975). It appears then that Al affects the mitotic cycle at the interphase stage.

When tolerant plants exposed to Al have expressed the ability to exclude Al from the root cells, mitotic activity continues and gradually progresses until it fully recovers and consequently, it reaches a rate similar to that of the control. No burst of mitotic activity was observed upon removal of Al as indicated by Henning (1975).

Binucleated cells induced by Al have been reported on cotton by Rios and Pearson (1964) and Huck (1972), by Fleming and Foy (1968) in wheat. Henning, however, indicated that what appeared to be multinucleated cells in Al-treated wheat roots were masses of

cytoplasm densely stained with hematoxylin. It is worth noting that this dye is not specific for staining nuclear material as is acetocarmine. In this study acetocarmine was used to stain mitotic figures. Therefore no cytoplasmic material was stained that could be regarded as nuclear material. The binucleated cells were found in sensitive Al-treated plants, but later they were observed in tolerant and control treatments. It would therefore be difficult to attribute pressure of binucleate cells to Al treatment in this study. Although the frequency of binucleated cells was not very high they were easily identified.

With the findings of McLeod and Jackson (1967), Reid et al. (1969, 1971), and Kerridge et al (1971), that the reaction of barley and wheat to Al in nutrient solution was well correlated with their reaction in acid soil, and the fact that this reaction is simply inherited (Kerridge and Kronstad, 1968 and Reid, 1971), it appears, then, that the genetic improvement of crop cultivars with a higher degree of tolerance to soil acidity offers a promising approach to the problem of acid soils.

The results of this study indicate that a methodology previously designed to screen wheat for reaction to Al in growth chamber can be adapted to the greenhouse. This is important in areas where growth chambers are not available, and suggests that plant breeders concerned with breeding for tolerance to soil acidity can follow a screening technique like the one used in this study to evaluate their breeding materials.

Results also indicate that tolerant cultivars of rye tolerated approximately 5 times more Al in solution than tolerant wheats. On the other hand, amphidiploids involving a tolerant rye exhibited a level of tolerance twice that of tolerant wheats. This suggests that tolerant rye cultivars offer a source of tolerance to Al for triticale superior to that found in tolerant wheats.

VI. SUMMARY AND CONCLUSIONS

A technique previously designed to screen cultivars and segregating populations for tolerance to Al utilizing nutrient solutions in growth chambers, was found to be efficient in differentiating among cultivars of wheat, rye, and triticale for tolerance to aluminum under greenhouse conditions. However, it appeared that environmental factors like air temperature and light intensity, which are more difficult to control in the greenhouse, increased the toxicity of Al by increasing the plant's rate of absorption.

Crosses between a sensitive wheat and a sensitive rye yielded amphidiploids with a level of tolerance similar to that of the least sensitive parent. When tolerance was derived from wheat the amphidiploid was equal to its parental wheat with respect to tolerance to Al. The amphidiploid derived from sensitive wheat x tolerant rye was less tolerant than the rye parent but as tolerant as tolerant wheats like Atlas 66. It was assumed that unless aneuploidy was involved, the genetic background on which the rye genotype operated modified the full expression of Al tolerance on the rye parent in the resulting amphidiploid. Crosses where a tolerant wheat and tolerant rye were involved yielded amphidiploids with a level of tolerance twice that of the tolerant wheat parent, but not as tolerant as the rye parent.

Based on the results obtained, the following conclusions were made:

Adequate tolerance levels to Al in triticale can only be derived from some hexaploid wheats and diploid ryes.

Genes controlling the reaction to Al in both wheat and rye were expressed in the F_1 hybrids but did not interact to provide a level of tolerance higher than that in the more tolerant parent.

Tolerance of rye was fully expressed in F_1 wheat-rye hybrids, but this was not the case in F_1 hybrids where tolerance had been introduced only from a tolerant wheat parent.

Once the level of tolerance provided by wheat had been overcome by the Al concentration, the genetic variability of the rye was expressed in the F_1 hybrids when the rye was more tolerant than the wheat.

The high level of tolerance introduced in F_1 hybrids by tolerant rye was reduced in the amphidiploid upon colchicine treatment. This was not the case, however, for F_1's where the tolerance was derived from tolerant wheats.

Failure of the rye tolerance to be fully expressed in the amphidiploids was due to the wheat genetic background that modified the expression.

Doubling of chromosomes of the F_1 hybrids had no effect on the reaction to Al of amphidiploids with respect to their parents and F_1 hybrids.

Inhibition of cell division, being more striking in sensitive plants, occurred within the first three hours of Al treatment.

Although cell division of tolerant plants decreased as time of exposure to Al increased, a point was reached at which cell division no longer decreased, but started to increase progressively.

Aluminum treatment induced a plant reaction in tolerant cultivars that allowed plant roots to tolerate the Al and continue to grow even in the Al solution.

Aluminum treatment did not differentially affect any specific stage of cell division.

Binucleated cells were observed in both control and Al-treated roots, therefore the induction of binucleated cells cannot be attributed to Al treatment exclusively.

BIBLIOGRAPHY

Adams, F., and Z. F. Lund. 1966. The effect of chemical activity of soil solution aluminum on cotton root penetration of acid soil. Soil Sci:101:193-198.

Adams, F. and R. W. Pearson. 1967. Crop responses to lime in the southern United States and Puerto Rico. In: R. W. Pearson and F. Adams (ed.) Soil acidity and liming, agronomy monograph 12. Amer. Soc. Agronomy, Madison, Wisconsin, pp. 192-196.

Ali, S. M. E. 1973. Influence of cations on aluminum toxicity in wheat. (Triticum aestivum Vill., Host). Ph.D. thesis, Oregon State University, Corvallis, Oregon.

Armiger, W. H., C. D. Foy, A. L. Fleming, and B. E. Caldwell. 1968. Differential tolerance of soybean varieties to an acid soil high in exchangeable aluminum. Agr. Jour. 60:67-70.

Asen, S., N. W. Stuart, and E. L. Cox. 1963. Sepal color of Hydranagea macrophylla as influenced by the source of nitrogen available to plants. Proc. Amer. Soc. Hort. Sci. 82:504-507.

Aslander, A. 1952. Standard fertilization and liming as factors in maintaining soil productivity. Soil Sci. 74:181-194.

Bhumbla, D. R. and E. D. McLean. 1965. Aluminum in soils: VI. Changes in pH-dependent acidity, cation exchange capacity and extractable aluminum with additions of lime to acid surface soils. Soil Sci. Soc. Amer. Proc. 29:370-374.

Blackeslee, A. F. and A. G. Avery. 1937. Methods of inducing doubling chromosomes in plants. J. Hered. 28:393.

Carolus, R. L. and B. E. Brown. 1935. Truck Crop Investigations. Virginia Truck Exp. Sta. Bull. 89.

CIMMYT. 1973-1974. Results of the 5th International Triticale Yield Nursery (ITYN). Inf. Bull. 24.

Clarkson, D. T. 1965. The effect of aluminum and some other frivalent metal cations on cell division in root apices of Allium cepa. Ann. Bot. N.S. 29:309-315.

Clarkson, D. T. 1966. Effect of aluminum on the uptake and metabolism of phosphorus by barley seedlings. Plant. Physiol. 41:165-172.

Clarkson, D. T. 1966. Interaction between aluminum and phosphorus on root surface and cell wall materials. Plant Soil 27:347-355.

Clarkson, D. T. 1968. Metabolic aspects of aluminum toxicity and some possible mechanisms for resistance. Brit. Ecol. Soc. Symp. 9:381-397.

Clarkson, D. T., and John Sanderson. 1969. The uptake of a polyvalent cation and its distribution in the root apices of Allium cepa. Tracer and Autoradiographic Studies. Planta 89:136-154.

Duggar, J. F. and H. J. Funches. 1911. Lime for Alabama soils. Alabama Agr. Exp. Sta. Bull. 161.

Eigsti, O. J. 1938. A cytological study of colchicine effects in the induction of polyploidy in plants. Proc. Nat. Acad. Sci. U.S. 24:56.

Fleming, A. L. and C. D. Foy. 1968. Root structure reflects differential aluminum tolerance in wheat varieties. Agr. Jour. 60:172-176.

Foy, D. C. 1974. Effects of Aluminum on plant growth. In: The Plant Root and Its Environment. E. W. Carson (ed.) Univ. Press of Va. Charlottsville.

Foy, C. D., W. H. Armiger, L. W. Briggle, and D. A. Reid. 1965. Differential aluminum tolerance of wheat and barley varieties in acid soils. Agr. Jour. 57:413-417.

Foy, C. D., J. C. Brown. 1964. Toxic factors in acid soils. II. Differential aluminum tolerance of plant species. Soil Sci. Soc. Amer. Proc. 28:27-32.

Foy, C. D., A. L. Fleming, and W. H. Armiger. 1969. Aluminum tolerance of soybean varieties in relation to calcium nutrition. Agr. Jour. 61:505-511.

Haas, A. R. 1936. Phosphorus nutrition of citrus and beneficial effects of aluminum. Soil Sci. 42:187-201.

Hartwell, B. L. and F. R. Pember. 1918. The presence of aluminum as a reason for the difference in the effect of so-called acid soil on barley and rye. Soil Sci. 6:259-277.

Harward, M. E. and N. T. Coleman. 1954. Some properties of H- and Al-clays and exchange resines. Soil Sci. 78:181-188.

Harward, M. E., W. A. Jackson, W. L. Lott, and D. D. Mason. 1955. Effects of aluminum, iron and manganese upon the growth and composition of lettuce. Proc. Amer. Soc. Hort. Sci. 66:261-266.

Henning, S. J. 1975. Aluminum toxicity in primary meristem of wheat roots. Ph.D. thesis, Oregon State University, Corvallis, Oregon.

Hewitt, E. J. 1947. Resolution of factors in soil acidity: relative effects of aluminum and manganese toxicities on farm and market garden crops. Ann. Rept. Agr. and Hort. Res. Sta., Long Ashton, Bristol. 82:96.

Huck, M. G. 1972. Impairment of sucrose utilization for cell wall formation in roots of aluminum-damaged cotton seedlings plant. Cell. Physiol. 13:7-14.

Ikeda, T., S. Higashi, S. Kagokashi and T. Moriya. 1965. Studies on the adaptability of wheat and barley on acid soils, especially in regard to its varietal differences and laboratory detection. Tokai-Kinki Natl. Agr. Exp. Sta. Bull. 12:67-69 (Japanese with English summary).

Jacobson, L., R. Overstreet, R. M. Carlson, and J. A. Chastian. 1957. The effect of pH and temperature on the absorption of potassium and bromide by barley roots. Plant. Physiol. 32: 658-662.

Jenny, H., T. R. Nielsen, N. T. Coleman and D. E. Williams. 1950. Concerning the measurement of pH, ion activities, and membrane potentials in colloidal systems. Science 23:202-205.

Johnson, R. E., and W. A. Jackson. 1964. Calcium uptake and transport by wheat seedlings as affected by alluminum. Soil. Sci. Soc. Amer. Proc. 28:381-386.

Kaltsikes, P. J. 1974. Methods for triticale production. Z. pflanzenzuchtung. 71:264-286.

Kerridge, D. C. 1969. Aluminum toxicity in wheat (Triticum aestivum Vill; Host) Ph.D. thesis, Oregon State University, Corvallis, Oregon.

Kerridge, D. C. and W. E. Kronstad. 1968. Evidence of genetic resistance to aluminum toxicity in wheat (Triticum aestivum Vill; Host). Agr. Jour. 60:710-711.

Kerridge, D. C., M. D. Dawson, and D. P. Moore. 1971. Separation of degrees of aluminum tolerance in wheat. Agr. Jour. 63: 586-591.

Kiss, A. 1974. Triticale breeding experiments in Eastern Europe. Proc. Cimmyt. - U of M. Int. Triticale Symp. (El Batan, Mexico) pp:41-50.

Lance, J. C. and R. W. Pearson. 1969. Effect of low concentration of aluminum on growth and water and nutrient uptake by cotton roots. Soil Sci. Soc. Amer. Proc. 33:95-98.

Larter, E. N. and S. Shigenaga. 1971. Further evidence on the derivation of univalents in hexaploid triticale. Can. J. Genet. Cytol. 13:895-898.

Lee, C. R. 1971. Influence of aluminum on plant growth and mineral nutrition of potatoes. Agr. Jour. 63:604-607.

Lee, C. R. 1972. Interrelationship of aluminum and manganese on the potato plant. Agr. Jour. 64:546-549.

Lin, C. and T. Coleman. 1960. The measurement of exchangeable aluminum in soil and clays. Soil Sci. Soc. Amer. Proc. 24: 444-446.

Lipman, C. B. 1938. Importance of silicon, aluminum, and chlorine for higher plants. Soil Sci. 45:460-464.

Low, P. F. 1955. The role of aluminum in the tritration of bentonite. Soil Sci. Soc. Amer. Proc. 19:135-139.

Magistad, O. C. 1925. The aluminum content of the soil solution and its relation to soil reaction and plant growth. Soil Sci. 20:181-226.

McLean, F. T. and B. E. Gilbert. 1927. The relative aluminum tolerance of crop plants. Soil Sci. 24:163-175.

McLeod, L. B. and L. P. Jackson. 1967. Aluminum tolerance of two barley varieties in nutrient solution, peat and soil culture. Agr. Jour. 59:363.

Martinez-Racines, C. P. 1976. Aluminum toxicity studies in rice (Oryza sativa L.) Ph.D. thesis. Oregon State University, Corvallis, Oregon.

Medappa, K. C. and M. N. Dana. 1968. Influence of pH, calcium, iron and aluminum on the uptake of radiophosphorus by cranberry plants. Soil Sci. Soc. Proc. 32:381-383.

Merker, A. 1971. Cytogenetic investigations in hexaploid triticale. Hereditas 68:281-290.

Mesdag, J. and L. A. J. Slootmaker. 1969. Classifying wheat varieties for tolerance to high soil acidity. Euphytica. 18:36-42.

Millikan, C. R. 1949. Effects on flax of a toxic concentration of boron, iron, on nickel in the nutrient solution. Proc. Roy. Soc. Victoria (N.S. Wales) LXI:25-47.

Miyake, K. 1916. The toxic action of soluble aluminum salts upon the growth of the rice plant. J. Biol. Chem. 25:23-28.

Moore, D. P. 1974. Physiological effects of pH on roots. In: The Plant Root and its Environment. E. W. Carson (ed.). University Press of Va. Charlottsville.

Moschler, W. W., G. D. Jones, and G. W. Thomas. 1960. Lime and soil acidity effects on alfalfa growth in a red-yellow podzolic soil. Soil Sci. Soc. Amer. Proc. 24:507-509.

Muntzing, A. 1939. Studies on the properties and the ways of production of rye-wheat amphidiploids. Hereditas 25:387.

Neenam, M. 1960. The effect of soil acidity on the growth of cereals with particular reference to the differential reaction of varieties thereto. Plant. Soil. 12:324-337.

Nuttonson, M. Y. 1958. Rye-climate relationships and the use of phenology in ascertaining the thermal and photothermal requirements of rye. American Institute of Crop Ecology. Washington, D.C.

O'Mara, J. G. 1953. The cytogenetics of triticale. Bot. Rev. 19: 587-601.

Osorio, E. A., J. C. S. Moreira, and E. P. Gomes. 1973. Evaluation of the potential of triticale in South Brazil. Agronomia 8:217-221.

Pearson, R. W. and F. Adams. 1967. Soil Acidity and Liming. Agronomy monograph 12. Amer. Soc. Agronomy, Madison, Wisconsin.

Pratt, P. F. 1966. Aluminum. In: Diagnostic criteria for plants and soils, (ed.) by H. D. Chapman, Riverside, California. University of California. 793 pp.

Randall, P. J. and P. B. Vose. 1963. Effect of aluminum on uptake and translocation of phosphorus by perennial rye grass. Plant Physiol. 38:403-409.

Rasmussen, H. P. 1968. Entry and distribution of aluminum in Zea mays. Electron microprobe x-ray analysis. Planta. 81:28-37.

Reid, D. A. 1971. Genetic control of reaction to aluminum in winter barley. Barley genetics II. Proc. 2nd Int. Barley Genetics Symp. WSU Press. Pullman, Wash. 409-413.

Reid, D. A., G. D. Jones, W. H. Armiger, C. D. Foy, E. J. Koch, and T. M. Starling. 1969. Differential aluminum tolerance of winter barley varieties and selections in associated greenhouse and field experiments. Agr. Journal 61:218-222.

Reid, D. A., A. L. Fleming, and C. D. Foy. 1971. A method for determining aluminum response of barley in nutrient solution in comparison to response in Al-toxic soil. Agr. Journal 63:600-603.

Rhue, R. D. 1976. The time-concentration interaction of aluminum toxicity in wheat root meristems. Ph.D. thesis. Oregon State University, Corvallis, Oregon.

Riley, R. and V. J. Chapman. 1957. The comparison of wheat-rye and wheat-acgilops amphidiploids. Jr. Agr. Sci. 49:246-250.

Rios, M. A. and R. W. Pearson. 1964. The effect of some chemical environmental factors on cotton root behavior. Soil Sci. Sco. Amer. Proc. 28:232-235.

Rorison, I. H. 1958. The effect of aluminum on legume nutrition. Proc. Univ. Nottingham. (ed.) by Hallsworth.

Sampson, M., Clarkson, and D. D. Davis. 1965. DNA synthesis in aluminum-treated roots of barley. Science 148:1476-1477.

Sampson, M. and D. D. Davis. 1966. Metabolically labile DNA in mitotic and non-mitotic cells of Zea mays. Life Sci. 5:1239-1247.

Sampson, M., Katoh Y. Hotta, and H. Stern. 1963. Metabolically labile DNA. Proc. Nat. Acad. Sci. 50:459-463.

Schmehl, W. R., M. Peech, and R. Bradfield. 1950. Causes of poor growth of plants on acid soils and beneficial effects of liming. I. Evolution of factors responsible for acid-soil injury. Soil Sci. 70:393-410.

Shigenaga, S., E. N. Larter, and R. C. McGinnis. 1971. Identification of chromosomes contributing to aneuploidy in hexaploid triticale, cultivar Rosner. Can. J. Genet. Cytol. 13:592-596.

Sivasubramaniam, S. and O. Talibudeen. 1971. Effect of aluminum on growth of Tea. (Camellia sinensis) and its uptake of potassium and phosphorus. I. Sci. Fd. Agr. 22:324-329.

Slootmaker, L. A. J. 1974. Tolerance to high soil acidity in wheat-related species, rye and triticale. Euphytica. 23:505-513.

Srivastava, J. P. 1973. Triticale in Asia. Proc. CIMMYT-U of M. Int. Triticale Sym. (El Batan, Mexico). pp. 87-92.

Vlamis, J. 1953. Acid soil infertility as related to soil-solution and soil-phase affects. Soil Sci. 75:383-394.

Wallihan, E. F. 1958. The influence of aluminum on the phosphorus nutrition of plants. Amer. Jour. Bot. 35:106-112.

Way, J. T. 1950. On the power of soils to absorb manure. Jour. Royal Agr. Soc. 11:313-379.

Wright, K. E. 1937. Effects of phosphorus and lime in reducing aluminum toxicity of acid soils. Plant Physiol. 12:173-181.

Wright, K. E. 1943. Internal precipitation of phosphorus in relation to aluminum toxicity. Plant Physiol. 18:708-712.

Yuan, T. L. and Fiskell. 1959. Aluminum studies: II. The extraction of aluminum from some Florida soils. Soil Sci. Soc. Amer. Proc. 23:202-205.

APPENDICES

APPENDIX TABLE 1. Lists of wheat related diploid species tested for their reaction to Al toxicity.

Species		Origin
T. monococcum	G75-1541	PI-167556
T. monococcum	G75-1558	Ein-4
T. monococcum	G75-1560	Ein-5
T. monococcum	G75-1564	Ein-7
T. monococcum	G75-1663	Ein-6
Ae. speltoides	G75-1653	PI-172685
Ae. speltoides	G75-1656	USA
Ae. speltoides	G75-1656	USA
Ae. speltoides	G75-1658	USA
Ae. speltoides	G75-1659	USA
Ae. squarrosa	G75-1684	Wis 2095 Japan
Ae. squarrosa	G75-1712	Wis 2038 Japan
Ae. squarrosa	G75-1704	Wis 2038 Japan
Ae. Squarrosa	G75-1685	Wis 2095 Japan
Ae. squarrosa	G75-1686	Wis 2112 Japan

APPENDIX TABLE 2. List of Tetraploid wheats tested for their reaction to Al toxicity.

Cultivar		Origin
T. dicoccum		
T. dicoccum	GH 75-1196	USA
T. dicoccoides		USA
T. dicoccoides	GH 75-1201	USA
T. dicoccoides	GH 75-1202	USA
T. dicoccoides	GH 75-2953	USA
T. carthlicum	GH 74-2962	USA
T. polonicum	GH 74-3199	USA
T. persicum	GH 75-2930	USA
T. persicum	GH 75-1185	USA
T. persicum	GH 75-2972	USA
T. persicum	GH 75-2912	USA
T. persicum	GH 75-1172	USA
PI-094718	GH 75-1143	USA
PI-134914		USSR
PI-134930		Portugal
PI-136575		Portugal
PI-174699		France
PI-168911		Mexico
PI-168912		Mexico
PI-168913		Mexico
PI-168914		Mexico
PI-168916		Mexico
PI-168917		Mexico
PI-168920		Mexico
PI-168921		Mexico
PI-168924		Mexico
PI-168925		Mexico
PI-168926		Mexico
PI-168927		Mexico
PI-168928		Mexico
PI-168928		Mexico
PI-168929		Mexico
PI-176296		India
PI-181258		Afghanistan
PI-182101		India
PI-182114		India
PI-182664		Syria
PI-182665		Lebanon
PI-182666		Lebanon
PI-182668		Lebanon
PI-184172		Yugoslavia
PI-184543		Portugal
PI-184642		Portugal

APPENDIX TABLE 2. Continued

PI-185300	Argentina
PI-185725	Portugal
PI-185742	Portugal
PI-185751	Portugal
PI-185754	Portugal
PI-189772	Tunisia
PI-189733	Tunisia
PI-189774	Tunisia
PI-189777	Tunisia
PI-189778	Tunisia
PI-190977	Spain
PI-190980	Spain
PI-190990	Spain
PI-191029	Morocco
PI-191104	Spain
PI-191108	Italy
PI-191149	Spain
PI-191163	Spain
PI-191190	Spain
PI-191193	Spain
PI-191194	Spain
PI-191205	Spain
PI-191209	Italy
PI-191373	Italy
PI-191375	Italy
PI-191405	Morocco
PI-191411	Morocco
PI-191412	Morocco
PI-191438	Italy
PI-191472	Italy
PI-191476	Spain
PI-192130	Portugal
PI-192136	Portugal
PI-192141	Portugal
PI-192148	Portugal
PI-192143	Portugal
PI-192151	Portugal
PI-192161	Portugal
PI-192168	Portugal
PI-192180	Portugal
PI-192195	Portugal
PI-192200	Portugal
PI-208903	Iraq
PI-208907	Iraq
PI-208909	Iraq
PI-208912	Iraq
PI-209274	Australia
PI-209275	Australia
PI-209276	Australia

APPENDIX TABLE 2. Continued

PI-209277	Australia
PI-210378	Iran
PI-210380	Iran
PI-210381	Iran
PI-219382	Iran
PI-210386	Iran
PI-210855	Iran
PI-208910	Iraq
PI-210946	Cyprus
PI-210950	Cyprus
PI-210952	Cyprus
PI-210957	Cyprus
PI-211677	Turkey
PI-211688	Turkey
PI-211697	Turkey
PI-218121	Pakistan
PI-213674	Argentina
PI-221406	Yugoslavia
PI-221407	Yugoslavia
PI-221408	Yugoslavia
PI-221412	Yugoslavia
PI-223150	Jordan
PI-223152	Jordan
PI-223154	Jordan
PI-260058	USSR
PI-260061	USSR
PI-262584	USSR
PI-262656	USSR
PI-262672	USSR
PI-272476	Hungary
PI-272539	Hungary
PI-272542	Hungary
PI-272543	Hungary
PI-272547	Hungary
PI-295017	Bulgaria
PI-295019	Bulgaria
PI-295020	Bulgaria
PI-295024	Bulgaria
PI-297835	Ethiopia
PI-297836	Ethiopia
PI-297837	Ethiopia
PI-297843	Ethiopia
PI-297843	Ethiopia
PI-306639	France
PI-306641	France
PI-051211	Israel
PI-061351	Japan
CI-11477	USA
CI-11943	USA

APPENDIX TABLE 2. Continued

CI-11946	Canada
CI-12065	USA
CI-12066	USA
CI-12726	USA
CI-12899	Canada
CI-12922	Canada
CI-12924	Greece
PI-264985	Greece
PI-264989	Greece
PI-264991	Poland
PI-274668	Poland
PI-274672	Ethiopia
PI-297840	Poland
PI-274674	Germany
PI-277125	China
PI-283853	Ecquador
PI-286539	Ecquador
PI-286544	Ecquador
PI-286546	England
PI-289604	Israel
PI-292031	Algeria
PI-007785	Algeria
PI-007794	UAR
PI-007016	Bolivia
PI-036390	RSA
PI-042423	RSA
PI-042424	China
CI-05083	China
CI-05094	Uruguary
PI-043341	Peru
CI-08161	Canary Islands
CI-13918	Lybia
CI-6807	Tanzania
CI-6649	

APPENDIX TABLE 3. List of hexaploid wheats tested for their reaction to Al Toxicity. Origin and reaction to several concentrations of Al. T=Tolerant, S=Sensitive, V=Variable.

	Concentration of Al in ppm								
Cultivar	1	2	3	4	5	10	15	20	Origin
Atlas 66						T	V	S	USA
Aberdeen 6535	S								USA
Anza	V	S							USA
Bb #2	S								Mexico
Blue Boy	S								USA
Brevor	S								USA
Cajeme	V	V	S						Mexico
Carazinho						T	V	S	Brazil
Chinese Spring	T	S							USA
Colonias						T	T	S	Brazil
Daws	S								USA
Druchamp 8925	V	S							
Frondoso						T	T	S	Brazil
Gaines	S								USA
Hope 7D	V	S							USA
Hyslop	V	S							USA
Inia 66	S								Mexico
Nrn 10/Bvr 14/6	S								USA
Noroeste	T	T	V	S					Mexico
Nugaines	S								USA
Nuri	V	S							USA
Penjamo 62	T	V	S						Mexico
Pitic 62	T	V	V	S					Mexico
Preludio						T	V	S	Brazil
Pulman 101	S								USA
Ramona	S								USA
Red Bobs	S								USA
Red Egyptian	S								USA
Saric	S								Mexico
7 Cerros	-	-	V	S					Mexico
Tavcari	-	-	V	S					Mexico
Thatcher	S								Canada
Tobari	-	S							Mexico
Trintani						T	V	S	Brazil
T. per 3/Ac. squarrosa 1	S								USA
T. per 3/Ac. Squarrosa 1	S								USA
T. per 1/Ac. Squarrosa 2144	S								USA
T. per 4/Ac. Squarrosa 2144	S								USA
T. per 4/Ac. Squarrosa 2144	S								USA
Yecora	S								Mexico

APPENDIX TABLE 4. List of diploid ryes tested for their reaction to Al toxicity. Origin and reaction to several Al concentrations. T=Tolerant, S=Sensitive, V=Variable.

Cultivar	Concentration of Al in ppm. 1	10	15	20	25	30	35	Origin
Abruzzi	T		T	V	V	S		Spain
Rye	T			T			T	Spain
Otterbacher	T		T	V				Austria
Rye 37	V	V	S	S				Afghanistan
Rye 176	T			T	V	V	V	
Rye 1002	V	V	S					Turkey
Rye 1003	V	V	S					Turkey
Rye 1133	V	V	S					Turkey
Rye 1134	T	V	T	S				Turkey
Rye 1455	T			T	V	V	S	Brazil
Rye 1456	T			T	V	V	S	Brazil
Rye 1442	T			T	V	S		Brazil
Rye 1443	T			T			T	Brazil
Rye 1444	T			T		T	V	Brazil
Rye 1445	T			T		T	V	Brazil
Antelope B/Seed	T			T			T	USA
Antelope W/Seed	T			T			T	USA
Blanco Rye	T			T			T	Brazil
Caribou W/Seed	T			T			T	USA
Caribou B/Seed	T			T		T	V	USA
Cougar	T			T		T	V	USA
Frontier	T			T		T	V	USA
Dakold W/Seed	T			T		T	V	USA
Dakold B/Seed	T			T		V	V	USA
Snoopy	T			T		V	V	Mexico
Snoopy Dwarf	T	T	T	T	V	V	-	Mexico
Harlan JR 1794	T	V	V	S				Turkey
Harlan JR 1994	T	V	V	S				Turkey
Gentry HS 14191	T	V	V	V	T			Pakistan
Smith EE 683	T	V	V	S				Afghanistan
Smith EE 743	T	V	V	V	T			Afghanistan
Gentry HS 15320	V	V	V	S				Iran
Lavaszpatonai	T			T		T		Hungary
Knowles PF 1548	T							Yugoslavia
K 7276	T			T		V		USSR
K 9573	T			T		V		USSR
K 9805	T			T		V		USSR
Hohennauer	T			T		V		Australia
Lungaver	T			S				Australia
Gayerobo	T			T				Brazil
1-22-141	T			T	V	V		Brazil
1-22-142	T			V	V	V		Brazil
1-22-143	T			T	S			Brazil
Wrens Abruzzi	T							Spain
Adams	T			T		T		Spain

APPENDIX TABLE 5. List of hexaploid and octoploid triticales tested for their reaction to Al.

Hexaploid	Concentration of Al in ppm 5	10	15	20	30	Origin
Chapala/snoopy	T	V	V	S		
Chapala/snoopy		T	V	S		
Chapala/snoopy	T	V	V	S		
Cisne/snoopy	V	V	S			
Cisne/snoopy	V	V	S			
Flamingo/snoopy	V	V	V	V	S	
Flamingo/snoopy	V	V	V	S		
Cocorit/snoopy	T	V	S			
Cocorit/snoopy	T	V	S			
Jori/snoopy	T	V	S			
Gaviota/snoopy		T	V	V	S	
Pinguino/snoopy		T	V	S		
Cajeme/snoopy			T	V	S	
Yemon/snoopy	T	V	V	S		
Giorgio/snoopy	T	V	V	S		
Brant/snoopy		V	V	S		
Brant/snoopy	V	V	S			
Albatross/snoopy	T	V				
Var/23 snoopy	T	V	S			
Polonicum/snoopy		T	V			
Antelope	T	S				
Antelope "s"	T	V	S			
Jaboli	T		S	S		
Leopardo	T		V	S		
Zebra	T		V	S		
Fox	T		V	S		
Deer	T		V	S		
Buffalo	T		V	S		
Reindeer	T		V	S		
Puppy	T		V	S		
Cinnamon				S		
Dach "s"			V	S		
Bgr-Cal			V	S		
VM-940"s"-Arms			V	T	T	
Kla "s"	V	S				
VM-940"s"-MY64			V	T	T	
Tcl Maya II	V	S				
Tcl Maya II-Arm			V	S		
Tcl Maya II-Arm			V	S		
Tcl Maya II-Arm			V	S		
Inia-Arm "s"	V	S				
Inia-Arm "s"			V	S		
Inia-Arm "s"			V	S		
Inia-Guarday Arm "s"			V	S		

APPENDIX TABLE 5. Continued

(Inia-Rye) Arm "s"			V	S		
(Inia-Rye) Arm "s"			V	S		
(Inia-Rye) Arm "s"			V	S		
Ciervo			V	S		
Octoploid	5	10	15	20	30	Origin
Tcl Maya II	V	S				Mexico
Cajeme/snoopy			V	V	V	Mexico
Penjamo 62/snoopy	T	V	S			Mexico
Inia 66/Rye	V	V	S			Mexico
(Inia-Rye) Rye	V	V	S			Mexico
Nugaines/Dakoid		T	T	V	V	U.S.A.
Daws/Antelope B		V	V	V		U.S.A.
Daws/Cougar		V	V	V		U.S.A.
X - 17761		V	S			Mexico
X - 17763		V	S			Mexico

APPENDIX TABLE 6. Mean root regrowth (mm) of three cultivars of wheat, two of rye and amphiploids derived from them.

Concentr. of Al.	Cultivar	Root Regrowth**	Standard Error	H.S.D.*
1 ppm	Hope 7D x Rye 1133	21.9a	8.0	9.2
	Penjamo 62 x Rye 1003	15.6ab		
	Hope 7D	12.5bc		
	Penjamo 62	11.8bc		
	Rye 1133	6.2bc		
	Rye 1003	5.3c		
	Inia 66 x Rye 1003	0.0c		
	Inia 66	0.0c		
4 ppm	Rye 1003	4.6c		
	Rye 1133	3.4c		
	Penjamo 66 x Rye 1033	3.4c		
	Hope 7D x Rye 1133	2.1c		
	Inia 66 x Rye 1003	0.0c		
	Penjamo 062	0.0c		
	Hope 7D	0.0c		
	Inia 66	0.0c		

* H.S.D. Honestly significant difference

** Means noted by the same letter were not significantly different at 5% level.

APPENDIX TABLE 7. Mean root regrowth (mm) of Atlas 66, Carazinho, Rye 37, Rye 1133 and the amphiploid derived from them.

Concentr. of Al.	Cultivar	Root Regrowth**	Standard Error	H.S.D.*
5 ppm.	Atlas 66	24.5 a	23.70	7.27
	Carazinho	21.6 a		
	Atlas 66 x Rye 37	19.1 ab		
	Carazinho x Rye 1133	14.1 ab		
	Rye 37	0.0 c		
	Rye 1133	0.0 c		
10 ppm	Atlas x Rye 37	7.8 a	1.40	1.76
	Carazinho x Rye 1133	7.3 a		
	Atlas 66	6.3 ab		
	Carazinho	4.7 ab		
	Rye 37	0.0 c		
	Rye 1133	0.0 c		

* H.S.D. Honestly significant difference

** Means noted by the same letter were not significantly different at the 5% level.

APPENDIX TABLE 8. Effect of Al on the average mitotic activity wheat, rye, and triticale at different times of exposure.

	PROPHASE			METAPHASE			ANAPHASE			TELOPHASE		
Time Hours	S	T	C	S	T	C	S	T	C	S	T	C
3	16.2	29.4	54.8	7.6	14.3	27.9	1.5	7.7	15.0	8.0	15.5	27.4
6	9.3	22.9	39.9	4.4	20.4	26.5	1.5	5.4	17.0	3.9	9.8	20.4
9	3.8	11.7	36.3	1.5	5.8	26.3	0.8	1.6	14.6	1.2	3.3	19.4
12	3.1	12.6	28.3	0.4	6.1	16.7	0.3	2.0	14.2	0.5	5.6	23.2
24	2.2	10.8	39.7	0.1	6.6	22.6	0.0	6.5	15.2	0.6	7.8	30.5
36	1.2	14.7	45.0	0.7	7.0	26.4	0.6	6.0	18.5	0.2	10.1	29.8
48	0.6	31.3	27.5	0.3	33.1	23.7	0.0	7.1	19.5	0.0	10.3	27.0

S = Sensitive; T = Tolerant; C = Control